MICROBIOLOGICAL REVIEWS, Dec. 1990, p. 381–431
0146-0749/90/040381-51$02.00/0

Vol. 54, No. 4

To Shape a Cell: an Inquiry into the Causes of Morphogenesis of Microorganisms†

FRANKLIN M. HAROLD

Department of Biochemistry, Colorado State University, Fort Collins, Colorado 80523

The Word is an edged tool, equivalent to the sculptor's chisel, for chipping form out of the obstinate material of consciousness.

Philip Glazebrook
Journey to Kars

INTRODUCTION

Of cellular morphogenesis it can justly be said that we know much but understand little. Thanks to the labors of biologists over many generations, a huge body of literature now records the form, anatomy, and life cycle of innumerable single-celled creatures, procaryotes as well as eucaryotes. However, the student of morphology will be hard put

† Dedicated to Arthur L. Koch, as a token of admiration and friendship, on the occasion of his 65th birthday.

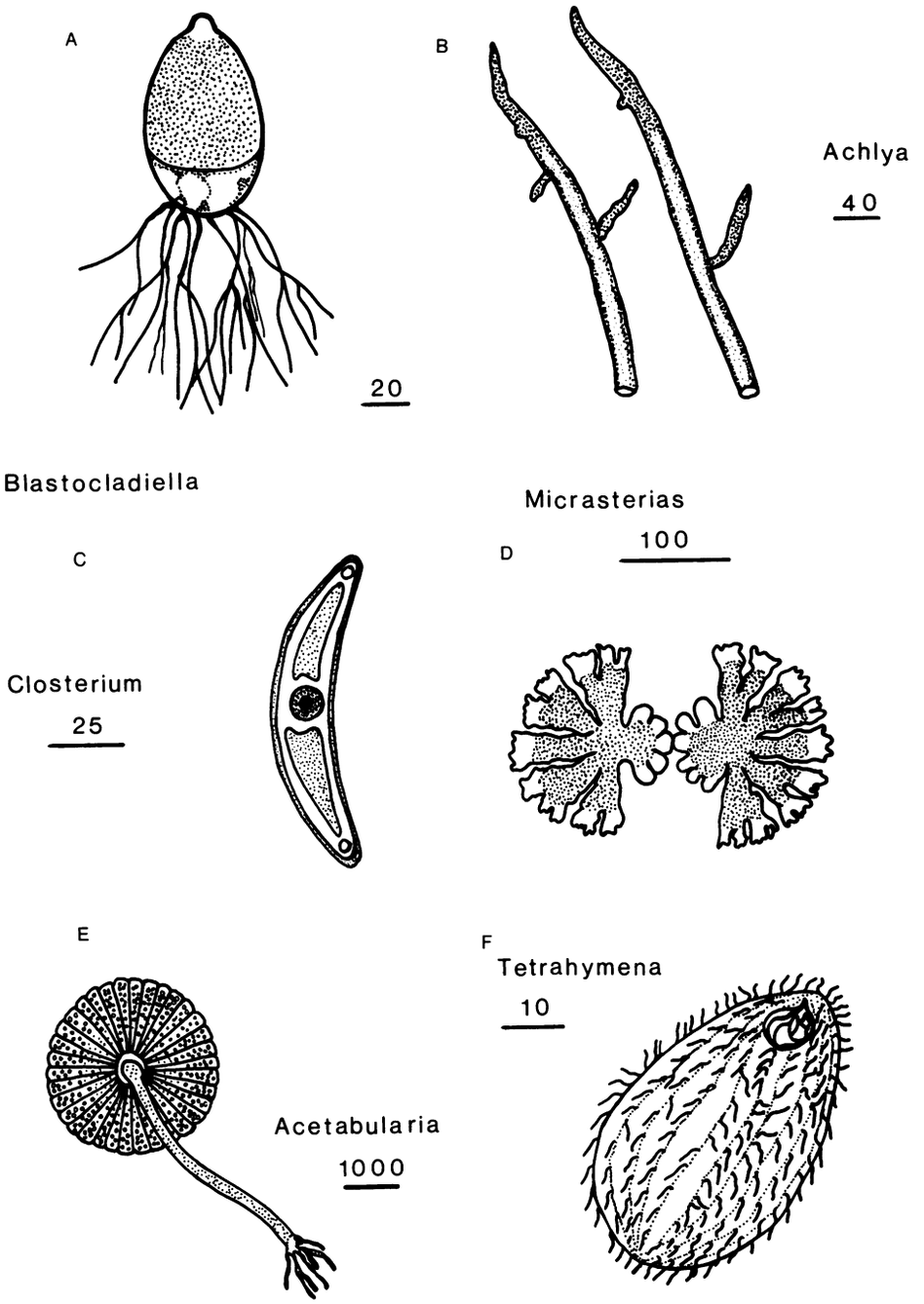


FIG. 1. Sampler of eucaryotic microorganisms. (A) *Blastocladiella emersonii*, developing sporangium. The discharge papilla has appeared, and a cross-wall separates the cytoplasm from the empty rhizoids. (B) *Achlya bisexualis*, hyphae with branches. (C) *Closterium littorale*, mature cell with central nucleus. (D) *Micrasterias denticulata*, midway through division; each daughter consists of one mature and one developing semicell. (E) *Acetabularia mediterranea*, a gigantic unicellular alga; note the reproductive cap and the rhizoid which contains the nucleus. (F) *Tetrahymena thermophila*, showing ciliary rows and buccal cavity. Scale bars in micrometers. Drawn by Ruth Harold after various sources.

to discover in this literature more than a very few explanatory principles; we have facts in abundance, but few general relationships with which to weave the particulars into a comprehensible pattern.

Reflect for a moment on the sampler of unicellular organisms illustrated in Fig. 1. How do these shapes arise as each organism grows, divides, and traverses its life cycle? How is form so faithfully transmitted from one generation to the next that a glance is often sufficient to distinguish one species from another? How is the original form regenerated after injury? And what do these forms mean: are they products of natural selection, frozen accidents of biological history, or expressions of higher-order morphogenetic laws? These riddles define the scope of the field; we have no satisfactory solution to any one of them, and to find the answers we shall plainly require much experimental infor-

mation that is not now available. However, data in themselves will not suffice: as the late Sir Peter Medawar once put it, no new principle will declare itself from beneath a heap of facts. It is timely, even now, to distil from the avalanche of observations whatever general principles can be discerned. That is the object of this article. I do not propose to solve any one of the many puzzles in microbial morphogenesis, but to help fashion a conceptual framework upon which to hang the facts garnered by research on particular organisms.

Let us consider at the outset what such a broad-gauged inquiry into cellular morphogenesis may hope to accomplish. In the tradition of classical morphology, a primary goal would be to identify and classify the basic shapes that cells can assume; to display morphological regularities corresponding to such familiar categories as mushrooms or tetrapods; and, if possible, to express the harmony of forms in mathematical language. Aside from the familiar classification of bacteria by their shapes, this approach has not commended itself to microbiologists, and I shall not pursue it here. Alternatively, and more in keeping with the spirit of the times, we can try to formulate causal principles to explain how biological forms arise and how they are transmitted from parent to offspring. The very diversity of microbial forms warns us immediately that there is no simple unitary principle of morphogenesis, in the sense that unitary principles do account for heredity, protein synthesis, or oxidative phosphorylation. On the other hand, it seems implausible that each organism is altogether unique. More likely, the multitudinous forms represent variations on a much smaller number of generative themes, whose discovery is the proper goal of research in cellular topobiology (63).

Why is it that we understand so much less about the manner in which cells shape themselves than we do of their metabolism or genetics? This huge lacuna in our comprehension of biology, rarely emphasized in textbooks, is ruefully attributed by those who recognize it to the complexity of the problem. Difficult it certainly is, but I suspect that the obstacles have been magnified by the way the quest is formulated. In today's emphatically molecular phase of biological thought, it is all but taken for granted that principles of morphogenesis will be expressed in molecular terms. Insofar as organisms are composed of molecules and obey the laws of physics and chemistry, all aspects of biology are rooted in the molecular dimension. It does not follow, however, that the molecular level is appropriate for the formulation of morphogenetic principles, just as the atomic level proved unsuitable when the objective was to understand metabolic pathways. Cellular forms are expressed on a scale 3 to 6 orders of magnitude above the molecular and represent the cooperative interactions of millions of molecules. To attempt to reduce morphogenesis to the molecular level seems to me as futile as to expect close scrutiny of the computer chips to explain the reservation system of an airline. Instead, I shall begin from the premise that every cell and organism is an integrated system that displays a particular pattern of structure and function. Form is one aspect of this pattern. Growth of a cell is not merely the accretion of molecules of the proper kind, but designates the enlargement or replication of the pattern. By the same token, development refers to the orderly transformation of one pattern of form and function into another. Growth, morphogenesis, and development thus appear to be fundamentally problems of biological order; as with other emergent properties of complex systems, the characteristics of the parts are often less informative than the rules of their articulation. The basic reason why our vast store of biochemical and genetic knowledge has yielded so meager an understanding of morphogenesis is that it does not directly bear on the problem.

In what follows, no attempt will be made to describe individual pathways of growth and development, or to survey all the variety of microbial shapes. Indeed the term microorganism will be used loosely to cover all free-living and single cells; interactions between cells will not be considered. The term morphogenesis will refer to the processes that generate the forms of cells in the course of their growth, division, or development; I will not discuss the forms of molecules and organelles. Since my object is to review what has been learned rather than what has been done, I shall emphasize concepts and ideas over facts or data; it will often be convenient to cite reviews rather than experimental papers. Responses to the literature extend through 1989. Ideas applicable over a reasonably broad range of organisms are not plentiful, nor are they necessarily recent, and they must be unearthed from beneath a mounting overburden of print. The task would have been hopeless but for the writings of others—especially those of John T. Bonner, Joseph Frankel, Brian C. Goodwin, Paul B. Green, Lionel F. Jaffe, Arthur L. Koch, Gunther S. Stent, D'Arcy Thompson, and Paul Weiss—which have shaped my reflections over the years. I can lay no claim to technical expertise in most of the subjects touched upon below, but since specialists are notoriously reluctant to venture beyond the bounds of their subject, amateur status may be accounted a virtue. Aside from the framework of this essay, little in it is original; I think of it as a distillate of other men's flowers. The article will have fulfilled its purpose if it encourages a few colleagues to look up from their clones or gels and resume the quest for an understanding of biological wholeness.

STRATEGY OF THE GENES

The forms of microorganisms are quite strictly inherited, in gross as well as in detail. Most biologists therefore take it for granted that form, like other aspects of cell structure and function, is determined by its genes: "The whole plan of growth, the whole series of operations to be carried out, the order and the site of syntheses and their coordination are all written down in the nucleic acid message." (135). No one claims that form is directly encoded in the genome; rather, the genome is thought to embody a program that specifies the manner in which form arises during growth or development. This conception shapes almost all contemporary research in developmental biology and is implied whenever one reads that a particular gene controls or determines a biological process.

The heuristic value of this hypothetical program hinges upon the meaning one assigns to terms such as controls or determines. As Goodwin (84) points out, in daily parlance these terms imply that "knowledge of the information in the genes is *sufficient* to determine the form that is produced by the products" (his emphasis). If the morphogenetic program is to be more than a truism, one must state explicitly what steps lead from linear nucleotide sequences on the nanometer scale to particular three-dimensional shapes 4 or 5 orders of magnitude larger. One should assess whether this sequence is accurately described as the execution of a program. One must also ask whether all features of a cell's form can be traced to the expression of particular genes or whether some are better ascribed to more global physical or informational processes. These abstract but important issues can be addressed empirically through the study of morpho-

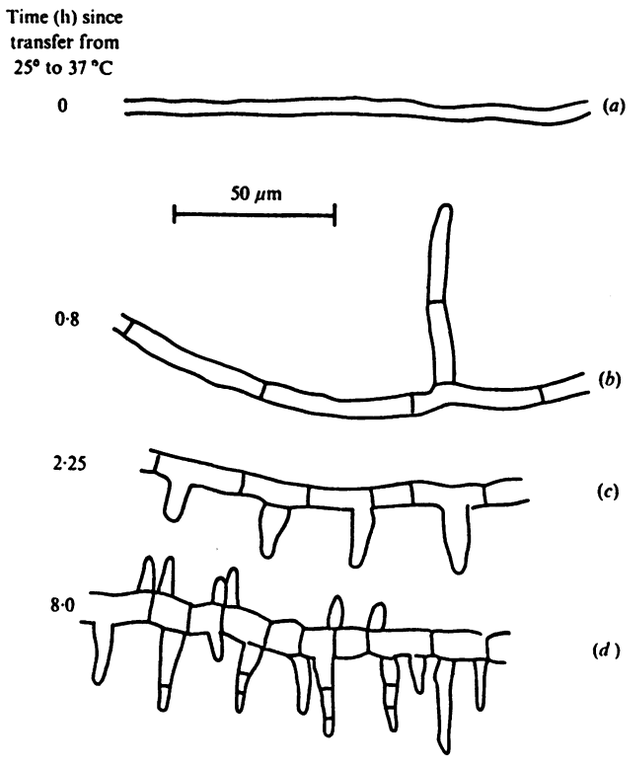


FIG. 2. Colonial mutant of *N. crassa*. When shifted to the restrictive temperature, linear extension gives way to intense branching. Reprinted from *Transactions of the British Mycological Society* (50) with permission of the publisher.

genetic mutants, i.e., organisms whose form has been altered as a consequence of mutation.

Mutants Defective in Morphogenesis

There are, in principle, two ways to select mutants defective in morphogenesis. One is to screen for mutants that fail to complete the normal cycle of growth and development or that generate abnormal shapes and then to try to work out what is the matter with them (58, 109, 217). The second is to isolate or construct mutants defective in the production of a protein suspected to be involved in morphogenesis and then to examine the consequences of the lesion. Suppressors of the original mutation are likely to identify genes coding for proteins that interact with the defective one (131). Yeasts and filamentous fungi proved especially amenable to such research and will serve here to illustrate what has been learned concerning the relationship between genes and form.

Morphogenetic mutants of filamentous fungi. The first breakthrough in the analysis of the relationship between genes and form was achieved more than 20 years ago by S. Brody, then a postdoctoral fellow in E. L. Tatum's laboratory. His objective was to discover the primary defect of colonial mutants of *Neurospora crassa*; these mutants fail to spread, as the parent strain does, but give rise to tight colonies of intensely branched hyphae (Fig. 2). Brody and Tatum (31) reported that in one of these, *col-2*, the enzyme glucose-6-phosphate dehydrogenase differs from that of the parent strain in substrate affinity, temperature sensitivity, and stability in general; they concluded that the *col-2* locus specifies the primary structure of the enzyme. Subsequently, mutants deficient in phosphogluconic acid dehydrogenase and phosphoglucomutase were found to generate similar (but not identical) phenotypes (30, 32, 189). These prescient papers, which have been all but forgotten because computerized scholarship fails to retrieve them, clearly make an important point: genes do not determine form directly but act through lengthy cascades of pleiotropic effects. Sadly, we still know little of the chain of causality that links a defect in glycolysis to the propensity for branch initiation. Brody (30) has thoughtfully considered the possibilities: reduced generation of NADPH, diminished levels of linolenic acid in the plasma membrane, perhaps abnormalities in secretion or in cell wall assembly. The subject remains open, a standing invitation to researchers eager to carve out a niche at the interface of biochemistry, genetics, and morphology.

A roster of morphogenetic mutants of filamentous fungi, whose primary lesion has been at least partially identified, is presented in Table 1. These will be discussed in a later section, together with the analogous yeast mutants.

Genetic control of the yeast cell cycle. In 1971, Hartwell published the first in a continuing series of studies on

TABLE 1. Filamentous fungus mutants defective in morphogenesis

Genus	Gene	Phenotype	Primary defect	Reference(s)
Neurospora	*col-2*	Colonial: slow-growing compact and densely branched colonies. Mutants with mutations at many loci exhibit this phenotype, with minor variations	Glucose-6-phosphate dehydrogenase	31
	bal		Glucose-6-phosphate dehydrogenase	
	frost		Glucose-6-phosphate dehydrogenase	
	col-3		Phosphogluconic acid dehydrogenase	
	col-10		Phosphogluconic acid dehydrogenase	30
	rg-1, *rg-2*		Phosphoglucosemutase	32, 189
	inos	Colonial on suboptimal inositol	Inositol phosphate synthase	30
	crisp-1	Premature and profuse conidiation	Adenylate cyclase	209, 274
Aspergillus	*ts6*	Deficient in chitin synthesis; tips swell and burst at restrictive temperature	Glucosamine synthesis	147
	sepA2	Lacks septa at restrictive temperature, but hyphae extend normally	Unknown	281
	tubA, *tubB*	Benomyl blocks mitosis and nuclear migration, but tip extension is normal	α and β subunits of tubulin	76, 203
	brlA	Switches from apical to budding growth	Possibly a DNA-binding protein	3

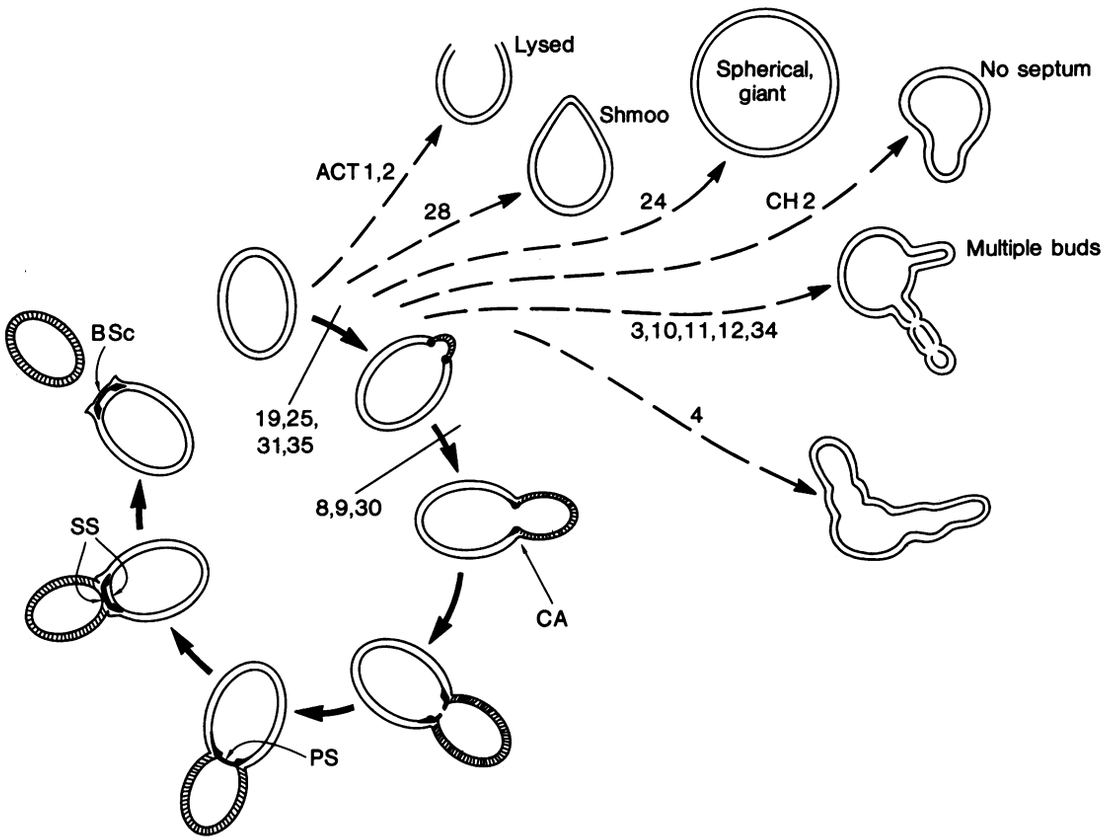


FIG. 3. Mutants of *S. cerevisiae* deficient in cell division and morphogenesis. Symbols: ——→, normal cycle of budding and septum formation; – – →, aberrant pathways. Abbreviations: CA, chitin annulus; PS, primary septum; SS, secondary septum; BSc, bud scar. For a description of the mutants, see Table 2.

temperature-sensitive mutants of yeast that are blocked at specific stages of cell division. These mutants, designated *cdc* for cell division cycle, were identified by the criterion that at the restrictive temperature each cell would cease to grow at the same point in the cycle. This would be the point at which the product of the defective gene is required. Consequently, an asynchronous population of a *cdc* mutant develops into a homogeneous one in which all the cells arrest with the same terminal phenotype. This phenotype may correspond to one of the normal landmarks of the cell cycle, or may be an abnormal form resulting from the continuance of some growth processes in the absence of the one blocked by the mutation (Fig. 3). The approach proved brilliantly successful. About 60 *cdc* mutants of *Saccharomyces cerevisiae* have been described, *Schizosaccharomyces pombe* has contributed as many again, and further mutants with lesions in cytoskeletal proteins and wall synthesis have been generated by gene disruption (for reviews see references 100, 109, 111, 131, and 217). Table 2 lists the best-studied mutants of bakers' yeast, particularly those in which the primary lesion has been identified. The stage at which each gene product is apparently required is indicated in Fig. 3, which also illustrates some of the abnormal forms produced in the absence of the gene product.

The use of these mutants to work out the sequence of events and their integration into an orderly cell cycle is described in the reviews cited above; the present discussion is restricted to the relationship between *CDC* genes and cell form. The cell cycle is genetically controlled in the sense that particular gene products are required at each stage and the sequence is aborted if one of them is lacking. However, the relationship between what the genes specify and the succession of shapes is evidently exceedingly indirect. In terms of their primary defects, the *cdc* mutants (Table 2) fall into three classes, which can also be recognized in Table 1. The first class comprises genes that specify structural materials or the enzymes required for their synthesis: tubulin, actin, chitin, DNA, 10-nm filaments. These mutants have supplied new and powerful instruments to dissect the functions of the gene products; were it not for these lesions, we would not have known that chitin synthase 2 deposits the septum (252), that actin plays a major role in bud emergence (199), or that microtubules are dispensable during budding (132). The second class consists of genes that code for enzymes in central metabolic pathways, such as pyruvate kinase and phosphoglucose isomerase; how defects in these enzymes arrest division at specific stages of the cell cycle is quite unknown. The third class contains genes whose products govern the rate of reaction at the branch points of chemical pathways: adenylate cyclase, protein kinase, calcium-binding proteins, and presumably many others.

The most intriguing of these mutants is the *cdc24* mutant, which is defective in bud emergence and polarization (255). At the permissive temperature, buds form well enough but at random loci, rather than being localized to the poles. At a restrictive temperature, mutant cells enlarge to six times the normal volume but cannot produce buds. Chitin and mannan are deposited at random all over the surface, chitin annuli are not made, secretion of acid phosphatase is delocalized, and the actin cytoskeleton is disorganized (2). Experiments

TABLE 2. *S. cerevisiae* mutants defective in morphogenesis

Gene	Terminal phenotype	Primary defect	Reference(s)
CDC4	Grows into uninucleate cells with multiple buds. Spindle-pole body (SPB) duplicated but not separated, DNA synthesis blocked	Unknown	2, 3
CDC3, *CDC10*, *CDC11*, *CDC12*	Grow as multinucleate cells with multiple abnormal buds, lacking chitin rings and 10-nm filaments	Synthesis of 10-nm filaments	2, 217
CDC8	Grows into singly budded cells with nucleus in the neck; DNA synthesis blocked	Thymidylate kinase	144, 248
CDC9	Like *cdc8*	DNA ligase	8
CDC19	Arrest as unbudded, uninucleate cells; SPB satellite absent	Pyruvate kinase	217
CDC21	Like *cdc8*	Thymidylate synthase	217
CDC24	Grows into huge, round, unbudded cells; secretion delocalized	Unknown, probably involves Ca^{2+}	205, 255
CDC25	Arrest unbudded in G_1, defective in Start	Positive regulator of cyclase adenylate	173
CDC28	Uninucleate, unbudded cells that grow into shmoos	Protein kinase	226
CDC30	In presence of glucose, arrest as uninucleate, singly-budded cells with long spindles	One of two isozymes of phosphoglucose isomerase	57
CDC31	Grows as uninucleate, singly budded cells; mitosis arrested with SPB; not duplicated	Protein related to calmodulin, may recognize a Ca^{2+} signal	15
CDC34	Like *cdc4*, multiple buds	Ubiquitin-conjugating enzyme, possibly tags histones	79
CDC35	Arrest as uninucleate, unbudded cells lacking SPB satellite	Adenylate cyclase	48
TUB-1, *TUB-2*	Accumulate as large-budded cells; DNA duplicated but chromosomes not separated	α and β subunits of tubulin, respectively	132
ACT-1, *ACT-2*	Unbudded cells that lyse and die; aberrant secretion	Actin	199
CHS-2	Cells enlarge, fail to divide, become misshapen and die; unable to make primary septum	Chitin synthase 2	252

described in the original publication (255) indicate that the *CDC24* product is instrumental in organizing the budding site. Subsequently, it is required to direct growth to that site and then into the bud and to determine the pattern of expansion within the bud. The function of the protein deficient in *cdc24* remains unknown, but is likely to involve Ca^{2+}. This was inferred by Ohya et al. (205), who isolated a mutant that is allelic to *cdc24* and exhibits a very similar phenotype when grown in the presence of 100 mM Ca^{2+}. The mutant has no obvious defect in either calcium uptake or extrusion and was therefore considered to be deficient in some calcium-binding regulatory protein. We shall return to these mutants below, in the context of pattern formation.

Is Morphogenesis the Expression of a Genetic Program?

At bottom, the genetic program is a metaphor. We ought to ask, not whether the idea is true but whether it is useful, and in this respect the results collected in Tables 1 and 2 are disconcerting. We now know many genes whose products are required for normal morphogenesis, and in a growing number the primary defect has been identified. However, successful completion of this demanding task brings one little closer to understanding either the normal shape or the mutant's aberrant one. There appear to be few true morphogenes (genes dedicated to specifying shape [58]), at least in microorganisms. (Such genes do exist in higher organisms, for example the *bicoid* gene of *Drosophila* species [61].) The great majority of morphologically abnormal microbial mutants represent defects in quite mundane metabolic or regulatory functions. The connection between genes and form is undeniable but oblique; even if we had on hand all the information encoded in the yeast genome, we could not infer the shape of the cell, let alone the succession of shapes during budding.

The reason is not difficult to fathom. Genes specify the primary structure of macromolecules, proteins in particular, and participate in controlling the timing and amount of macromolecule production. The primary structure is not irrelevant to spatial localization, as illustrated by self-assembly of supramolecular complexes (see below) and by the sequences that direct proteins to particular organelles. It remains true, however, that genes chiefly specify cellular composition. Morphogenesis is quite another matter, since it is the product of multiple interactions by many kinds of molecules on a scale several orders of magnitude higher than the molecular; it is especially a matter of large-scale spatial organization. It should come as no surprise that form, like other complex traits, should be linked quite indirectly to the genetic repository.

Is the genetic program a useful figure of speech to describe this relationship? As Stent (265) has pointed out, a program implies the existence of a directing entity separate from the events themselves—the text of a play, for instance, or the countdown of a shuttle launch. Stubblefield (267), in a thoughtful paper, explicitly formulated the hypothesis that embryonic development in metazoa is controlled by a program encoded in the genome, and it would not be difficult to rephrase Stubblefield's hypothesis to account for the regularity and stability of cellular morphology. Stubblefield postulated the existence of control DNA, "a set of genetic elements activated in a specific sequence, one at a time. . . . The complete series of control units would constitute the encoded (and inherited) development program of an organism." (267). Control units are thought to exert their effects

by producing segments of RNA (or even DNA) that are translated into proteins or combined with other proteins to induce, enhance, or repress the activity of specific genes. A genetic program need not be mindlessly robotic: like the launch program, a control unit will test internal and external factors and take control of gene expression only when the test results are favorable.

A simple criterion for the existence of a genetic program in Stubblefield's rigorous sense is whether progression requires transcription of genes at particular stages. This is evidently the case in the development of many embryos (*Drosophila* embryos for example [133]), for which the concept of a genetic program seems quite appropriate. In unicellular organisms also, developmental pathways often call for stage-specific expression of previously quiescent genes, especially when the organism redirects its efforts from vegetative growth toward new goals such as mating or sporulation; the *Aspergillus brlA* gene, whose transcription is sufficient to switch the growth habit from apical to the budding mode, is a case in point (3). However, morphogenesis does not as a rule rely upon that kind of genetic control. The assembly of bacteriophages is not regulated by sequential transcription of genes and does not depend on the order in which the constituent proteins make their appearance. Instead, the orderly progress of assembly is ensured by the constraint that the subunits cannot associate at random: protein n becomes competent to bind protein o only after it has joined a complex that already contains proteins k, l, and m. Cell division in *Escherichia coli* is an intricate process involving at least 20 genes (58, 127), but there is no evidence that any one is transcribed at a particular stage of the division cycle: their products, rather than their expression, appears to be required. The same appears to be true of the yeast cell cycle: here again there is no evidence for stage-specific expression of the numerous *CDC* genes, except for certain histone genes and that for thymidylate kinase (182). The germination of *Fucus* zygotes (167) and the transformation of *Naegleria* amoebae into flagellates (74) both require new gene expression, but the requisite proteins are produced well before the shape changes take place. An extreme instance is the germination of the *Blastocladiella* zoospore. In response to an environmental signal, the swimming zoospore settles down and retracts its flagellum; the nuclear cap breaks down with the release of ribosomes; the mitochondrion fragments and gamma particles are activated; a primary cell wall is laid down and the germ tube initial emerges; all this takes place in the course of a few minutes, yet this radical transfiguration of the cellular order occurs in the absence of gene transcription or translation (259).

Enough is now known to suggest that the metaphor of a genetic program obscures rather than illuminates the quest for understanding of forms and their genesis. Gene products do, of course, play essential roles in morphogenesis—as catalysts, regulatory elements and structural materials. However, form does not appear to be hard-wired into the genome in some explicit, recognizable fashion. It seems rather to arise epigenetically, implicitly (one is tempted to say fortuitously) from the chemical and physical processes of cellular physiology. As Stent (265) put it, speaking of the development of the nervous system, it is a historical process in which one thing leads to another: given the same constraints, like causes will produce like consequences. Morphogenesis, it appears, belongs to the large class of processes that are regular and predictable but not programmatic, such as the ecological succession after a forest fire or the daily ebb and flow of city traffic.

All this has been said before and never better than by C. H. Waddington. I borrowed the title of this section from his book (293), and his is still the most graphic illustration of the relationship of genes to form. Waddington spoke of the epigenetic landscape (Fig. 4A). The trajectory of the ball, rolling down the deepest valley toward the front, represents the normal course of morphogenesis; a relatively minor change in the topography, or a judicious nudge, would deflect the ball into the adjacent valley, representing an aberrant morphology. And the genes? Figure 4B depicts the same landscape seen from beneath. It is a surface, held up by props and stays. These are the gene products, and the genes themselves are the pegs in the ground. It will be obvious that severing any one strand (mutation) will elicit changes in topography (and hence ultimately in form) that depend on all the interacting strands, those that are still exerting tension as well as the one that was severed.

Let me put this another way and have done. The interesting events in morphogenesis—how forms actually arise and how a dividing or developing cell models itself upon itself—take place on a higher plane than that of the genes. It is not sufficient to identify morphogenes and to work out their primary functions. The pertinent level is the epigenetic one; at the end of the day, morphogenesis is not really reducible to particular genes because it represents the end result of multiple pathways: cyclic, parallel, and interdigitating. Although grounded in the actions of molecules, it must be addressed as a problem in physiology.

PERSISTENCE OF FORM

If it is true that the forms of unicellular organisms are not direct expressions of recognizable genetic programs, the problem of the inheritance of form presents itself in a new and sharper light. To be sure, the faithful reproduction of the gene complement ensures the constancy of the gene products and hence the stability of cellular composition; self-assembly of supramolecular complexes carries order to the level of membranes and organelles. But how is it that the interplay of numerous gene products in physiological concert takes the same gross form, generation after generation, subject only to minor perturbation by environmental conditions? If not the genes, what stabilizes the product within narrow limits? Perhaps the gravest objection to the genetic program metaphor is that it distracts attention from the real issues: how the shapes of cells are produced and how they are inherited.

Structural Inheritance in Ciliates

A significant clue emerged 25 years ago, when Sonneborn and colleagues discovered that in ciliates certain structural alterations can be transmitted for hundreds of generations by nongenetic mechanisms. This undoubted instance of the inheritance of acquired characteristics leaves both the theory of evolution by natural selection and the central dogma quite unscathed, but has important implications for the inheritance of form.

To recapitulate briefly a tale well told by others (6, 17, 261), occasionally the conjugation of ciliates goes awry such that one exconjugant comes away with a small patch of its partner's cortex. The foreign patch winds up oriented such that the polarity of its ciliary rows is opposite to that of the host's rows (Fig. 5). The inverted ciliary rows persist and propagate themselves, with the result that after a few fissions the cells contain one or more entire rows of inverted polarity

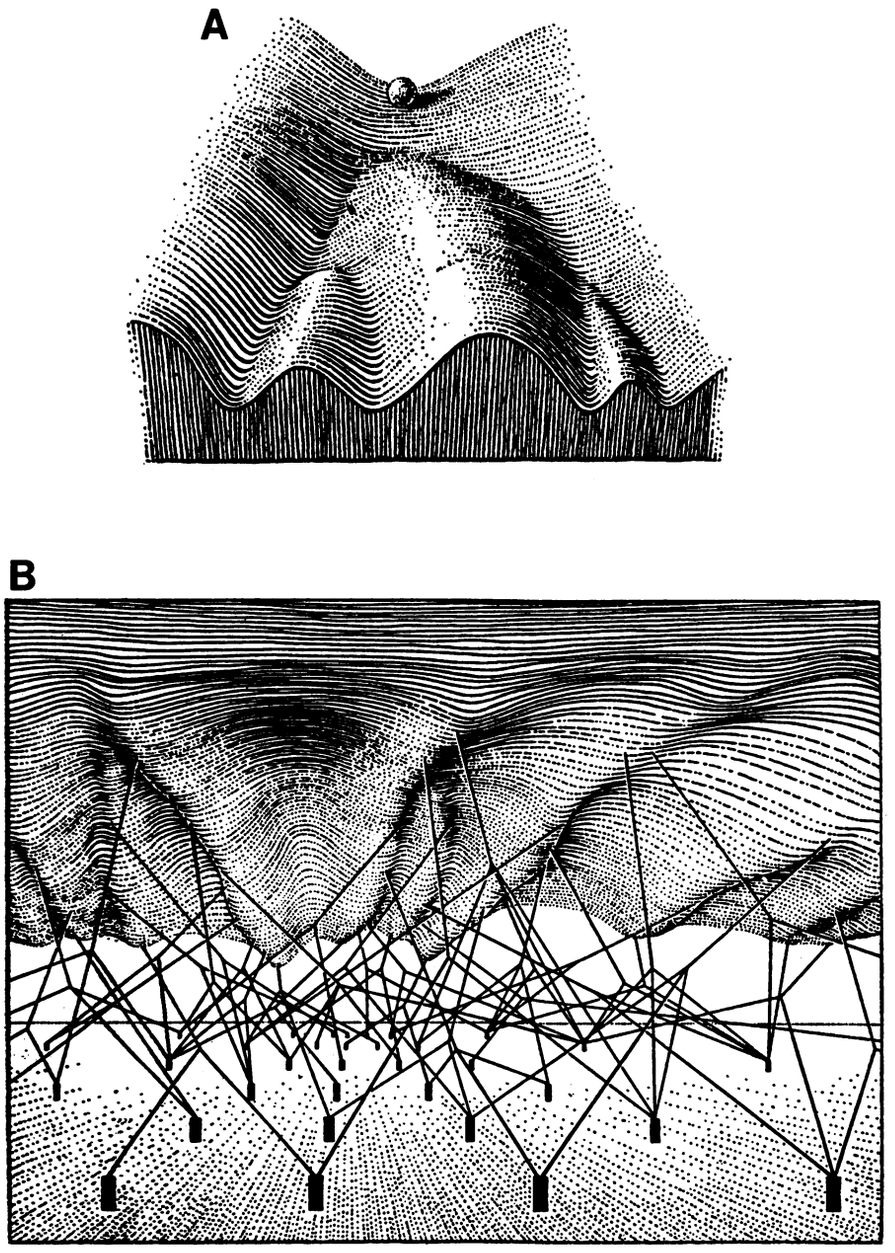


FIG. 4. Epigenetic landscape. The course of morphogenesis is represented by the path of the ball as it rolls downhill toward the observer. It will normally keep to the observer's left, but may be diverted into alternative channels by genetic or environmental changes. (B) System of interactions underlying the epigenetic landscape. The pegs in the ground represent genes, and the strings leading from them correspond to the activities of their products. Reprinted from reference 293 with permission from Unwin-Hyman.

(18, 261). Cilia that sprout from such rows beat in the reverse direction, and this confers upon the cell a characteristic twisty mode of swimming. Inverted rows are inherited faithfully from one generation to the next. They are not absolutely stable, throwing off normal lines at a low but significant frequency; nevertheless, with periodic selection of twisty cells for subcloning, inversions can be propagated for many generations, 800 in one instance. Sonneborn and colleagues took pains to eliminate the possibility, implausible to begin with, that a genetic mutation is responsible for the inversions of ciliary rows. By repeatedly backcrossing the inverted line to a normal one, they effectively replaced the genome (and, of course, the cytoplasm) of the inverted line by the normal one. At no time did the progeny of the normal cells acquire the inversion, nor did the progeny of the inverted line lose this characteristic. There is no escaping the conclusion that the inversion is propagated vegetatively in a fashion that depends neither on nuclear genes nor on components of the exchangeable portions of the cytoplasm. The vegetative transmission of inverted ciliary rows was subsequently documented again for *Tetrahymena* species by Ng and Frankel (197). The recent discovery that basal bodies of *Chlamydomonas* species, and presumably those of ciliates, contain DNA that codes for ciliary proteins (98) does not alter this conclusion.

There is no mystery about the persistence of ciliary

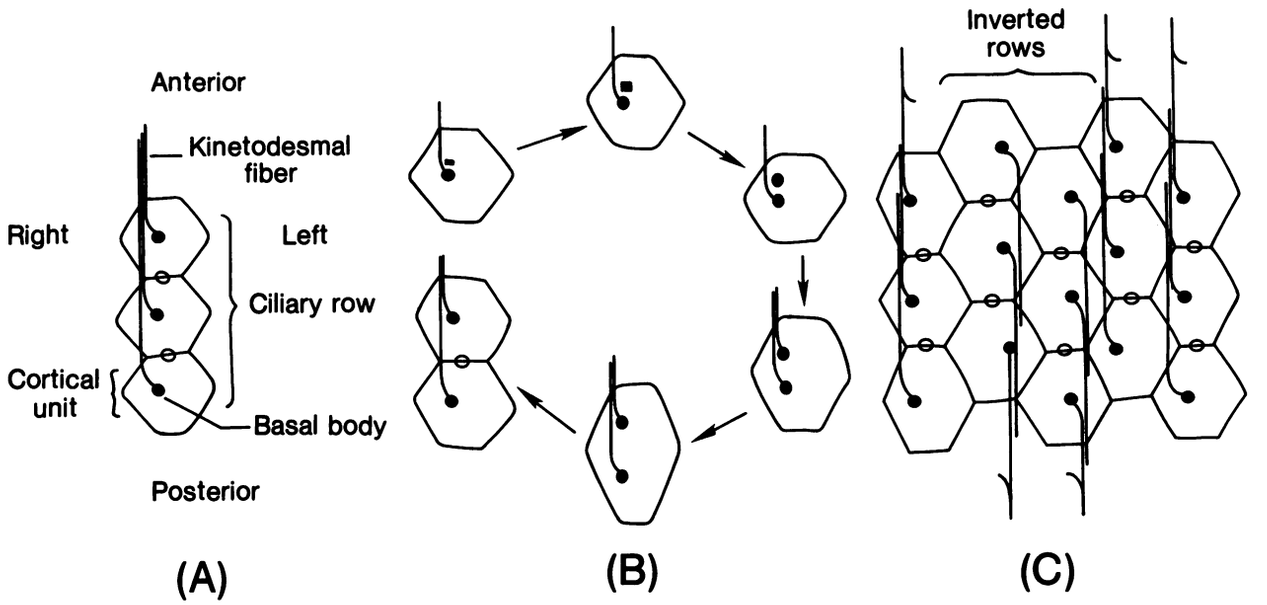


FIG. 5. Schematic representation of simplified ciliary units. (A) Frontal section. The asymmetry of the units and their packing pattern are evident. (B) Replication of a ciliary unit. A new basal body rudiment (rectangular element) appears within a preexisting ciliary unit in a precise spatial relationship to other structures. (C) Portion of a ciliate cortex with two inverted ciliary rows. Reprinted from the *Journal of Protozoology* (194) with permission of the publisher.

inversions in the absence of genetic change: the secret resides in the manner of ciliate growth. A clone of cells can be thought of as a continuously elongating cylinder: growing cells extend longitudinally while division cuts across transversely, producing daughter cells of coherent polarity (see Fig. 30). Among the elements that are continuous from one generation to the next are the ciliary rows. Rows grow linearly by the insertion of new basal bodies, which normally arise anterior to an existing one. Inverted rows consist of ciliary units that are normal in every respect, save only their orientation with respect to the axis of the cell. They elongate in the normal fashion, propagating the inversion because the position of new units is determined solely by the local geometry of existing units (Fig. 5). Clones bearing inverted rows tend to revert to the normal state by the loss of the abnormal rows: inheritance mediated through structural continuity is less stable than that transmitted through the genome.

A second and even more dramatic instance of structural heredity (17) is the propagation of doublets, which can arise in various ciliates including *Paramecium* and *Tetrahymena* species (6, 194, 260). By various tricks, two cells can be induced to fuse back to back, generating a homopolar doublet with two oral apparatuses, two sets of contractile vacuole pores, double the number of ciliary rows, and a common cytoplasm (Fig. 6 and 7). All the organelles are perfectly normal and are disposed relative to each other in the normal way. Doublets propagate as doublets for hundreds of generations, although they do tend to revert to singlets and periodic reselection is required to maintain the doublet state. By genetic tests, Sonneborn (260) documented that the difference between singlets and doublets is not determined by a difference in nuclear genes, by the state of expression of such genes, or by a difference in the exchangeable portion of the cytoplasm. Rather, it reflects the continuous and coherent propagation of surface organization.

Gene Mutations Can Alter Large-Scale Order

The counterpoint to structural inheritance of pattern is the role of genes, exemplified by mutations that affect pattern formation. The most remarkable of these is called *janus*, because, like the Roman god, it looks two ways (142). In *Tetrahymena* clones homozygous for *jan*, many of the cells lack a dorsal half but possess instead a second ventral one complete with a second oral apparatus and additional contractile vacuole pores (Fig. 7). At first sight, *janus* mutants resemble homopolar doublets, but in fact their organization is quite different. The first, or primary, oral apparatus is normal in structure and function. The secondary oral apparatus is generally not operative and is structurally abnormal; its position is not precisely opposite to the primary oral apparatus but slightly offset, dividing the cell circumference into a longer and a shorter arc. As far as global organization is concerned, the duplicate half cell is a mirror image of the normal half (note the tilt of the membranelles and the location of the contractile vacuole pores in Fig. 7C). However, at the level of the ciliary units themselves, the symmetry relations are the same as in jan^+ cells. Incidentally, *jan* mutations and their phenotype are also more stable than doublets. Evidently the *janus* gene affects some aspect of global cellular organization, and it is quite astonishing that a single mutation should suffice to invert it (70).

So what is the matter with *janus* mutants? At the biochemical level, no information whatsoever is available, but considerable progress has been made in identifying the breakdown of global organization that generates the *janus* phenotype. The experiments are intricate, and interested readers must refer to the original publications (see especially references 70, 71, and 142 and a summary by Frankel et al. [72]); suffice it here to indicate what they suggest regarding the interplay between genetic specification and structural transmission. The placement of organelles appears to be

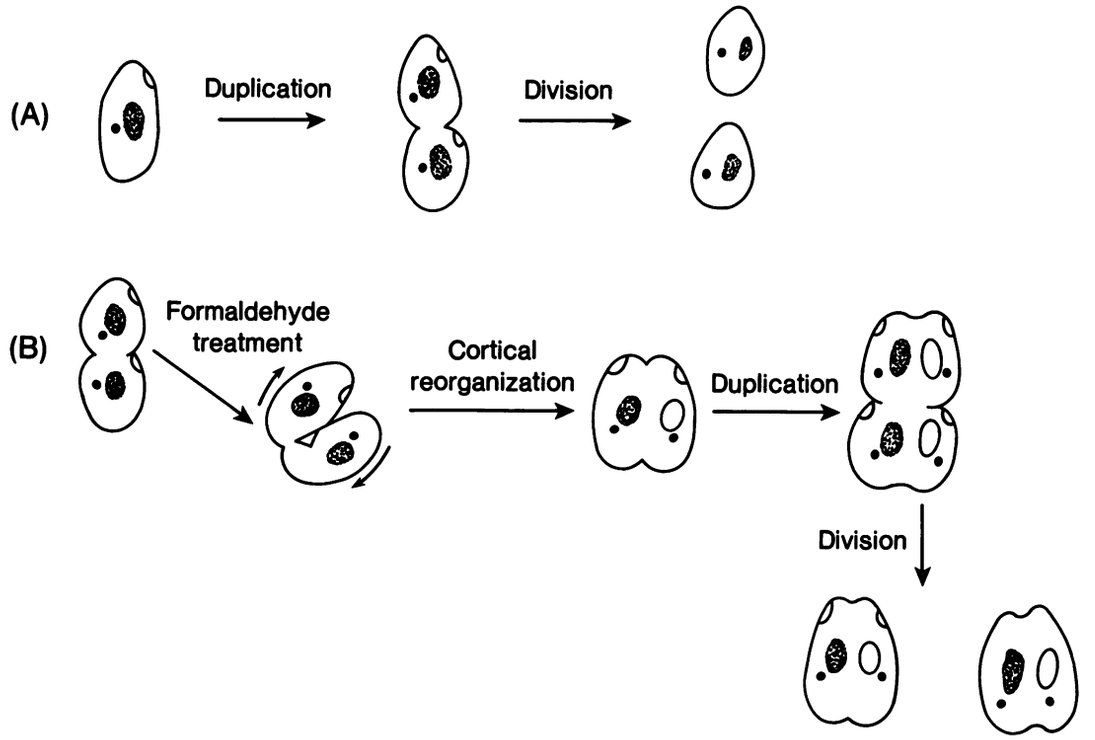


FIG. 6. Singlet and doublet ciliates reproduce by binary fission with retention of their global organization. Modified and reprinted from the *Journal of Protozoology* (194) with permission of the publisher.

determined by positional values along a circular gradient orthogonal to the axis of the cell (see the last section). The *jan* gene would be involved in maintaining these values, and the mutation would result in the loss of certain ones (e.g., the numbers 10 and 11 on a clock face). Normally, positional values propagate clonally as the cell elongates prior to division, and stable positioning of organelles around the circumference is thereby assured. In the mutants stability is jeopardized, forcing the cell to reorganize. The *janus* phenotype can be seen as a consequence of a particular mode of reorganization that involves the intercalation of allowable numbers in place of those lost, according to an explicit mathematical rule. An understanding of how organelles find their proper place will evidently depend on the appreciation of both levels: that of the gene products and their molecular actions, and that of their interplay to generate behavior that obeys mathematical rules.

Varieties of Spatial Memory

The geometrical considerations invoked by ciliatologists have found no echo in the work on other microbial systems. It is quite likely that, thanks to their complex surface organization and clonal mode of propagation, ciliates are unique and have capabilities not found in other unicells. However, it is also possible that structural continuity is inconspicuous rather than rare and deserves more consideration than it has received so far.

Next to ciliates, diatoms and desmid algae have the most elaborate surface organization. Dividing cells of the alga *Micrasterias* frequently produce morphological variants (Fig. 8): some lack one or both wings, whereas others display an extra wing at right angles to the plane of the normal two. Variants reproduce their kind but tend to revert to the normal biradiate form; they are traditionally referred to as mutants, but there seems to be no evidence of genetic change (see reviews by Kiermayer [149] and by Kallio and Lehtonen [145]; a much clearer account is given by Pickett-Heaps [212]). Could the variants be analogous to ciliate doublets that reproduce their form by structural inheritance? Recent work has shown that in uniradiate variants the distribution of membrane-associated calcium is altered, suggesting that the variants lack half of the prepattern that directs growth of the wings (184). What may be a related clue comes from the work of Ueda and Noguchi (288), who mapped the distribution of microfilaments in *Micrasterias* cells. Bundles extend from the tip of each lobe in the mother half cell, across the isthmus, and into the corresponding lobes on the same side of the daughter half cells. If these microfilament bundles play a role in the mechanism of morphogenesis, one can see how the loss of one lobe with its attendant microfilaments may preclude the initiation of the corresponding lobe in the daughter half cell, so that the morphological abnormality is carried over into the next generation (288).

Even if high-fidelity structural inheritance proves to be restricted to the most elaborately sculptured cells, one should expect less sophisticated modes of spatial memory to be common. In any growing or developing organism, new gene products come into a preexisting spatial context defined by structures laid down in the preceding cycle. These should influence, or even determine, the placement and orientation of new additions. Diverse structures can serve as spatial markers, illustrating a significant but little-studied aspect of the strategy of the genes.

A striking illustration from the bacterial world is the wall band of streptococci (see Fig. 10), which is discussed in the

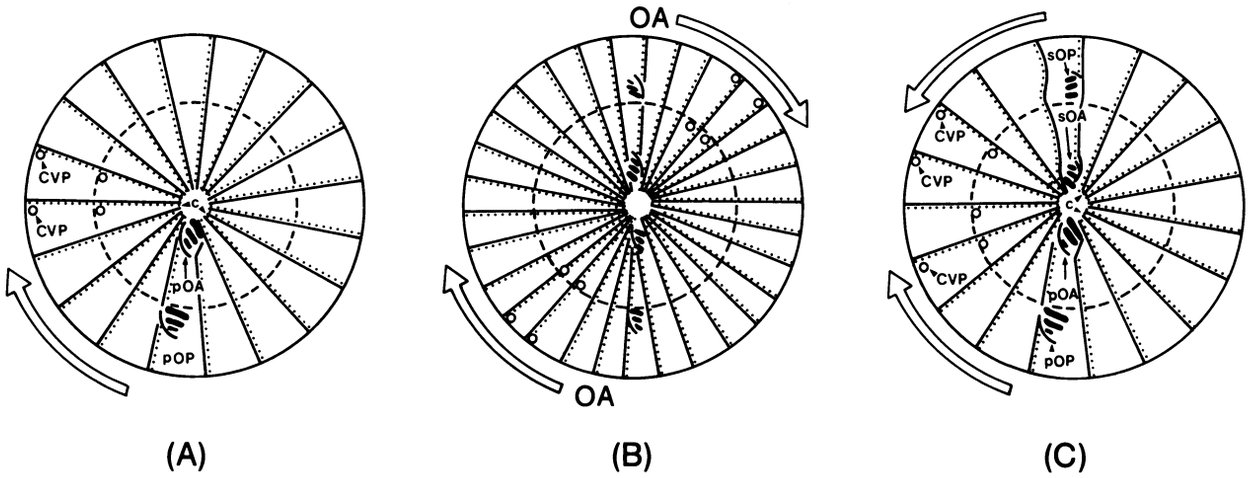


FIG. 7. Cell geometries in *Tetrahymena* cells. The diagrams show polar projections of dividing cells; the anterior pole is in the center, and the fission zone is indicated by the dashed circle at the equator. The external arrows emphasize the symmetry relationships between the oral apparatus and the contractile vacuole pores. Abbreviations: POA and POP, primary oral apparatus and primordium, respectively; SOA and SOP, secondary oral apparatus and primordium, respectively; CVP, contractile vacuole pore. The dotted lines represent the ciliary rows. (A) Normal singlet. (B) Homopolar doublet. (C) *janus*. Modified and reprinted from reference 72 with permission from Alan R. Liss, Inc.

next section. Suffice it here to note that a streptococcal cell bears a raised equatorial band. The cells grow by inserting new cell wall polymers into a zone formed by the splitting of the wall band; each daughter cell inherits one of the wall bands, which marks the boundary between the old surface and the new. Wall bands are immortal, each one descending from a prior one. Similarly, *Bacillus subtilis* constructs poles and side walls alternately; rigid poles set the diameter of the cell cylinder and vice versa.

It seems likely that the cell wall (and structures associated with it) often supplies a foundation that localizes and orients new structures, but there are not many clear examples. In *S. cerevisiae*, the neck of the early bud is delimited by a band of 10-nm filaments; the structures are indispensable, for mutants defective in the genes that code for the filament proteins fail to form buds (217). In due course, this becomes the site of the chitinous septum and eventually of the bud scar. When a *Micrasterias* cell divides, the primary wall bears three loci that will eventually mark the deep furrows delimiting the lobe and lateral wings (Fig. 8A) (149). Kropf et al. (168) found quite recently that protoplasts of the *Fucus* embryo can form a polar axis, but wall synthesis is required to fix it in space. However, the ability of most protoplasts to regenerate indicates that whatever spatial cues are supplied by the wall, their participation is not indispensable.

One class of organelles that unquestionably supply spatial

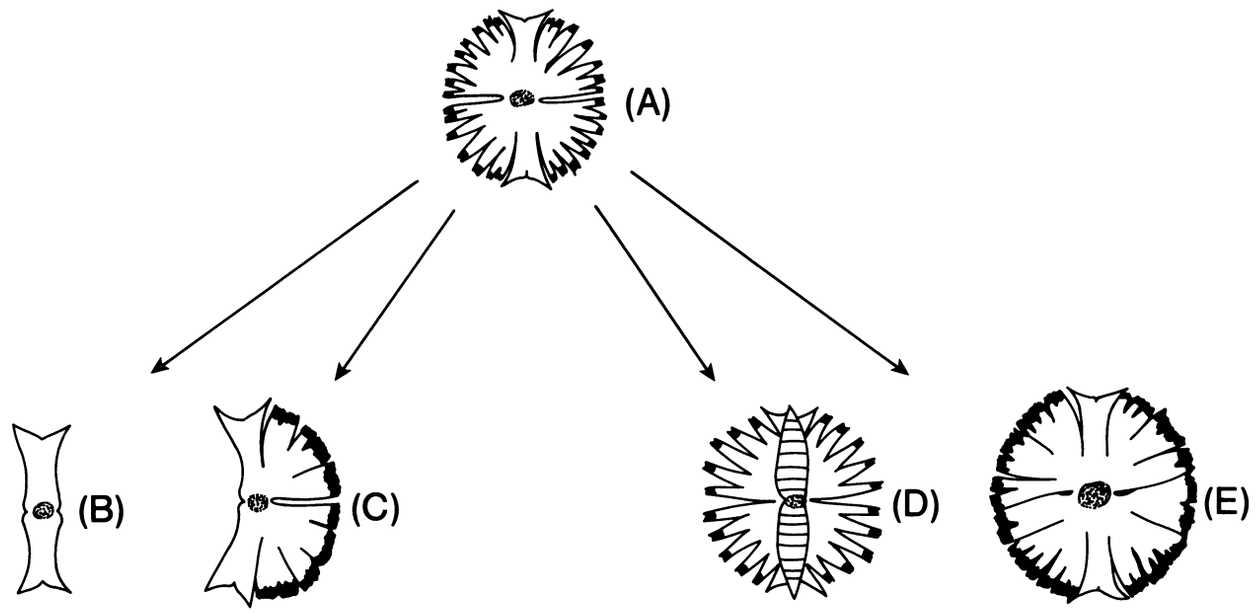


FIG. 8. Variants of *Micrasterias* sp. (A) The normal biradiate haploid. (B) Aradiate haploid variant. (C) Uniradiate haploid variant. (D) Triradiate diploid; the third wing comes out of the plane of the page. (E) Biradiate diploid. Modified and reprinted from reference 212 with permission from Sinauer Associates.

cues features the microtubule-organizing centers, a diverse set of structures that share the capacity to nucleate microtubule synthesis. Examples include flagellar basal bodies, the spindle-pole bodies of fungal and algal nuclear membranes, the centrosome of animal cells, and analogous sites in various walled and unwalled organisms (29, 97, 175, 285). Microtubule-organizing centers are thought to determine the number of microtubules that sprout from a given locus, their overall orientation, and their molecular polarity. By contrast, the length of individual microtubules and the extension or shrinkage of each are subject to local, dynamic control (152). Microtubules perform several roles in cellular physiology. They supply the scaffolding for flagellar axonemes and for a host of feeding structures in ciliates (89, 285). They also serve as tracks for the translocation of cytoplasmic vesicles from their site of production to the cell surface (290). Quite recently it became clear that mitochondria, the Golgi apparatus, and even the endoplasmic reticulum are, so to speak, strung out along a microtubule framework (273, 277). Yet microtubules are often quite ephemeral structures that are disassembled during cell division and in response to various perturbations. The microtubule-organizing centers survive and are the foundation upon which the microtubule framework is reconstituted.

Illustrations of the role of microtubule-organizing centers as spatial markers abound. A classic one comes from the work of Bouck and Brown (19, 33) with the unwalled golden-brown alga *Ochromonas danica*. The organism maintains a distinctive teardrop shape with the aid of a double array of microtubules, one set to support the anterior beak and the other the tail. Both sets disassemble upon treatment with colchicine or high pressure, and the cell rounds up; removal of the drug or release of the pressure allows microtubules to sprout again, with restoration of the original shape. A more subtle illustration is the role of flagellar basal bodies in maintaining cellular asymmetry during the division of *Chlamydomonas* cells, thereby ensuring the correct positioning of cellular organelles (128). Microtubules are especially prominent in organizing animal cells, whose polarized form and behavior depend directly on the integrity of the microtubule array. Microtubules grow out of the centrosome, a unique region adjacent to the nucleus. Polarized movement is preceded by repositioning the centrosome ahead of the nucleus (253). The position of the centrosome may also be what accounts for the curious observation that the daughters arising from the division of nerve cells have similar shapes, but with mirror-image symmetry.

Cell walls and microtubule-organizing centers are probably not the only organelles involved in spatial continuity. Localized ion channels (tethered to the wall or to the cytoskeleton) have been spoken of but not documented. In *Drosophila* embryos, the future head is identified by stable maternal mRNA, which is translated locally to generate a protein that serves as a morphogen (61). Whether such mechanisms are also found in microorganisms remains to be seen. In any event, the question of how spatial continuity is maintained during growth and development must be posed and answered for each organism individually.

Self-Organization

The relationship between genes and form was first explored 50 years ago, by taking advantage of the remarkable anatomy of the unicellular marine alga *Acetabularia mediterranea* (Fig. 1E). The organism is gigantic as cells go. The stem is several centimeters in length; at one end is a holdfast or rhizoid which contains the single nucleus, and at the other is a hat-shaped cap in which the gametes form. The shape of the cap is characteristic of the species. The cap regenerates after amputation, and this operation can be repeated several times; the nucleus likewise can be removed or transferred from one plant to another. By performing experiments of this kind, Hämmerling found that the structure of the cap is specified by cytoplasmic determinants now thought to be mRNA (99). The ultimate source of these determinants is the nucleus: when the nucleus from one species was transferred to an enucleated plant of another species, the cap produced after repeated cycles of regeneration was characteristic of the donor of the nucleus (99).

The conclusion that cellular forms are ultimately determined by the genome is, of course, all but universally accepted, but consensus rests upon a narrow base of observations, especially the extensive researches of J. B. Gurdon on nuclear transplantation in amphibian embryos. I am aware of only one further study at the cellular level, performed with fibroblasts. By treating the cells with cytochalasin followed by centrifugation, one can separate the nucleus (encased in a thin sheath of cytoplasm—a karyoplast) from the remainder of the cell (the cytoplast); karyoplasts and cytoplasts can also be fused to reconstitute viable cells. Hightower et al. generated cybrids from mouse-cell karyoplasts and human cytoplasts; within 48 h, the form of the cybrids had become indistinguishable from that of the donor of the nucleus (121).

In the 1960s, with cytoplasmic inheritance in the air, the possibility that cytoplasmic heritable factors supplement the nuclear ones was widely debated, but was ultimately rejected (17) for lack of convincing evidence. Structural inheritance in ciliates obviously is a mode of heredity, but one operating on a shorter time frame than the genetic one and on quite another level of organization. This applies a fortiori to the less rigorous kinds of spatial memory exemplified by the position of microtubule-organizing centers: they are subject to superior nuclear control and are ultimately dispensable. The only permanent determinants of form appear to be nuclear ones. However, it must be clearly recognized that the observations bear only on the differences between two related shapes: on the details that distinguish the cap of *A. mediterranea* from that of *A. crenulata*, not on cap formation per se. Whether gross cellular forms can be meaningfully said to be determined by the genome is simply not known.

I cannot help feeling that in our haste to tidy things up by crediting everything to DNA ("The whole plan of growth, the whole series of operations to be carried out . . . " [135]), we have swept out of sight the very problem we set out to solve. A cell is a system; it exhibits properties that emerge as a consequence of its state of organization and that cannot be simply attributed to one part or another. The genome is one component of the system, an essential and prominent one, but a cell is not an aggregate of its gene products. The form of a cell is one of its system properties, produced by a hierarchy of self-organizing processes, and can be understood only as an expression of this functional unity. Morphogenesis is not a problem in molecular biology, but in the physiology of highly organized systems. It should become more tractable when we achieve a clearer sense of what cell shaping entails: what elements are organized in space, what forces are deployed, and how localization is achieved. What little has been learned of these matters is the subject of the remainder of this review.

D'ARCY THOMPSON'S LEGACY

The objects that surround us in daily life, most of them man-made, were produced by shaping a natural or artificial material to suit some function or purpose. They owe their form to the application of physical forces, most commonly mechanical ones. The idea that biological forms likewise reflect the action of physical forces on formless protoplasm is a venerable one, although somewhat unfashionable today. D'Arcy Thompson's book, *On Growth and Form*, first published in 1917, remains the classic statement of this point of view: "The form. . .of any portion of matter, whether it be living or dead, and the changes of form which are apparent in its movements and in its growth, may in all cases alike be described as due to the action of force. In short, the form of an object is a 'diagram of forces' in this sense, at least, that from it we can judge or deduce the forces that are acting or have acted upon it" (p. 11 of reference 276). Some forces act between molecules, whereas others exert effects upon larger objects. Consequently, the statement that form reflects the action of forces past or present is not a hypothesis that can be falsified by experiment: it is a truism. Thompson's insight is nonetheless of enormous heuristic value, for it challenges us to inquire just what forces have impinged on a given cell and what contribution they have made to its form.

Self-Assembly: Phage Model of Morphogenesis

If there is a single principle of morphogenesis universally acknowledged by biochemists and cell biologists, it is self-assembly. The term is used quite loosely to designate phenomena ranging from the molecular level to the organismic, but always refers to structures that arise by the spontaneous association of their component parts and whose shape is wholly specified thereby. Despite the ambiguity of the term spontaneous, let alone "wholly," it is not difficult to recognize a class of biological events that can be usefully subsumed under this rubric and others that cannot. Bouck and Brown (20) and Anderson (5) have surveyed the varieties of self-assembly.

Examples will be found in any textbook. Nascent protein molecules fold spontaneously into their active configuration as they come off the ribosome and often refold correctly after denaturation. The same is true of RNAs held together by stretches of complementary bases, and of chitin or cellulose molecules that aggregate into fibrils. The only caveat is that correct folding requires suitable ionic conditions and sometimes posttranslational modification; these are hints that in reality the specification of form is somewhat more subtle (5).

Macromolecules of diverse kinds often associate into structures of higher order with little need for additional energy or information. Historically this insight stems from the discovery that tobacco mosaic virus can be dissociated into RNA and capsid proteins and that the purified components reassociate to reconstitute virions of normal shape and infectivity. Subsequently, Wood (304) and King (151) analyzed the biogenesis of the intricate bacteriophage T4 and found it to proceed by a succession of self-assembly steps. (For the moment I shall set aside the complications, including several enzyme-catalyzed steps and a set of scaffolding proteins that help assemble the head but are not retained in the finished structure.) It is important to note that the sequence of steps in virus assembly does not reflect the order in which the building blocks are produced but is intrinsic to the constituents themselves. Phage proteins cannot associate at random but must add onto a complex of the proper conformation, created by the prior association of at least two other proteins; it is the requirement for conformational switching that ensures the order of assembly. Thanks to this clever feature, it is valid to conclude that all the instructions required to generate the virus particle are supplied by the building blocks themselves; the final form is implicit in the information encoded in the viral genome.

The formation of biological structures by the assembly of subunits, with the shape of the product wholly or very largely determined by the subunits themselves, is unquestionably one of the most widespread modes of morphogenesis at the subcellular level. Microtubules assemble spontaneously from tubulin monomers, and microfilaments assemble spontaneously from actin. Myosin molecules aggregate into bipolar structures with the heads clustered at either end, just like the thick filaments of muscle. Bacterial flagella, ribosomes, nuclear membrane pores, clathrin cages, the glycoprotein subunits of algal cell walls, and the histones of nucleosomes supply further examples. Astonishingly, the eucaryotic nucleus itself can be reconstituted from its dissociated components and must be considered capable of self-assembly (196). Also, that most basic of biological shapes, the phospholipid bilayer vesicle, arises by self-association in the absence of informational macromolecules.

The assembly of molecules into regular, reproducible structures entails an increase in order, i.e., a diminution of entropy, yet it normally proceeds without input of either information or energy. What drives the interaction is usually the increase in entropy arising from the liberation of water molecules excluded from the complex. The hydrophobic effect (270) is especially prominent in the formation of lipid bilayers. Additional factors, notably charge interactions and hydrogen bonds between complementary surfaces, play a role in the association of macromolecules. In sum, self-assembly reactions are spontaneous in the thermodynamic sense: they move toward equilibrium.

Given the ubiquity of self-assembling entities in biology, can one regard the whole cell as the product of still further spontaneous molecular associations? Is cellular morphogenesis akin to crystallization, an entropic process moving down the thermodynamic hill toward equilibrium? In my view, the beginning of wisdom regarding cellular morphogenesis is to discard this simplistic belief. The forms of cells and the arrangement of their parts in space cannot be attributed solely to self-assembly of macromolecules by intermolecular forces on the nanometer scale, but commonly require large contributions from energy-consuming processes organized on the micrometer scale. The point deserves to be put in a little detail, for the assumption that self-assembly is the key to morphogenesis seems to have been held by no less a thinker than Monod (see p. 95 in reference 191) and is deeply embedded in contemporary molecular philosophy.

Consider, for example, the construction of biological membranes. The structure of integral membrane proteins favors their insertion into the lipid bilayer in an active configuration, a feature ascribable to the primary amino acid sequence. The primary structure may also include targeting sequences, which serve as an address to direct the protein to its destination. By contrast, the orientation of membrane proteins is not specified by their primary structure, but relies on the manner in which membrane biogenesis is organized. The bacterial cell supplies ribosomes and precursor proteins to the cytosolic surface of the plasma membrane and the appropriate lipids as well. The role of cellular architecture is

even more prominent in eucaryotic cells, with their elaborate machinery designed to deliver membrane-bound vesicles to the correct compartment. Clearly, molecular structure and the intermolecular forces implicit therein are insufficient to specify the organization of cellular membranes; there must also be an input of spatially organized work.

The walls of bacteria and fungi make another case in point. The shape of these cells is maintained by the wall and is determined by the spatial configuration of wall deposition during growth. However, the walls of *E. coli*, *Streptococcus* species, yeasts, or *Neurospora* species are not composed of subunits that assemble like the pieces of a Lego set. Rather, they resemble woven fabrics: the entire murein sacculus of a bacterial cell can be regarded as a single giant molecule (157, 297). The chemical composition of each wall reflects the operation of the cascade of enzymes and can ultimately be traced back to genomic specifications. But the form of the wall reflects the manner in which the work of assembly is organized in space and the physical characteristics of the fabric, a point to be considered more fully in later sections.

Again, take the cytoskeleton of eucaryotic cells. It is true that microbutules form by the self-assembly of prefabricated tubulin subunits. But the length of an individual microtubule and its propensity to elongate or shrink are determined by local dynamic factors that include the pH, Ca^{2+} ions, and other signals, as well as the presence of particular auxiliary proteins (152). Actin microfilaments, like microtubules, come in a variety of higher-order structures: cables, fibers, hexagonal arrays, and loose meshworks. All these modes of organization are compatible with the capacity of actin to assemble spontaneously into filaments but are not specified by the structure of the actin molecule; they are, instead, outcomes of local dynamic processes that remain poorly defined (73).

There is probably no need to belabor the point. In what follows, I shall take it for granted that morphogenesis at the cellular level is fundamentally different from the self-assembly of viruses. Molecular structures predispose molecules toward particular functions, and intermolecular forces do play a large role in the biogenesis of standard cell components: their ribosomes, microtubules, and bilayer membranes. However, this is not the level with which we are concerned here. The domain of cellular morphogenesis, with a size range of 1 to 100 μm, revolves around the organization of standard parts into diverse functional organisms. The processes responsible generate form by moving not toward equilibrium but away from it, thanks to a continuous input of metabolic energy. Our object now is to define more precisely how energy is converted into organization on the cellular scale.

Surface Tension

If cells are not shaped by forces between molecules, what alternatives can we consider? Thompson (276) devoted but one chapter to the forms of unicellular organisms, which turns on the proposition that many common biological shapes result from surface tension. This force is a consequence of the mutual attraction between molecules in a liquid; it acts parallel to the surface. In objects governed by surface tension, the shape will be that which has the smallest surface area compatible with volume and mechanical constraints.

The sphere is the simplest and most familiar shape of this type, but by no means the only one. Soap bubbles, whose properties are governed by surface tension in the film of soapy water, can be made to assume diverse shapes with the aid of structural supports: a bubble blown upon a pair of rings may be cylindrical as long as its length does not exceed its circumference. More esoteric forms, collectively designated as Plateau's surfaces of revolution, include the catenoid (derived from a parabola), the unduloid (derived from an ellipse), and a strange surface called a nodoid, which cannot be fully realized in three-dimensional space. What makes this biologically relevant is that many cells and unicellular organisms exhibit forms compatible with the law of minimal surface area. Spheres are especially common (bacterial and fungal protoplasts, many algal cells, eggs), as are cylinders (fungal hyphae and algal filaments), but unduloids and catenoids can be found among the protists, and *Paramecium*, with its reentrant gullet, has been described as part of the nodoid surface (276).

That unicellular organisms often exhibit the smallest surface area for their volume must not be construed to mean that the operative force is surface tension sensu stricto. In fact, as J. T. Bonner points out in his editorial introduction to Thompson's chapter, it was already clear in Thompson's day that the surface tension of biological membranes is too small to determine cell shape. However, cells are usually subject to contractile forces, collectively referred to as membrane tension, that operate to shrink their surface area. From the viewpoint of morphology, the physical nature of the force is less important than the observation that surface layers often behave as though they were fluid and assume a configuration that minimizes the surface area for any given volume and mechanical constraints. In recent years, this simple principle has proven to be a wellspring of insight into cellular morphogenesis, particularly of walled organisms.

Surface Stress Theory of Bacterial Morphogenesis

The shape of a bacterial cell is determined by its wall; broken walls retain the shape of the cell from which they came, whereas enzymatic removal of the wall generates a spherical protoplast. By the mid-1970s, bacterial physiologists had concluded with some regret that self-assembly by itself could not account for the shapes of bacteria (53, 118, 249). Bacterial walls are not self-assembling structures. They are not composed of subunits whose structure is directly specified by the genome, but are woven or plaited from a kind of stiff fabric, and their form reflects the manner in which the wall is laid down as the cell grows and divides. Moreover, since all eubacterial walls are chemically much alike, it appears that the chemical composition of the wall does not determine its form.

The chief constituent of the wall, at least from the viewpoint of shape determination, is the layer of peptidoglycan (or murein), a mixed polymer of carbohydrates and amino acids unique to bacteria (235, 278). The several strands are cross-linked by covalent bonds, such that the entire peptidoglycan sacculus may constitute a single macromolecule, stiff but not rigid, that has the size and shape of the cell. Such a sacculus can be enlarged during growth only by cutting existing bonds and inserting new units. The problem of enlarging the wall in a controlled manner is exacerbated by the fact that a growing cell is a vessel under pressure: the turgor pressure of gram-negative bacteria is of the order of 5 atm (0.5 MPa), comparable to the pressure of a racing bicycle tire (156), and that of gram-positive bacteria is near 20 atm (2 MPa). The stress is borne chiefly by the peptidoglycan layer, and it is obvious that random scission could easily cause the wall to fail and the cell to burst. Growth and

division of bacterial cells thus becomes a problem in biological engineering, analogous to the problems faced by human engineers in constructing a tunnel or repairing a ship holed below the waterline.

In a nutshell, the surface stress theory proposes that turgor is not only the problem but also part of the solution. Hydrostatic pressure supplies the driving force for surface enlargement by exerting tension upon the wall, which counteracts the cohesive forces that hold the wall together. Cells yield to this force by controlled expansion at particular loci, through the insertion of new units into the existing wall. Bacteria lack mechanoproteins; their shapes are determined by the manner and the sites of surface expansion, within the constraints imposed by the law of minimal surface area. Bacterial morphologies can therefore be understood, and even calculated mathematically, from the same physical principles that govern the shapes of soap bubbles. The diversity of bacterial shapes reflects the different modes of localizing the deposition of new wall, and the cleavage of old wall, in space and in time. The surface stress theory has been expounded in detail by its creator, A. L. Koch (155–157). My objective here is to recapitulate briefly how the idea explains bacterial shapes and why it is important.

The attribution of scientific ideas is always a problem. Seldom does one spring full-blown from a single brow, and Koch has been at pains to acknowledge the contributions of his collaborators, particularly T. J. Beveridge, I. D. J. Burdett, R. J. Doyle, M. L. Higgins and N. Nanninga. Nevertheless, the surface-stress theory bears the unmistakable stamp of a single mind; it seems to me a pardonable simplification to credit it to Koch alone.

Gram-positive cocci. Of all the bacteria, streptococci have the simplest mode of growth and division, and the spatial transformations that this entails are well known (53, 249). Briefly, cells of *Enterococcus hirae* are spheroidal, resembling a football, with a raised equatorial wall band that marks the site of the future septum (Fig. 9). The poles are not hemispherical but significantly more pointed. When the cell begins to grow, the wall band splits; the septum is initiated between the daughter bands and invades the cytoplasm, eventually cleaving it into two compartments. Concurrently, as the cell enlarges the growing septum is progressively externalized and distributed between the two emerging daughter cells (Fig. 10). Conveniently, in streptococci the wall is stable and does not turn over. This has made it possible to discern that new wall material is added predominantly to the growing edge of the septal ring and, to a lesser degree, at the point of splitting. In other words, new wall is initially deposited as septum, becoming pole as the septum is externalized; a cell consists of two poles, one newly made and one inherited from the previous division cycle.

The next step in the quest for understanding is to explain how streptococci divide in the absence of mechanoenzymes and to account for the peculiar shape of the poles. Koch's point of departure was the observation that a pair of fused soap bubbles mimic the shape of the streptococcal growth zone. Koch et al. inferred that the form of the poles is determined not by the kinetic parameters of the enzymes that generate the wall (as Shockman and his colleagues had thought), but by the law of minimal surface area, and proceeded to calculate the shape that poles should have if that premise were correct (161). The argument is mathematical and cannot be reproduced here, but it is worthwhile at least to indicate its general nature. To expand the surface of a soap bubble, work must be done against the cohesive force of surface tension. This work is performed by an increase in

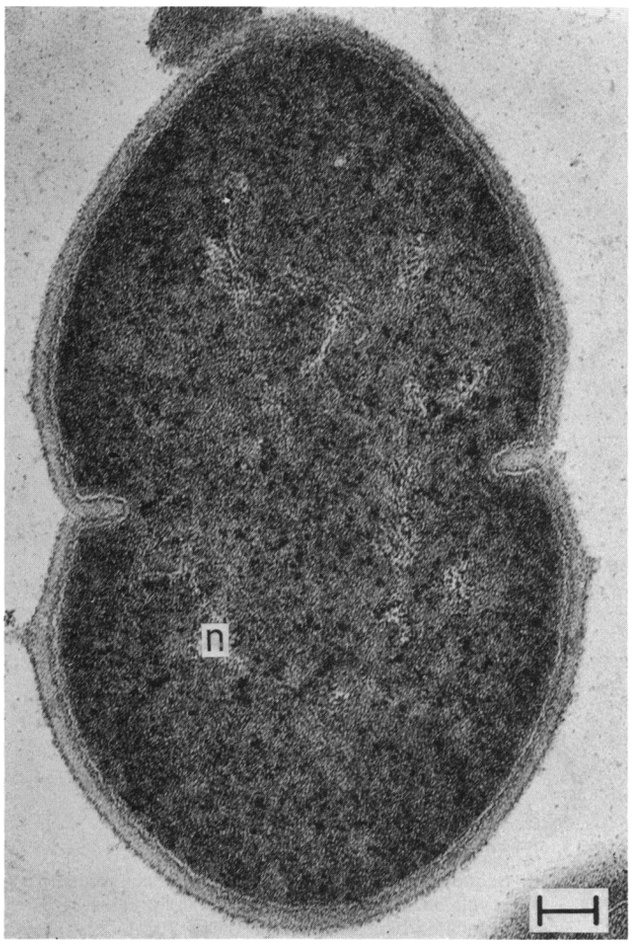


FIG. 9. Cross-section of *E. hirae* with a single growth zone. Abbreviation: n, nucleoid. Bar, 0.1 μm. Photomicrograph courtesy of M. L. Higgins.

the pressure of the gas inside the bubble, and its magnitude is given by PdV (where P and V are the gas pressure and volume, respectively). The newly formed surface area, dA, will be the smallest area compatible with the new volume; the work done in creating it is TdA, where T is the surface tension. The central assumption of Koch et al. is that the energy conservation equation, $PdV = TdA$, holds for cells as well as soap bubbles, with T representing the energy required to create a unit area of new surface. When speaking of cells, P represents the hydrostatic pressure (turgor), which is presumed to be the force that drives enlargement of the surface.

Many cellular shapes would in principle be compatible with the energy conservation equation; constraints or boundary conditions determine the shape created by any particular cell. For *E. hirae*, the following constraints were found to be necessary and sufficient (Fig. 11). (i) Growth is confined to a narrow zone adjoining the ingrowing septum. (ii) Septal material is laid down under stress-free conditions; it comes under tension just before it becomes part of the external wall, but undergoes negligible stretching. (iii) The septal wall is exactly twice as thick as the external wall. (iv) The hydrostatic pressure, P, the tension, T, and biosynthetic activity all remain constant. The changes in volume and in area, dV and dA, respectively, can be obtained from geometric considerations. The absolute values of P and T are

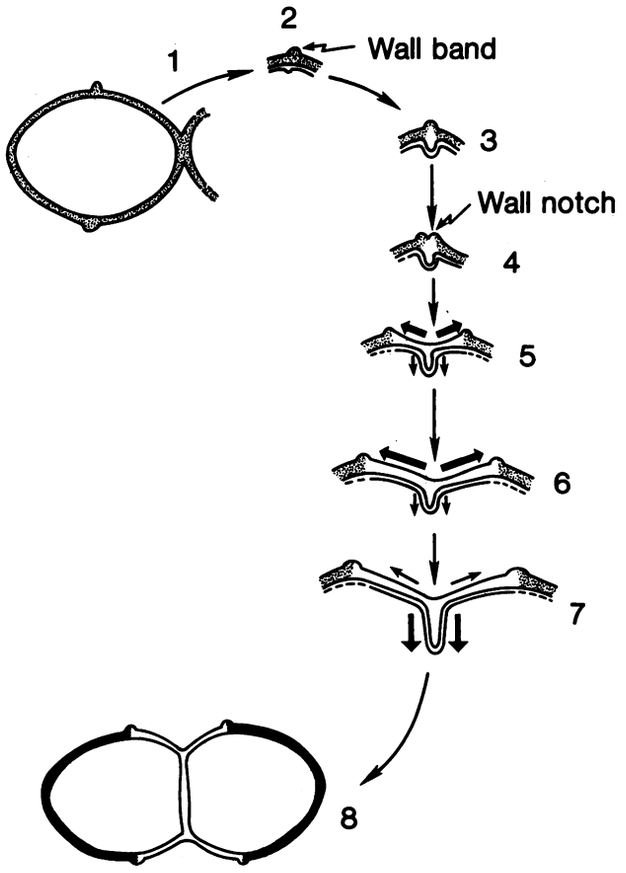


FIG. 10. Sequence of events during surface enlargement in *E. hirae*. 1. The cycle begins with the activation of a preexistent site of wall synthesis located beneath the wall band. 2 to 6. The nascent septum grows down into the cytoplasm, while a notch in the wall marks the beginning of its duplication. As the septum elongates, new wall is externalized in response to turgor pressure, forming an equatorial zone of newly made (unstippled) peripheral wall. 7 and 8. When surface expansion halts, the septum continues to elongate, finally dividing the original cell into two compartments. Modified and reprinted from *Annals of the New York Academy of Sciences* (249) with permission of the publisher.

not known, but their ratio could be calculated by making use of the cell dimensions painstakingly measured by Higgins and Shockman (120). This value is then inserted into an equation that specifies the height of the pole for any given

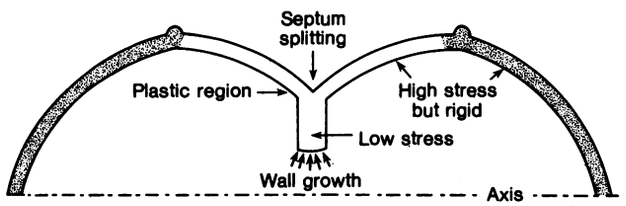


FIG. 11. Wall growth in *E. hirae*, as envisaged in the surface stress theory. The driving force for expansion is turgor pressure, which is uniform at all points. Old wall is rigid enough to retain its shape despite the hydrostatic pressure. Surface expands only by addition of new wall (unstippled) to the septum. At the site of splitting, the wall is plastic and assumes the form dictated by surface tension. Reprinted from the *Canadian Journal of Microbiology* (156) with permission of the publisher.

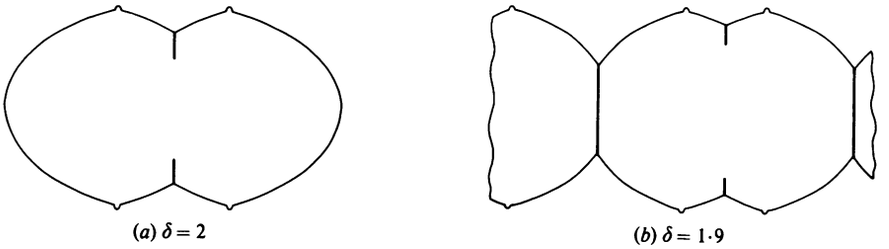
radius of the cell. The remarkable agreement between the observed shape of streptococcal poles and that calculated from biophysical principles is impressive and gratifying; it testifies to a depth of understanding not commonly encountered in the study of living forms.

Under favorable conditions, division progresses to the point of cell separation, but as a rule streptococci grow as chains of cells, individuated but attached by their septa. Chain formation was readily accommodated by the hypothesis that cell division and pole formation result from forces analogous to surface tension. Whenever the septal thickness is less than twice that of the external wall, formation of the new pole cannot proceed to completion; the cells will then fail to separate, and any further increase in volume must initiate a new growth zone (161) (Fig. 12).

Thus far, the argument has sidestepped an obvious difficulty: given that the wall is fairly stiff, how can it respond to internal pressure as though it were a fluid film? Addition of new wall units requires that the existing fabric be cut, but random cuts would endanger the osmotic integrity of the cell; the danger is particularly acute because mechanical stress lowers the activation energy of covalent bonds, rendering them more susceptible to hydrolysis. Koch et al. therefore envisage that new units are added to the wall in a stress-free environment (such as the septum) and that existing bonds are hydrolyzed only when that stretch of wall becomes part of the external surface and comes under tension. Local hydrolysis then allows the wall to expand, pulling the new unit into place. During this instant the wall responds to pressure in a plastic manner that is mathematically equivalent to fluid behavior. A central prediction of the surface stress theory, thus far not experimentally verified, is the existence of a class of hydrolases that cleave bonds only when they are stretched.

Gram-positive rods. *B. subtilis* cells are 3 to 5 μm in length and nearly 2 μm in diameter, with rounded blunt caps. The wall is thick, about 25 nm across; peptidoglycan makes up about half its mass. Its shape can be understood as a consequence of surface stress on the postulate that formation of the poles is quite different from that of the cylindrical side walls (40, 162) (Fig. 13).

Poles are formed, as in streptococci, by the externalization of wall material initially laid down as planar septum in a stress-free environment. However, the poles of *B. subtilis* are closer to hemispherical than those of *E. hirae*, indicating that the septal material stretches when it is bisected and comes under tension, while the streptococcal wall is more rigid. (The difference has been attributed to certain negatively charged groups in the wall of *B. subtilis*, which are replaced by uncharged groups in streptococci.) Forces analogous to surface tension can also generate the cylindrical cell body, provided the system satisfies the rather strict conditions for blowing cylindrical soap bubbles. The diameter of the cylinder is determined by rigid supports, consisting of the two poles. Stable extension of the cylinder is possible only if new material is added to the wall in a diffuse manner, rather than in a localized growth zone. Finally, P and T must remain constant, with a relationship such that $Pr/T = 1$ (r is the radius of the cylinder). Under these conditions, insertion of new peptidoglycan units will naturally cause the cylinder to elongate without bulging. According to Koch et al. (162), bacilli exemplify inside-to-outside growth (Fig. 13). New peptidoglycan units are continually inserted into the wall, in a relaxed conformation, at the external face of the plasma membrane. As the material moves outward through the wall, it comes under tension and stretches to accommodate elon-



(a) $\delta = 2$ (b) $\delta = 1{\cdot}9$

FIG. 12. Shapes of streptococcal cells calculated from the premises given in the text. (a) If the thickness of the septum, δ, is twice that of the wall, the model leads to repetitive cell division. As the primary growth zone enlarges in volume, the septal radius decreases until the cells separate. (b) When δ is taken to be 1.9, the model predicts that division cannot be completed. At some point in the development of the primary growth zone, septum splitting must cease; further growth of the cytoplasm will force the formation of secondary growth zone which, in turn, cease developing after a time. The end result is a chain of cocci with closed septa. Reprinted from the *Journal of General Microbiology* (161) with permission of the publisher.

gation; eventually it ruptures and is sloughed off into the medium. This ingenious scheme explains, at least in principle, how cylindrical cells of constant diameter can arise without recourse to templates, scaffolding, or unprecedented properties of wall constituents.

The cells divide by constructing the septum precisely in the middle of the cell; DNA is segregated into the two compartments thus created. DNA segregation and surface expansion have been linked ever since Jacob et al. (136), in their influential replicon hypothesis, proposed that the duplicated points of origin are pushed apart by growth of the membrane between them. Their original version became untenable when the fluidity of the plasma membrane was recognized. The principle can be rescued, however, by proposing that the duplicated origins of replication become attached to conserved portions of the cell wall, specifically the poles. The renovated replicon model has been developed in detail (163) and can account not only for the segregation of the chromosomes, but also for centering the septum: the midpoint of the cell may be determined by the mechanical tension that develops in the duplicating chromosomes as their attachment sites move apart.

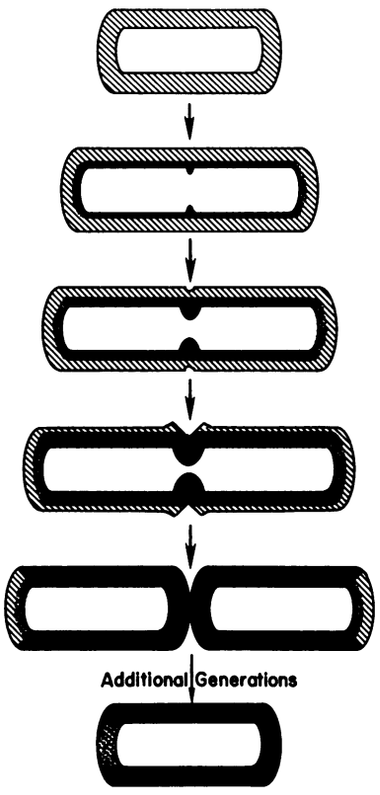


FIG. 13. Growth and division of *B. subtilis*. Note the elongation and turnover of the side walls by inside-out growth; poles turn over slowly. Symbols: ▨, old wall; ■, new wall. Reprinted from *Critical Reviews of Microbiology* (59) with permission of the publisher.

Wall growth and cell division in *B. subtilis* have been studied extensively during the past two decades, and much of the evidence is broadly consistent with the hypothesis outlined above; reference to the experimental papers will be found in reviews by Doyle and Koch (59) and Burdett (39). It is clear that poles are conserved as the cells grow and divide, but the side walls are not. The side walls grow continuously, new material being added to the inner aspect of the wall and old material being sloughed off at the surface. The model requires that poles and side walls be formed at different times, and this also appears to be the case. Recent additions are the demonstration that the planar septum can stretch while splitting, generating two cup-shaped poles despite the presence of enough vancomycin to prevent additional wall synthesis (160); that the terminus and origins of DNA replication are indeed associated with the poles (262); and that new wall is incorporated uniformly rather than in defined zones, as had been thought before (187).

Gram-negative rods. It is ironic that *E. coli*, the most intimately familiar of all organisms, presents special difficulties for students of morphogenesis. The cells grow as rods, 1 to 5 μm in length with rounded ends; their width varies quite markedly with the growth rate. Superficially, *E. coli* cells resemble those of *B. subtilis*, but they cannot grow and divide in the same way because of the differences in envelope structure. Gram-positive bacteria have a thick peptidoglycan layer external to the plasma membrane; gram-negative bacteria have two lipid membranes with the stress-bearing peptidoglycan sandwiched in between. In *E. coli* the peptidoglycan layer is probably but one or two molecules thick, in a partly extended configuration (there is some disagreement about this [157]). Such a layer would be too thin to extend by inside-to-outside growth. By the same token, the cells cannot construct poles by laying down a stress-free septum that is subsequently split. Instead, the septum forms by invagination of the plasma membrane and the murein layer within a specialized region of the cell, defined by a pair of annuli on either side of the forming septum (51, 176).

Growth and division of gram-negative rods can be under-

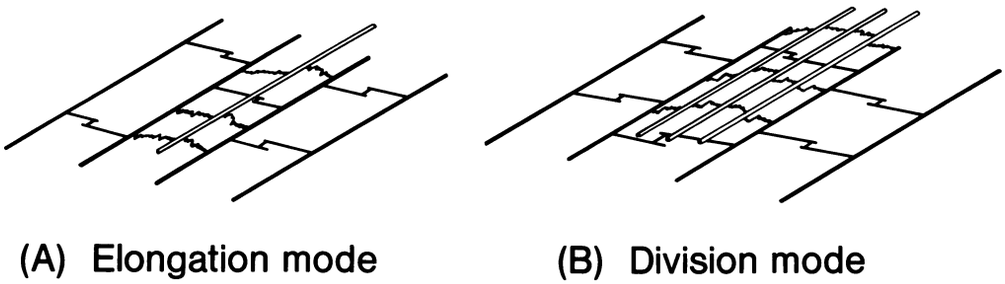


FIG. 14. Surface expansion in gram-negative bacteria, as envisaged in the surface stress theory. (A) Elongation depends on the insertion of one or two new strands at a time. (B) Septum formation calls for the insertion of rafts of prefabricated, prelinked glycan chains. Regions of wall in which this mechanism applies will incorporate material more rapidly and grow inward to form the septum. Reprinted from the *Canadian Journal of Microbiology* (156) with permission of the publisher.

stood as a variant of the model proposed for *B. subtilis* (156, 157, 159). Side walls can extend in a cylindrical fashion provided that the incorporation of new wall elements is diffuse rather than zonal. The thin peptidoglycan layer is uniformly under stress; it is therefore important that new units be linked to the wall before any stress-bearing bonds are cleaved. Septum formation is more difficult to understand: why should the expanding surface invaginate despite high hydrostatic pressure? Once again, the physics of soap bubbles provides a clue: the septum can grow inward provided that the cells locally alter the magnitude of T, the parameter that expresses the amount of work that must be done to enlarge the surface by a unit amount. Calculations show that local diminution of T by a factor of 2 would suffice to elicit invagination. Note the difference: the forms of streptococci and bacilli were deduced from the premise that T and the hydrostatic pressure, P, remain constant throughout the division cycle; to explain division in gram-negative bacteria, T must be assumed to vary locally.

Local variation of T implies that the mechanism of wall synthesis must be altered locally such that less energy is required to enlarge the surface. One possible solution is that side walls form by the insertion of single linear oligopeptidoglycan chains, whereas at septal sites the cell secretes rafts of prefabricated, cross-linked units. In this way, cleavage of a single stress-bearing bond would expand the surface by a much larger amount (Fig. 14); it is tempting to suppose that the periseptal annuli described by Cook et al. (51) delimit such a zone of altered mechanism.

Some aspects of this proposal are testable at present. There seem to be no significant chemical differences between side walls and poles or between cells of different shapes (55, 60). Most, though perhaps not all, investigators agree that side wall growth is diffuse (41, 155, 157, 303) or at least not confined to a narrow zone. Septum formation, by contrast, is associated with intense local wall deposition. Koch (157) makes a strong argument that the wall cannot be laid down in the form of neat hoops (42) but has an irregular structure partly ordered by preferential cleavage of bonds exposed to hoop stress. Most remarkably, De Jonge et al. (55) have obtained evidence that during cell elongation peptidoglycan strands are inserted singly, whereas during constriction multiple strands go in en bloc. However, as yet there is no way to look for smart hydrolases whose activity is responsive to tension and which cleave only bonds protected by attached new peptidoglycan strands.

In search of simplicity. The virtues of bacteria as elementary exemplars of universal biological processes are familiar to molecular biologists and to bioenergeticists, but less so to morphologists. The surface stress theory deserves consideration as an example of what a structuralist approach to this field can hope to achieve and what its limitations are likely to be.

What Koch's reflections supply is a thermodynamic framework for morphogenesis, not a molecular one. The theory accounts in principle for the ability of bacteria to divide and generate diverse shapes despite the absence of mechanoenzymes and in the face of internal hydrostatic pressure; it explains how the work is done. The general framework is compatible with a range of forms and must be supplemented with specific boundary conditions; these alone lend concreteness to the model proposed to account for any particular shape. These specifics are concerned with the manner in which the scalar driving force, turgor pressure, is localized to produce a particular form, and they include persistent structures that provide spatial memory from one generation to the next. Specified constraints can often be subjected to experimental scrutiny, and the plausibility of the explanation as a whole can be assessed by comparing actual shapes with those predicted by a mathematical model.

The surface stress theory is based on two cardinal assumptions, whose general falsification would require that the theory be abandoned. The first is that turgor pressure supplies the driving force for surface expansion in all its variety. Microbial physiologists have long been aware that turgor pressure is required for growth, but that alone does not prove that it is the mechanism by which metabolic energy is transduced into mechanical work. There are no simple and satisfactory methods for measuring the hydrostatic pressure in bacteria; this restricts research into its functions. The second premise is the existence of hydrolytic enzymes that selectively cleave wall polymers at sites subject to mechanical stress; for *E. coli* it must be assumed that only bonds protected by new units linked to the old will be cleaved. Such smart hydrolases are likely to be present in minute quantities and will be hard to assay; at present they remain a logical necessity rather than a demonstrable reality. The theory also requires the spatial localization of enzymes (more exactly, of enzymatic activity) responsible for wall biogenesis. Localized biosynthesis has been demonstrated in some instances (e.g., *E. hirae*), but in general it is not clear just what is localized and how. Specific recognition of complementary proteins (self-assembly) probably plays a role.

To calculate possible forms, Koch et al. applied the energy conservation equation $PdV = TdA$; in effect, they assumed that surface expansion is a near-equilibrium process. That is necessary as a computational device, but I question whether it is true in the living cell. As a rule, organisms generate ample driving force and regulate the performance of work

kinetically (just like an automobile, whose performance is constrained by power output only under extreme demand). I would expect this principle to apply to bacterial enlargement as well: if turgor supplies the driving force for surface enlargement, the energy supply will probably be plentiful but compliance will be regulated.

The surface stress theory accounts in principle for the forms of cocci and rods (155, 156) and has recently been extended to prosthecate bacteria (158). Its application to fungal hyphae, and to apical growth in general, will be taken up below. However, it is not certain whether it is universally applicable, even in the bacterial realm. Spiral forms are presently beyond its reach, and its application to the archaebacteria (many of which have walls composed of self-assembling subunits) is in doubt. Square bacteria (294), which probably do not maintain turgor, fall outside its scope altogether.

A more fundamental limitation is that models based on surface stress, even when well validated, do not immediately predict structural or molecular detail. They do not, for instance, tell us the function of gene products required for division (54, 127) or what ails the many mutants whose morphology is abnormal (16, 58, 59, 272). Each of these poses a puzzle that may some day be resolved within the framework of the theory, or else may call it into question. However, the surface stress theory has a breadth that a more molecular hypothesis can never attain, and that lends it unique heuristic value. We have no alternative answer to offer anyone who asks how bacteria shape themselves.

VECTORIAL PHYSIOLOGY

Nowhere in the microbial world is there greater diversity of forms and functions than among the lower eucaryotes; common principles are obscured by an exuberance of variations that reflect profound evolutionary divergence (178, 256). Consequently, the protists and the fungi are the cellular topobiologist's proper study.

One element shared by many of the fungi and algae is the presence of a wall that maintains—even defines—the form of the cell. Morphogenesis in these organisms comes down to molding the wall. Their shapes express the physical properties of the wall and the manner in which the cell accomplishes wall expansion and localizes it in space. This chapter is based on the premise that the forms of many walled cells can be understood as variations upon a general theme, localized compliance with global force. As in bacteria, the driving force for surface expansion appears to be hydrostatic pressure. Cells yield to this global force in a localized manner by stretching and by synthesizing new wall. Both processes depend on the secretion of enzymes and/or of precursors into the wall in a localized manner. The multitudinous forms thus represent variations on the way that secretion is organized in space.

The title of this section draws attention to the hierarchy of spatially ordered processes that underlie secretion and other directional aspects of cell function or behavior. In the case of bacteria, it is believed to distribute molecules around the cell; this will not do for the far larger eucaryotic cells, which require special devices to mobilize resources from a volume of protoplasm, to direct them toward a particular site, and to perform localized or oriented work. Mitchell (190) originally coined the term vectorial metabolism to emphasize that biochemical reactions can have a direction in space, usually defined in reference to a membrane. Vectorial physiology designates functional ensembles that are directional on a larger scale, that of the whole cell. Cytoplasmic transport and contractile processes linked to the cytoskeleton are especially prominent in attempts to understand how cells shape themselves.

The proposition that the forms of walled cells represent diverse modes of localizing compliance with hydrostatic pressure is not so much a hypothesis to be challenged by exceptions as a guide to reflection and experimentation. It focuses attention on relevant questions and allows one to organize the mass of disparate observations in a coherent manner. Testable hypotheses emerge only when the general idea is applied to particular organisms and given specific content. For any morphogenetic process, we can then assess the adequacy of our understanding by asking how surface expansion is effected and how it is localized.

Apical Growth of Fungal Hyphae

The hypha is the archetype of fungal form, a tube of approximately constant diameter ending in a rounded or tapered tip; the filament may or may not be subdivided by septa into individual cells. Hyphae also provide the paradigm of apical growth: extension is confined to the tip, which makes up but a small fraction of the length. When conditions are favorable, hyphae branch, putting forth new tips from regions that were previously quiescent; branching is equivalent to division in cellular organisms. The branches extend and proliferate in turn, giving rise to the familiar pattern of a fungal colony spreading over a nutrient surface. The adaptive value of this mode of growth can scarcely be doubted: like motility, apical growth continually makes fresh nutrients available to the growing region at the hyphal anterior. Cellular morphogenesis in the filamentous fungi revolves around the problem of tip extension and tip formation. Other issues, such as the placement and diverse forms of septa and reproductive structures (49), must be set aside here.

Extension by polarized secretion. Hyphal forms depend on the generation of a rigid wall, but do not obviously reflect the chemical composition of that wall. The polymers present vary significantly between taxonomic groups, yet morphologically the chitinous hyphae of *Neurospora* and *Aspergillus* species differ only in detail from the cellulosic ones of *Achlya* species. Moreover, *Aspergillus* mutants whose chitin content is reduced require an osmotic stabilizer but grow with typical hyphal form (147). Fungal forms can also not be credited to an orderly process of subunit assembly: hyphal walls, and also those of yeasts, consist of an irregular meshwork of fibrils (generally chitin or, in the oomycetes, cellulose) embedded in an amorphous matrix in which little order can be discerned.

What fungal hyphae have in common and share with root hairs, pollen tubes, and algal rhizoids is the manner by which wall deposition is organized in space. The synthesis of cytoplasmic macromolecules is dispersed over the length of the hypha, but new cell wall is laid down chiefly in the immediate vicinity of the tip. Judging by pulse-labeling experiments, the rate of polymer synthesis over the apical 1 μm may be 50 times greater than at a position 50 μm behind the tip (13, 80). What takes place in the tapered, apical extension zone is only partially understood, and the details vary substantially between taxonomic groups. The sweeping account that follows should be supplemented with more detailed reviews, notably those of Trinci (280), Grove (93), Gooday (81), and a particularly thoughtful article by Wessels (298).

The ground-breaking insights into the physiological basis

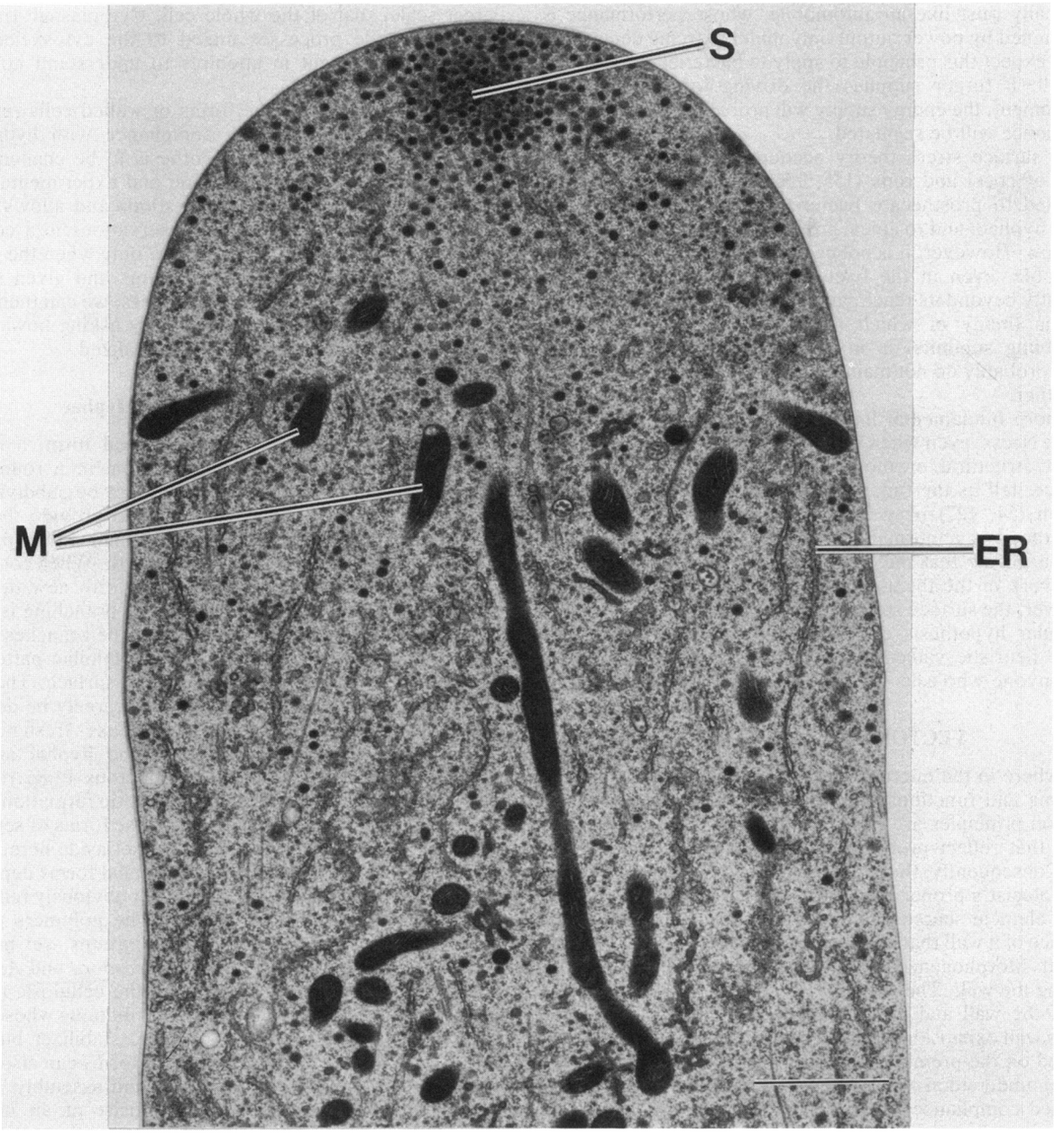


FIG. 15. Longitudinal section through the hyphal tip of *Sclerotium rolfsii*, showing cytoplasmic organization along the axis of the hypha. Abbreviations: M, mitochondria; ER, endoplasmic reticulum. The Spitzenkörper (S) appears as a dense aggregate of vesicles. Bar, 1 μm. Photomicrograph courtesy of R. W. Roberson. Reprinted from *Protoplasma* (231) with permission of the publisher.

of hyphal extension stem from ultrastructural studies undertaken two decades ago by Girbardt (78) and by Grove and Bracker (94). These investigators discovered that the apex lacks mitochondria, nuclei, and the other standard organelles, but is filled with membrane-bound vesicles. In some organisms these vesicles are organized into a coherent structure, the Spitzenkörper (apical body [Fig. 15]), and in others the vesicles are clustered at random (Fig. 16); however, dense aggregates of apical vesicles are characteristic of all tip-growing organisms. In living preparations the vesicles fuse with the apical membrane. The distribution of apical vesicles corresponds nicely to the gradient of wall synthesis (82) (Fig. 17), reinforcing the presumption that apical vesicles supply lipids, enzymes, and/or precursors for the production of new apical membrane and wall. In addition, there must of course be forward migration of the cytoplasm and its organelles (183), but it is universally agreed that what shapes tip and hypha is formation of the wall.

Considering the obvious importance of secretory vesicles in fungal growth, we are remarkably ill-informed regarding their structure, contents, manufacture, transport, and ultimate disposition. The vesicles are thought to arise in the endoplasmic reticulum far behind the tip, pass through Golgi equivalents, and then be transported vectorially to the apex, where they are exocytosed. It is uncertain whether vesicles are carried forward by general cytoplasmic streaming or

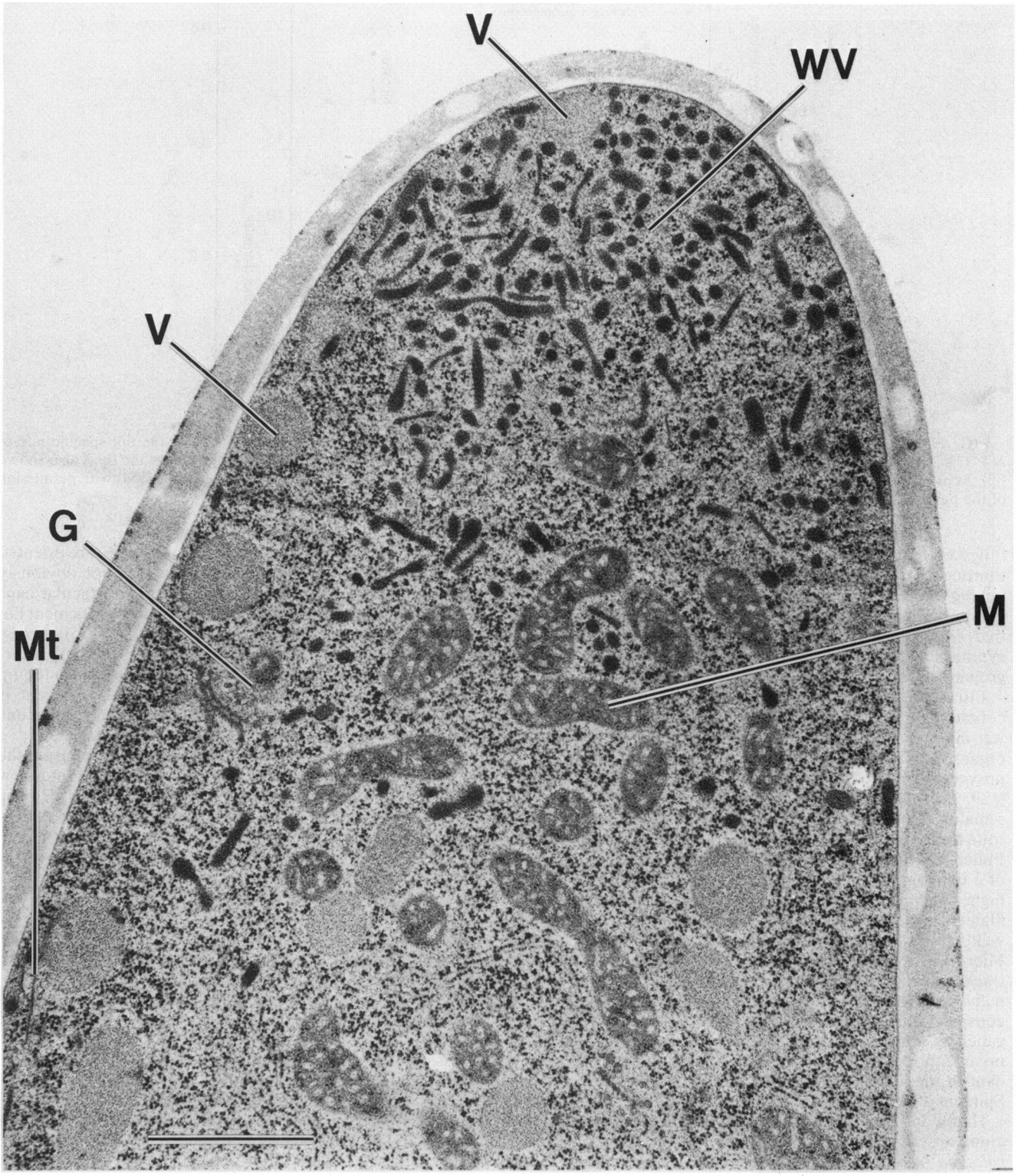


FIG. 16. Longitudinal section through the hyphal tip of *Saprolegnia ferax*. Note the aggregate of elongated, opaque wall vesicles (WV) and the absence of a discrete Spitzenkörper. Other abbreviations: V, vacuole; M, mitochondria; G, Golgi body; Mt, microtubule. Bar, 1 μm. Photomicrograph courtesy of I. B. Heath. Reprinted from *Protoplasma* (116) with permission of the publisher.

travel on specialized tracks. Hyphae contain at least two kinds of vesicles, which differ in size, form, and electron density, but it is uncertain just what any class of vesicles contains. Informed opinion discounts the belief that they carry cell wall precursors, but favors the presence of enzymes intended for secretion and for the biosynthesis of new wall and membrane. The most extensive information comes from yeasts (see following section); data from filamentous

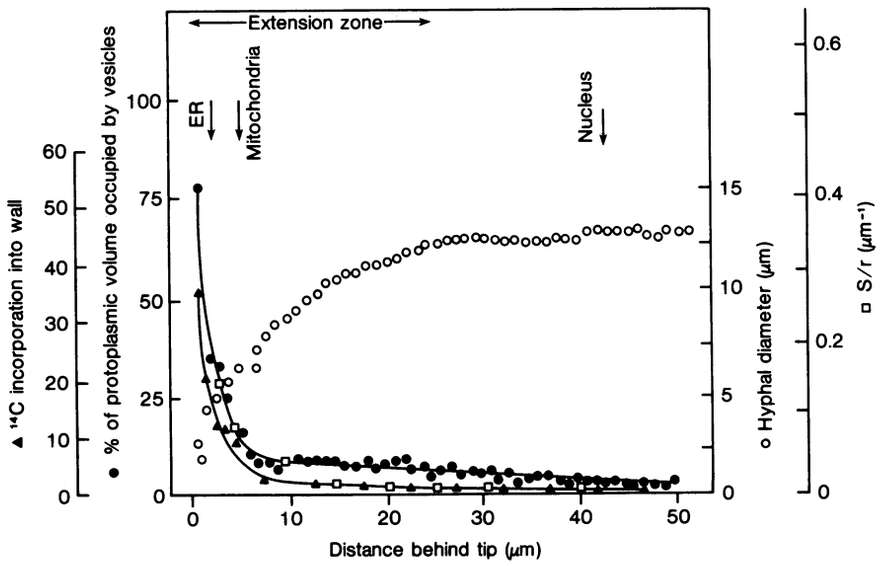


FIG. 17. Correlates of tip extension in *N. crassa*. Three pertinent parameters are plotted against distance from the tip: specific rate of *N*-[^{14}C]acetylglucosamine incorporation (▲), precursor vesicle concentration (●), and the ratio *S*/*r* (□). The diameter of the tip is also shown (○). Reprinted from *Symposium of the Society of General Microbiology* (82) and the *Journal of General Microbiology* (154) with permission of the publisher.

fungi are limited, but one unique class of vesicles has been thoroughly characterized. Chitosomes (vesicular bodies containing latent chitin synthase) have now been isolated from many fungal species (10, 21). It is likely, if not quite proven, that chitosomes serve as the transport vehicle for chitin synthase and that they accumulate with other vesicles at the growing apex.

Ultrastructure of hyphal tips. Biochemistry will, in due course, reveal the chemical processes that make a tip, but we must look to cytology for insight into their spatial organization. Preservation of apical ultrastructure has improved greatly with the introduction of freeze-substitution (129, 130), but it is fair to say that the organization of tips remains incompletely understood. Two recent studies illustrate the state of the art. For *Sclerotium* cells, Roberson and Fuller (231) documented a typical Spitzenkörper consisting of a tight aggregate of several kinds of vesicles (Fig. 15); at higher magnification, the core of the Spitzenkörper appears filamentous and vesicle free. The mitochondria are located well behind the tip, Golgi bodies and nuclei even more so. Microtubules, thought by some investigators to provide tracks for organelle transport, traverse the apex longitudinally to abut upon the plasma membrane, but are not conspicuously associated with either vesicles or larger organelles. Microfilaments are visible in places, but their organization is not obvious. Endoplasmic reticulum is abundant in the apical region and is closely associated with the Spitzenkörper.

Heath and co-workers have provided a detailed and judicious description of tip organization in the oomycete *Saprolegnia ferax* (113, 115, 183). It should be mentioned here that oomycetes, although still claimed by mycologists, are more closely related to certain algae (96, 178), which may account for their divergent anatomy. *Saprolegnia* hyphae have no Spitzenkörper, but the tip is filled with vesicles, especially the dense, elongated wall vesicles (Fig. 16). Behind the tip, wall vesicles tend to occur in the peripheral cytoplasm. Mitochondria and Golgi, excluded from the tip, favor the central cytoplasm. Microtubules occur in the tip proper but are more abundant farther back; they are oriented longitudinally but tend to be short and are not obviously associated with wall vesicles. Actin forms spectacular caps in the cortical cytoplasm of growing tips, an arrangement not reported in true fungi. Heath and Kaminskyi (115) suggest that wall vesicles are produced in the Golgi bodies, travel radially to the periphery, and are then transported to the tip in association with cortical actin cables; the argument is closely reasoned, but the evidence is not as direct as one would wish.

Shape of hyphal tips. As a growing hypha forges across the microscope field, its tip continuously reincarnates, at a new location, the exact shape it had a moment earlier. Having recognized a growing hypha as a system of polarized exocytosis, we can attempt to explain the shapes of tip and hypha: how does the tapered extension zone arise, propagate itself, and leave behind a lengthening tube of constant diameter? One possibility that has surely crossed every observer's mind is that the apex is, in fact, pushed along passively by expansion of a growing zone just behind the tip. This proposition was considered and firmly dismissed by the German botanist M. O. Reinhardt (227): the tip is continuously made afresh. (Reinhardt's paper of a century ago still makes illuminating reading, if only for his straightforward explanation of the reason why tips subjected to osmotic stress burst, not at the apex, but at the base of the extension zone: the tangential stress upon the wall is lowest at the apex and rises with the expanding radius.) Reinhardt also formulated a hypothesis that explains how the tapered tip comes about and why the faster a hypha grows the more pointed is its tip. He proposed that tips grow by the intussusception of new material in the apical region. The rate of intussusception is maximal at the tip, declining toward zero at the base of the extension zone; in modern idiom, exocytosis is maximal at the apex. Turgor pressure, in Reinhardt's view, is required to appress the plasma membrane to the wall, but performs no morphogenetic function.

Reinhardt's model was adopted and amplified by Bartnicki-Garcia (9) in his influential unitary model of apical

growth. Bartnicki-Garcia formalized the view that extension reflects the incorporation of precursor materials, through the agency of apical vesicles, into preexisting wall of uniform rigidity. This requires continuous scission of established bonds, effected by autolytic enzymes (lysins) that maintain a "delicate balance" between hydrolysis and synthesis at the apex. The shape of the tip, then, reflects the gradient in the rate of net wall deposition and can vary with that rate from hemispherical to an elongated half-ellipsoid of revolution (90, 227, 282).

The conception just outlined meshes well with the surface stress theory of bacterial morphogenesis (see previous section) and is the foundation for a biophysical analysis of tip extension put forward by Koch (154). On the assumption that extension is slow enough to be treated as a local equilibrium process, the energy conservation equation $PdV = TdA$ can be applied. P, the turgor pressure, which was assumed to provide the driving force for surface expansion, is presumably constant throughout, but to generate the observed shape, T, the work done in expanding a unit of surface area, must vary from a low value at the tip to infinity at the base of the extension zone. By plotting the ratio S/r (where S is the slope of the surface at any point and r is the diameter at that point, obtained from photomicrographs) against distance behind the tip, one obtains a measure of the variation of T with distance. In other words, a system that expands its wall surface at any point at a rate proportional to S/r will automatically construct a cylindrical tube with a tip of the appropriate shape. The variation of S/r with distance is again in excellent agreement with the distribution of apical vesicles and of the incorporation of radioactive precursors (Fig. 17). What this means is simply that, given the assumption that the shape of hyphal tips minimizes the surface area, the pattern of wall growth suggested by the distribution of apical vesicles is sufficient to generate the observed shape.

The above studies assumed, more or less explicitly, that apical wall is rigid, but mycologists have long suspected (following the pioneering researches of N. F. Robertson) that newly formed wall is plastic and rigidifies within 1 to 2 min. Saunders and Trinci (241) used this premise to calculate the shape that a tip should have: "The wall is now considered to be elastic, and the shape is determined not by the pattern of addition of material but instead by the wall adjusting itself in response to turgor pressure so as to adopt a shape which minimizes the surface energy." They derived an equation that relates wall elasticity to tip shape as expressed by the axes of an ellipsoid of revolution. (In their paper, R_1 and R_2 must be understood to refer to the principal curvatures, not the principal radii. The text is confusing on this point [A. R. Trinci, personal communication].) In principle, if the elasticity of the tip wall could be determined, the shape of the tip would be calculable. In practice, the equation allows one to use measurements of tip shape to compute how elasticity varies with position. It explains, for example, why the shape of hyphal tips varies with the growth rate such that vigorous tips have long, pointed extension zones while sluggish ones are rounded. In a hemispherical tip, the elasticity is uniform over the entire surface; the greater the taper, the sharper the gradient of elasticity. It will be intuitively clear that, if rigidification were simply a function of the time elapsed since a particular patch of wall was deposited, growth rate and taper should be positively correlated. This view, also, is consistent with the distribution of apical vesicles and the incorporation of radioactive precursors in pulse-labeling experiments. Overall, given the premise that exocytosis is maximal at the apex, realistic shapes can be calculated on the presumption of either rigid or plastic apical wall.

However, it is not necessary to postulate that exocytosis is actively restricted to the tip. In a most stimulating contribution, Bartnicki-Garcia et al. (11, 12) explored the hypothesis that the vesicles which supply the material for surface expansion are "released from a postulated vesicle supply center and are capable of moving to the surface in any random direction. The position and movement of the [vesicle supply center] becomes the critical determinant of morphogenesis." When the vesicle supply center remains stationary, vesicles reach the cell surface uniformly in all directions, and a spherical shape is generated. "Any displacement of the [vesicle supply center] from its original central position distorts the spherical shape. A sustained linear displacement of the [vesicle supply center] generates the typical cylindrical shape of fungal hyphae with their characteristic rounded tips." (12).

These premises were incorporated into equations that define both the shape and the diameter of a hypha by two parameters that have obvious physiological significance: the number of vesicles discharged by the supply center, and its rate of linear displacement. Computer simulation generates astonishingly realistic images of growing hyphae, germinating spores, tips, and some buds. The model also suggests that the Spitzenkörper, whose nature has long been mysterious, serves as the distribution center for apical vesicles. Best of all, the model wonderfully concentrates the mind by discarding the conventional assumptions of localized exocytosis and vectorial vesicle translocation. It would now seem desirable to model the traditional hypothesis and find out whether it, also, can account for the shapes of hyphae and tips.

So how do hyphal tips grow? The above discussion of tip shape illustrates both the strengths and the limitations of biophysical analysis. Simple forms are compatible with more than one generative mechanism, and therefore computer simulation can tell us what is possible but not what is true. So the ball bounces back into the physiologist's court, who must learn by experimentation what goes on in an extending tip, particularly how surface expansion comes about and how it is localized. A little reflection reveals many areas in which our understanding is insecure at best.

One of the largest gaps centers on the origin of the apical vesicles. It is generally believed on cytological grounds that the vesicles bud off distal Golgi units and travel to the apex, but there seems to be no compelling or even direct evidence for this. The very fact that vesicles accumulate subjacent to the apex argues that something more is required. If, indeed, vesicles arise far behind the tip, one must assume that exocytosis is the rate-limiting step in secretion and postulate either local binding of the vesicles or a vectorial transport pathway distinct from bidirectional cytoplasmic streaming. Alternatively, we can entertain the possibility that apical vesicles are in fact produced apically, perhaps even in the Spitzenkörper. In a remarkable study of the ultrastructure of *Chara* rhizoids, Bartnik and Sievers (14) emphasized the abundance of endoplasmic reticulum within and around the apical body. Roberson and Fuller (231) recorded a similar observation, which would be consistent with the Spitzenkörper's being a locus of vesicle production (I hasten to add that neither set of investigators is to be blamed for this minor heresy). Incidentally, the existence of hyphae that contain a Spitzenkörper and of others that do not may bespeak two different modes of generating secretory vesicles.

In this context, the spatial regulation of exocytosis ur-

gently requires clarification. Most published models assume, implicitly or explicitly, that exocytosis is restricted to a tightly circumscribed locus; a more diffuse site would give rise to isotropic rather than apical expansion. At present, we know nothing of the mechanisms that would localize exocytosis; the great virtue of the model developed by Bartnicki-Garcia et al. (11, 12) is that it questions their very existence.

Another critical but unsettled issue is the rigidity or plasticity of the nascent apical wall. If the wall is rigid at all times, intussusception of new units depends on local hydrolysis to create sites for their reception. It is difficult to see how a tip can maintain a stable balance between hydrolysis and synthesis unless one postulates smart hydrolases that cleave only protected bonds (156, 161). Indeed, there is little hard evidence that lysins play an obligatory role in tip extension (298). The proposal that nascent wall is plastic rather than rigid circumvents the difficulty. Moreover, the admirable researches of Wessels and colleagues have supplied a plausible chemical basis for its progressive rigidification. The wall of *Schizophyllum commune* consists of a complex of chitin and certain β-glucans. The studies of Wessels et al. suggest that chitin and glucan are deposited separately at the apex, becoming cross-linked into an insoluble complex by a time-dependent reaction as they fall behind the extending tip. The tip would be plastic because of the absence of cross-links between the polymers; rigidification corresponds to the formation of covalent bonds and consequent crystallization of chitin and glucan chains (250, 291, 298). Procedures to measure and manipulate the mechanical properties of apical wall would be helpful.

Fungal physiologists all but take it for granted that hyphal tips extend in response to turgor pressure, but few attempts have been made to examine this proposition critically. It is also not clear how the nascent, plastic apical wall withstands the hydrostatic stress. The apical actin cables and meshwork found in some hyphae may provide ancillary mechanical support; however, one would then expect cytochalasins to induce apical bursting, which is not the case. All these questions underscore how little we understand about the deposition of wall at a growing apex.

Branching and chemotropism. From the physiological perspective, initiation of a branch and germination of a spore can be described as the establishment of a polarized secretion pathway. Many investigators have reported that localized accumulation of vesicles precedes emergence of the new tip (93), but their contents are not known; wall lysins are favored candidates, since dissolution of the existing wall must be a prerequisite to branching and germination.

Is local wall dissolution sufficient to initiate a branch? Remarkably, the idea has a lot to recommend it. Mullins and his associates have collected considerable circumstantial evidence linking the secretion of cellulase in *Achlya* cells to the emergence of a branch (193, 275). They favor the proposition that a branch begins as a localized herniation of wall weakened by hydrolases; only later would the branch-initial acquire organelles and precursor vesicles and organize itself into a tip. This may also be the basis for the induction of branching by cytochalasins and various ionophores, an effect observed in *Achlya* species and other fungi: the inhibitors seem to disrupt the cortical cytoskeleton, which may cause hydrolase-bearing vesicles to stall and discharge their contents (103, 280).

Chemotropism, the reorientation of a growing tip in response to a nutrient gradient, is rare in true fungi but common among the water molds, including *Achlya* species. In principle, reorientation can come about either by bowing or by bulging (Fig. 18) (the terms are due to L. F. Jaffe, cited in reference 91). Bowing is the common response of plant shoots, *Phycomyces* sporangiophores, and algal rhizoids (251); it results from the retardation of surface growth on the near side of the gradient. However, in tip-growing organisms, bulging, mediated by acceleration of surface growth on the near side, is a much more likely response. Recent research from this laboratory showed that *Achlya* hyphae make two spatially localized responses to amino acid gradients: reorientation of the tip and initiation of a branch (247). These can only represent bulging, presumably due to localized, receptor-controlled exocytosis of vesicles involved in wall softening or wall biosynthesis. Localized fusion can potentially provide a unifying principle that links tip extension, reorientation, and branching; future research will tell whether this is an empty speculation or a fruitful hypothesis.

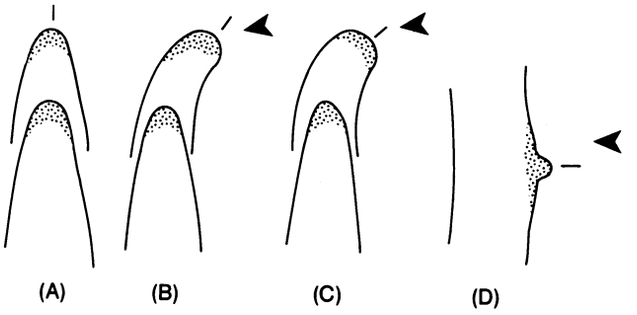


FIG. 18. Bowing, bulging, and branching: patterns of localized secretion. (A) Extension; secretion is symmetrical around the growth center (line). (B) Bowing; secretion is inhibited on the high side of the gradient (arrowhead), but the growth center remains in its original location. (C) Bulging; the growth center shifts to the high side of the gradient. (D) Branching; a new growth center develops on the high side of the gradient.

Budding and Cell Division in Yeasts

The cell division cycle of yeasts is one of the simpler morphogenetic pathways, intensely studied from the biochemical and genetic perspectives; but how the succession of forms comes about is no more clearly understood than the genesis of a hyphal tip. In fact, budding and apical extension are closely allied, and transitions between them are common. Many fungi switch from apical extension to the generation of a round blast at an early stage of conidiation (49); and cells of the pathogenic yeast *Candida albicans* can grow either in a yeastlike form or as a hypha, depending on environmental circumstances. We shall focus here primarily on the division cycle of *S. cerevisiae*, on the premise that conclusions will be more broadly applicable. The account that follows is indebted to reviews by Pringle and Hartwell (217), Byers (43), Cabib et al. (47), Schekman (243), and Wheals (299).

Figure 3 diagrams the essential features. A newborn cell, ovoid and somewhat smaller than its mother, enlarges uniformly until it attains a characteristic size that depends both on its genotype and on the environmental conditions. A small protuberance then appears at or near one of the poles; this represents the beginning of a bud. At this time wall synthesis, hitherto uniformly distributed over the surface of the growing cell, becomes localized to the bud, chiefly to its distal end. The base of the bud is delimited by a ring of filaments that prescribe the site where the septum will later develop. As the bud enlarges, the nucleus of the mother cell

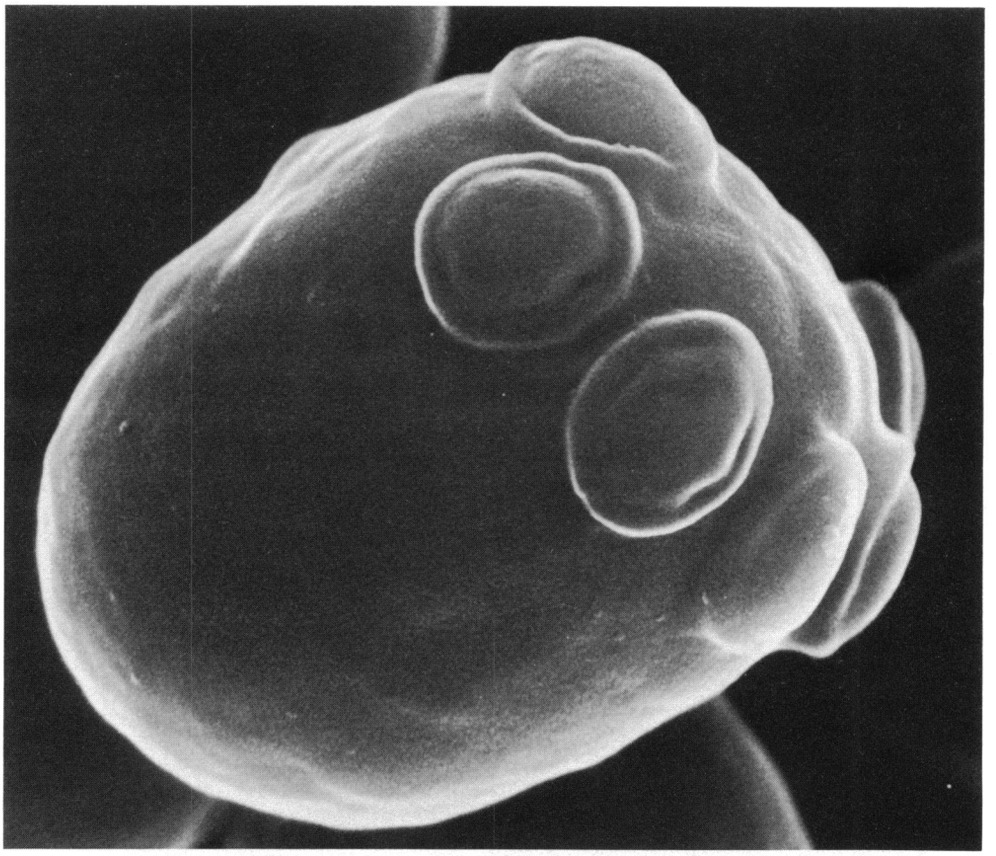


FIG. 19. Scanning electron micrograph of an *S. cerevisiae* cell. This haploid parent cell has produced at least six daughters as shown by the polar contiguous array of circular bud scars. Bar, 0.2 μm. Photograph by Anna Cosney and John Forsdyke, courtesy of A. E. Wheals.

migrates into the neck and then divides. Cytokinesis follows; it does not involve constriction, as in animal cells, but results from the growth of a septum and two plasma membranes across the neck. Remarkably, although the bulk of the wall of both mother cell and bud consists chiefly of glucans and mannoproteins, the primary septum is made of chitin. Septation begins early in the cycle, with the deposition of a chitin annulus within the neck of the incipient bud; chitin synthesis then halts, resuming after the cell has entered cytokinesis. When the primary septum has been completed, mother and daughter cells augment it with the addition of glucans and mannoproteins to both surfaces. Eventually the two cells separate; the primary septum remains with the mother cell, which carries a prominent bud scar (Fig. 19).

What concerns the student of morphogenesis is how surface growth is accomplished and localized; these matters remain quite obscure. At first glance, the yeast division cycle is not unlike that of streptococci (Fig. 9 and 10), but the resemblance is deceptive and the modes of wall expansion are entirely different. Yeasts have no wall bands and do not divide by zonal growth followed by splitting of the septum. Instead, localized expansion of the bud is supported by the mother cell through secretory vesicles, much as in apical growth. Moreover, the septum is chemically distinct rather than a precursor of wall substance. The differences illustrate the well-known principle that the manner of wall growth cannot be inferred from the final form (90, 91).

Localized and delocalized wall growth. Virgin cells enlarge more or less isotropically, inserting new wall uniformly over the surface (65, 66, 279). The wall is chemically quite simple, consisting chiefly of mannoproteins (exposed at the surface) and β(1→3) glucan chains partly organized into fibrils. Digestion with endoglucanase causes the wall to disintegrate, and it is therefore believed that the glucan chains confer strength and flexibility upon the wall, much as do steel rods embedded in amorphous concrete. Chitin is only a minor component of the yeast wall, but there are indications that a skeleton of chitin fibrils is a prerequisite for the proper assembly of the more abundant constituents (64). Expansion of the wall entails, at least, the synthesis of glucans and mannans. The biochemical pathways are known, but how the products are incorporated into the existing wall is not. It is presumed that glucanases or other hydrolases generate sites for the insertion of new wall elements and that turgor pressure provides the driving force for surface expansion, but the argument relies to an uncomfortable degree upon extrapolation from the manner of budding. The role of glucanase is particularly questionable (see below), and alternative candidates would be very welcome.

With the initiation of the bud, growth becomes localized and vectorial. Budding is analogous to branch initiation and spore germination; in all cases, secretory vesicles accumulate at the presumptive site of outgrowth. These are thought to contain glucanase and other hydrolases which, when secreted locally into the wall, soften it and allow it to bulge (172, 179, 180). The evidence is certainly suggestive, but the argument is weakened by the report of Santos et al. (240) that mutants deficient in exoglucanase bud normally. It is also unclear how the cell determines the locus of budding, which is not a random event. In an influential paper, Byers

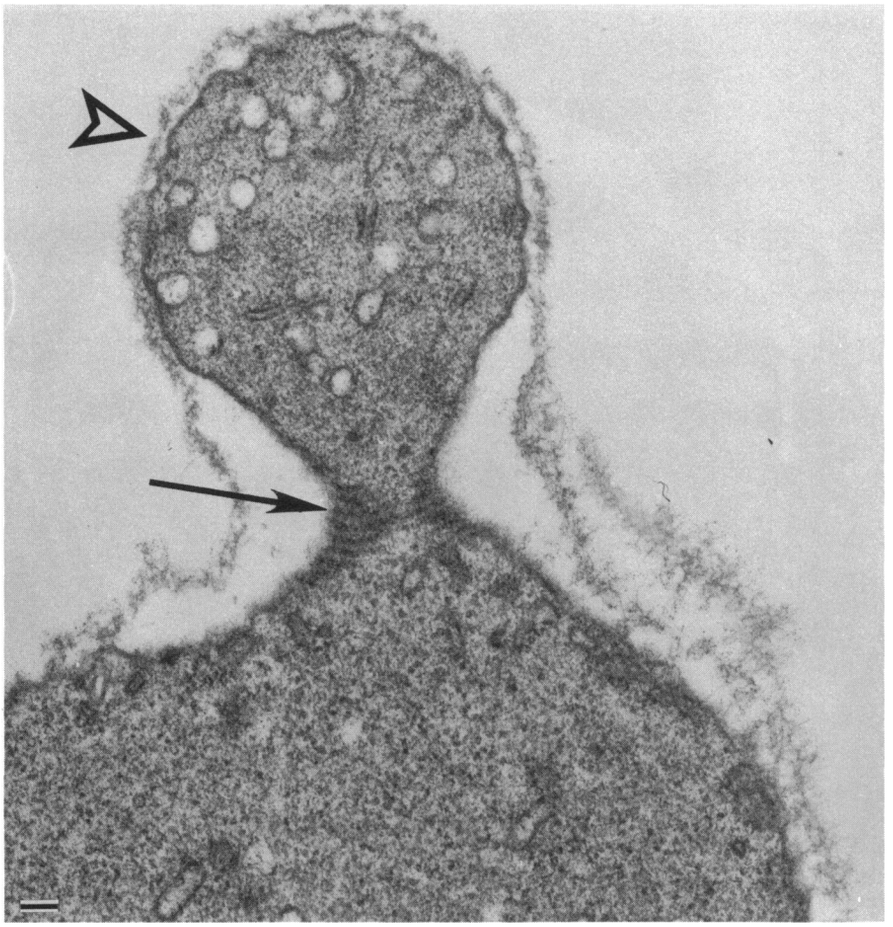


FIG. 20. Early bud of *S. cerevisiae* filled with secretory vesicles (arrowhead). Note the ring of 10-nm filaments at the base of the bud (arrow). Bar, 0.1 μm. Photomicrograph courtesy of B. Byers. Reprinted from the *Journal of Cell Biology* (45) with permission of the publisher.

and Goetsch (44) reported that microtubules extend from the spindle pole on the nuclear membrane toward and into the nascent bud; they suggested that these may guide secretory vesicles to the site of exocytosis. However, more recent studies with inhibitors of microtubule assembly (137, 224) and with mutants deficient in tubulin synthesis (132) have shown unambiguously that microtubules are required for mitosis and nuclear migration, but not for budding. Instead, there is mounting evidence that actin microfilaments play a critical though unidentified role (2, 62, 199). A calcium-binding protein is also involved: mutants of the class *cdc24*, which lack this protein, grow isotropically into enormous spherical cells but fail to initiate buds (205, 255). This links budding in yeasts to the large but chaotic body of evidence that implicates calcium ions in pattern formation, which is discussed in the final section.

The base of the bud is normally defined by a ring of chitin approximately 1 μm in diameter, whose deposition is one of the earliest events. Surprisingly, the chitin annulus may be dispensable, since polyoxin D, an inhibitor of chitin synthase, does not prevent initiation of the bud (46). What is obligatory is a ring of 10-nm filaments (Fig. 20), whose formation is another very early event (44, 258). Mutants lacking this ring, classified as *cdc3*, *cdc10*, *cdc11*, and *cdc12* (Table 2) cannot form buds. The filaments contain neither actin nor tubulin, nor do they appear to be related to intermediate filaments (J. R. Pringle, personal communication); their function is quite unknown.

Once the bud begins to grow, parallels with tip extension become obvious. The distal parts of the bud are filled with vesicles (Fig. 20), whose nature and transport are presently the subject of intensive investigation (243; see also the remarkable three-dimensional reconstructions of yeast cells undergoing morphological changes in response to mating pheromone [7]). The vesicles are components of a secretory pathway that carries membrane proteins and exoenzymes from the endoplasmic reticulum via the Golgi to the cell surface. Enzyme secretion and the production of new surface go together, and both are localized to the distal part of the bud (66, 283). The secretory vesicles are known to contain invertase and other enzymes destined to be released into the medium; acid phosphatase, which remains in the periplasm; and integral membrane proteins, including the proton-translocating ATPase. The vesicles have been purified and characterized. Remarkably, exoenzymes and proton-translocating ATPase occur together in the same vesicle (22, 126, 295). Whether enzymes involved in wall biosynthesis or cleavage are also carried in these vesicles is unfortunately not clear. Chitosomes, carrying latent chitin synthase, lack secretory enzymes and apparently represent a distinct class of vesicles (88).

From the standpoint of morphogenesis, the spatial localization and temporal control of secretory processes are especially critical, for this is probably what determines whether growth takes the form of a bud or a hypha. Once outgrowth has begun, synthesis of wall substance (as judged

by the incorporation of [^{14}C]glucose into glucan and mannan) is confined to the bud, while the mother cell ceases to expand. The wall of the bud is all made afresh, not recruited from the mother cell. Isotope incorporation is initially focused at the tip of the bud but later becomes approximately uniform (65, 66, 143, 255, 279). More recently, Soll and co-workers (257, 264) examined surface expansion in *Candida* species directly by monitoring the position of latex particles bound to the surface. They estimated that 70% of surface expansion takes place at a tiny apical zone, while the remainder is uniform. When the bud has attained about two-thirds of its final circumference, the apical site shuts down, leaving isotropic expansion to complete construction of the bud. By contrast, in hyphae the apical site remains active indefinitely and the contribution of delocalized expansion is minimal. The processes responsible for surface expansion were not identified by Soll and co-workers, but must ultimately reflect secretory activity. The simplest interpretation is that changes in the rate and locus of vesicle exocytosis mold the shape of the bud. Apical extension would differ from budding in that in the former exocytosis is sharply focused, whereas in the latter it is relatively delocalized. Alternatively, it may be possible to accommodate the observations in the model of Bartnicki-Garcia et al. (11, 12), which postulates that translocation of a vesicle supply center is the key event in switching from isotropic to localized secretion (see the preceding section); in this case, one should really expect to find an ultrastructural equivalent of the supply center.

Septum formation. The ultimate event in the division cycle is construction of the septum, which is chemically and architecturally quite distinct from growth of the bud. As mentioned above, whereas the bulk wall is composed largely of glucans and mannoproteins, the septum is made of chitin; virtually all the chitin found in yeast cells occurs in septa (47). Formation of the septum begins early on with the deposition of the chitin annulus. Its subsequent completion by ingrowth of a chitinous diaphragm is closely integrated with other steps in the division cycle: closure normally awaits bud enlargement, nuclear migration, and mitosis, but mutants are known in which septum formation is dissociated from its usual prerequisites. Whereas bud enlargement most probably reflects localized yielding of the wall to applied hydrostatic pressure, the septum of yeasts (and of fungi in general) forms in a stress-free environment. The forces that shape the septum must be quite different from those that mold the bud.

A latent chitin synthase is present in the yeast plasma membrane. The enzyme is distributed uniformly over the cell surface, and until quite recently septum formation was envisaged as the spatially localized activation of this enzyme, probably by proteolysis (47). It is now clear that septum formation is mediated by a separate, hitherto unknown enzyme designated chitin synthase 2 (242, 252). This enzyme, like the better-known chitin synthase 1, occurs as a zymogen and requires proteolytic activation; spatially localized activation may be what regulates septum production. Vesicles accumulate at the site of the growing septum, but their contents are not known; one wonders whether chitosomes bearing the new synthase are among them. Incidentally, it is possible that generation of the early chitin annulus, which apparently serves as a template for the work done by chitin synthase 2, requires yet a third chitin synthase.

At the end of the day, we do not understand what makes a bud grow and separate in an orderly manner from its mother cell. As in the case of hyphal tips, the contributions of turgor pressure, wall plasticity, localized exocytosis, and enzymes that manipulate polymers all remain topics for speculation and further research. The largest lacunae in our understanding concern directional processes and events localized in space or in time. The limitations of both our technology and our conceptual framework make these the hardest gaps to fill.

Germination of Fucoid Embryos

The marine brown algae *Fucus* and *Pelvetia* attain a length of several meters and hardly qualify as microorganisms. They nevertheless hold a special place in this review, and in developmental biology generally, because the germination of fucoid zygotes has provided a favorite exemplar of biological pattern formation for nearly a century. The genesis of patterns will be the subject of the following section; my concern here is with the changes in cell shape during germination and the remodeling of the wall which these entail.

Within a few minutes following fertilization, the naked zygote deposits a thin, fragile primary wall by the exocytosis of cortical vesicles. During the next 6 h the wall thickens and becomes sufficiently stable to retain its shape when isolated. Cell walls at this stage contain some 20% cellulose in ordered lamellae, 20% fucans (polymers rich in fucose and sulfated to various degrees, which seem to be required for adhesion of the embryo to its substratum), and 60% alginic acid (a polymer of mannuronic and glucuronic acids). Concurrently, the zygote accumulates salts, chiefly KCl, which raise the intracellular osmotic pressure above that of seawater by about 5 atm (0.5 MPa) (211, 222, 223). Its form is still spherical, without any outward sign of polarization, but by 12 h after fertilization the *Fucus* embryo has developed a slight bulge or protuberance (Fig. 21). The future rhizoid grows progressively more elongated and acquires a definite axis of symmetry, while the volume of the cell changes little if at all. Around 22 h the embryo lays down a cross-wall that demarcates two unequal cells which have different developmental fates. The rhizoid cell, which comprises the outgrowth, is destined to become the holdfast; in the early stages of development this is the growing part, for the first objective of the embryo is to secure firm footing. The larger thallus cell is destined to become the frond. Upon completion of the first cleavage the pattern of the future plant is established, including its permanent polar axis. What makes this system so attractive to developmental biologists is that the zygote is unpolarized: the locus of germination, and hence the polar axis, is determined by environmental vectors such as unilateral light, which are under the control of the investigator. Just how the embryo goes about changing its shape has attracted much less attention than the selection of its axis.

The unpolarized, symmetrical nature of the early embryo is evident not only in its outward form but also in its ultrastructure. However, even before outgrowth of the surface begins, signs of polarity appear at the level of intracellular organelles. Osmiophilic granules begin to accumulate near the presumptive rhizoid pole, and fingerlike projections radiate from the nuclear membrane toward that site. The perinuclear region fills with Golgi vesicles, and in time a secretory pathway arises that directs vesicles to the site of germination (25, 221; Fig. 21f). The flow of secretory vesicles was tracked by monitoring a specific constituent, the sulfated fucan called fucoidin, which is deposited at the tip of the growing rhizoid. Fucoidin serves no essential role in

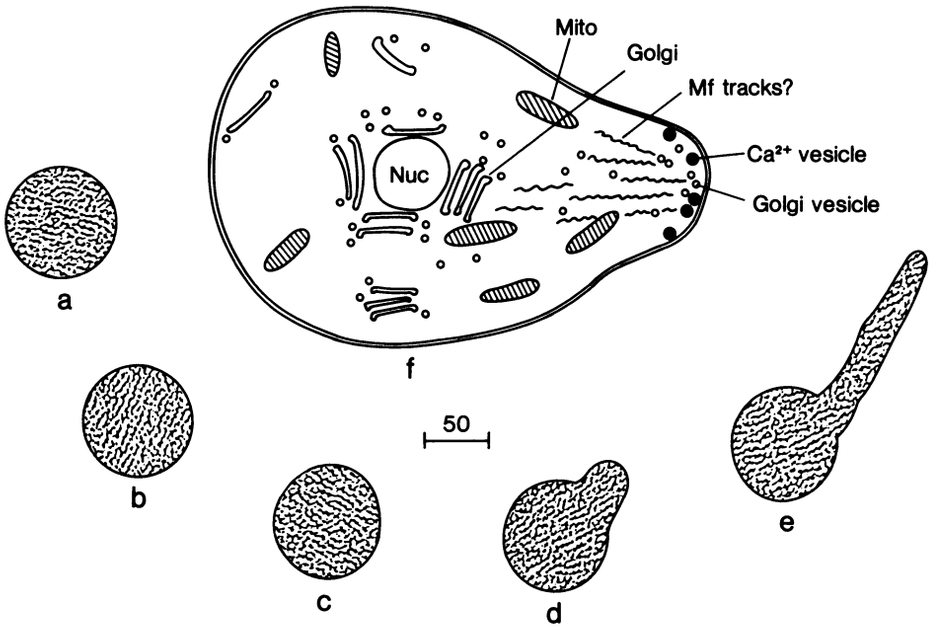


FIG. 21. Shape transformation during early development of *Fucus distichus*. (a) Unfertilized egg. (b) Zygote at 6 h, ungerminated. (c) Zygote at 15 h, beginning to germinate. (d) Germinated, unicellular embryo at 20 h. (e) Fourteen-celled embryo at 72 h. Outlines from photographs by Kropf and Quatrano (169); bar, 50 μm. (f) Polarized zygote (speculative sketch). Indications are that Golgi vesicles travel to the site of outgrowth, that mitochondria become axially oriented, and that several kinds of vesicles accumulate in the presumptive rhizoid. The "tracks" thought to guide vesicles to the site of exocytosis are especially speculative. The nucleus remains in the cell center; chloroplasts (not shown) are dispersed throughout the cell. Drawn with advice from S. H. Brawley, D. L. Kropf, and R. Quatrano, none of whom bear responsibility for sins of omission or commission.

germination: cells grown in a medium containing methionine in place of sulfate cannot make fucoidin, yet they do generate rhizoidal outgrowths (albeit more slowly) and they presumably establish the secretory pathway that carries vesicles to the rhizoid. Vesicle translocation is inhibited by cytochalasin, but exocytosis itself is unaffected (23, 24). We can therefore consider germination of fucoid embryos to be a special instance of apical growth.

Just what substances are carried to the site of germination, aside from fucoidin, is not clear. Some of the vesicles appear to carry alginate and perhaps other wall precursors; cellulose is presumably deposited in situ by secreted cellulose synthase, although there is no direct evidence of this (25, 211). By analogy with other organisms, one may expect localized loosening of the wall in the rhizoidal region, and there is some indication that alginase is secreted there (292). Turgor pressure is likely to supply the driving force for surface remodeling; the finding that addition of sucrose to the seawater can prevent outgrowth (222) is consistent with that presumption. Putting together a web of inference and speculation, it is reasonable to see the establishment of directional secretion (Fig. 21f) as the central event in polarization. Regrettably, the disposition of the Golgi apparatus is not certain, and neither is the nature of the tracks that are presumed to link it to the rhizoid tip. Studies with cytochalasins and colchicine indicate that actin is involved (24, 165, 222) and have been interpreted as evidence that the tracks consist of microfilaments. The involvement of microtubules is presently being reinvestigated.

In fucoid zygotes, as in other walled cells, the wall maintains the form; protoplasts are spherical. It now appears that the cell wall is also required to fix the polar axis. The idea that formation of a polar axis and its fixation are distinct and dissociable phenomena grew out of early experiments on the orientation of outgrowth by a long pulse of light (222). Unilateral light causes the rhizoid to emerge from the shaded half. A second pulse of light can impose its own polarity, overriding the first pulse, if given early enough, but by 11 h after fertilization the axis has become fixed and is no longer subject to reorientation by subsequent light pulses. Kropf et al. (168) have now shown that *Fucus* protoplasts can form an axis, but it remains labile indefinitely unless wall regeneration is permitted. The findings are consistent with the notion that axis formation involves the establishment of an oriented secretory pathway, which is then stabilized by anchorage to the cell wall. As in other walled cells, it appears that turgor pressure drives wall growth and secretion localizes it.

Mechanical Design and Morphogenesis in Algal Cells

In fungi, the characteristic mode of surface expansion is apical growth; diffuse intercalation of new wall over the entire surface does occur, as in yeasts, but is less prominent. In plants and algae, although apical growth is not unheard of (pollen tubes, root hairs, algal rhizoids), diffuse surface growth is the norm. This mode of growth generates a surprising variety of shapes, thanks to the mechanical anisotropy of the cell wall.

How *Nitella* shapes a tube. Much of our insight into these matters grew out of research on the giant internodal cells of freshwater algae. In *Nitella* species these tubular cells, some 5 cm in length and 0.5 mm in diameter at maturity, arise as 20-μm segments carved out of the growing apex. Subsequent enlargement by a factor of 5,000 or more consistently favors an increase in length over diameter by a ratio of 5:1. *Nitella* internodal cells, like other plant cells, are under turgor: indeed, turgor pressure is thought to supply the driving force for extension, and it is well known that in a cylinder under

pressure, the stress in the transverse direction is twice as large as in the longitudinal one. How, then, does the cell confine its expansion to the longitudinal direction?

The explanation was found in the physical structure of the cell wall (90, 91, 268). The *Nitella* cell wall, like that of other algae and higher plants, is chemically complex, consisting chiefly of cellulose microfibrils embedded in an amorphous matrix of hemicellulose, pectins, and protein. The manner of their association and the degree of covalent cross-linking remain uncertain. The mechanical strength of the wall is attributed to the cellulose because inhibitors of cellulose synthesis cause growing cells to burst. It became clear early on that the cellulose microfibrils are deposited in a regular manner, with an orientation transverse to the long axis. These cellulose hoops reinforce the cylinder, resisting expansion in girth, but can slide apart to allow expansion in length. One can trace this process by monitoring the orientation of cellulose microfibrils with polarized light. New microfibrils deposited on the external surface of the plasma membrane are oriented transversely to the long axis. As the wall ages and stretches in the course of growth, microfibrils reorient until, in the oldest and outermost part of the wall, they are almost longitudinally arrayed.

The morphogenetic significance of transversely oriented cellulose microfibrils was clearly demonstrated by disrupting their organization. As will be discussed below, the orientation of cellulose microfibrils is determined by a cortical array of microtubules. Colchicine, a powerful inhibitor of microtubule assembly, does not inhibit cellulose synthesis but causes the fibrils to be deposited at random. Consequently, soon after addition of the drug, the ratio of growth rates (in length versus girth) shifts dramatically in favor of radial expansion. It is important that only the newly deposited wall is affected by drug treatment; this is clearly also the stress-bearing part. Upon removal of the colchicine, deposition of transverse microfibrils resumes and the ratio of growth rates shifts back to favor longitudinal extension (90, 268). One arrives, then, at a clear sense that the wall is mechanically designed so as to yield to turgor stress in the longitudinal rather than the radial direction.

The progressive change in microfibril orientation from transverse to nearly longitudinal is evidence that the wall is physically stretched as the cell grows. The wall maintains a constant thickness all the same, because extension is accompanied by the intercalation of new wall material diffusely over the entire surface (90, 268). At least two processes contribute to wall synthesis. Matrix components are deposited by the exocytosis of Golgi vesicles that carry precursors to the surface (300). Cellulose, by contrast, is produced in situ by a cellulose synthase located in the plasma membrane (36, 56). The enzyme has long been elusive, and satisfactory cellulose synthesis in vitro has yet to be achieved; the enzyme can be visualized, however, in the form of ordered arrays of intramembrane particles located at the ends of cellulose microfibrils. The discovery of similar arrays in Golgi vesicles suggests that in the case of cellulose it is the enzyme, rather than the precursors, that is transported to the membrane in vesicles.

Cellulose appears in plant walls in the form of characteristic lamellae or bands of parallel microfibrils (see Fig. 24) whose orientation influences the mechanical parameters of the wall and ultimately molds the shape of the cell. Production of these lamellae is itself somewhat convoluted. Cellulose-synthesizing complexes are believed to be mobile in the plane of the plasma membrane. Each subunit is an active catalytic entity that spins out a single glucan polymer. These associate spontaneously into cellulose microfibrils whose diameter is proportional to the number of contributing catalytic units. Synthesis and polymerization of the cellulose microfibrils exert force upon the complexes, setting them in motion. What cortical microtubules may do is to constrain the movements of cellulose synthase complexes into parallel lines, probably by quite indirect mechanisms (see below). The specification of cell shape by the cytoskeleton is thus quite circuitous.

The ordered deposition of cellulose in transverse hoops accounts for the mechanical properties of the *Nitella* cylinder, but we have still to explain how new wall elements are inserted among the old ones as the cell grows. Physical stretching of the wall by hydrostatic pressure is clearly involved, but compliance is not purely mechanical and remains poorly understood. Stretching and loosening of the wall depend upon metabolic activity, probably because they involve the breaking and making of bonds by hydrolysis or transglycosylation. Protons play a key regulatory role in this process. Acidification of the wall by the extrusion of protons from the cytoplasm is effected by an H^+-ATPase, which is a target of the growth hormone auxin (268). Under certain growth conditions, the *Nitella* cell localizes these proton pumps to discrete circumferential bands, and wall extension is confined to these bands (188). Evidently, one mechanism that can determine the site of surface expansion is the localization of wall loosening.

Ordered cellulose deposition generates diverse forms. Many unicellular algae, such as *Nitella* spp. and higher plants, generate form by constraining turgor pressure with an ordered array of wall microfibrils. In most cases the fibrils are made of cellulose and their production is aligned upon a framework of cortical microtubules (97, 174, 175).

A compelling case in point is that of the filamentous algae *Mougeotia* spp., from which one can readily prepare protoplasts that grow, regenerate walls, and eventually recover their elongated, cylindrical form. Reconstitution of the cylindrical shape depends upon the progressive construction of an array of parallel microtubules subjacent to the plasma membrane; these, in turn, guide the deposition of cellulose hoops that define the shape of the emerging cylinder (Fig. 22). Treatments that prevent assembly of the microtubule array lead to the deposition of random wall fibrils, surrounding spherical cells. Inhibition of cellulose synthesis does not block the formation of the microtubule array, yet the protoplasts remain spherical (75, 177). Clearly, the cellulose microfibrils determine the form of the cell, while the microtubules specify the arrangement of these fibrils.

Closterium spp. are unicellular algae, crescent shaped with pointed wings and a nucleus positioned at the midpoint. Division cleaves the cell transversely into two semicells, each with an original pointed end and a new blunt one; the blunt end expands to regenerate the original form (Fig. 23). In the old half of the cell, wall microfibrils are arranged in randomly overlapping lamellae, while in the growing semicell, microfibrils are neatly laid down transversely to the axis of the cell; a sharp boundary separates the two halves (Fig. 24).

The parallel microfibrils in the expanding semicell are aligned upon an array of parallel microtubules that pass transversely around the cell. No cortical microtubules are present in the old semicell. If the formation of the microtubule array is perturbed with colchicine, wall microfibrils are laid down at random and the growing semicell bulges out spherically (Fig. 23). After some hours microtubules disappear from the cortex of the new semicell; secondary wall is

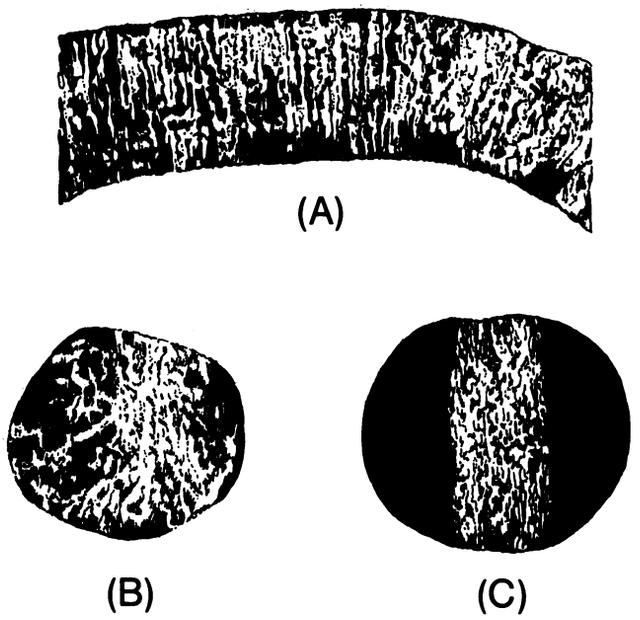


FIG. 22. Microtubules and microfibrils in regenerating *Mougeotia* protoplasts, showing cortical microtubules in a filamentous, intact cell (A) and cortical microtubules (B) and wall microfilaments (C) in protoplasts allowed to regenerate for 21 h. Note the partial recovery of the cylindrical form and the locational correspondence between microtubules and microfibrils. Sketch based on photomicrographs of Galway and Hardham in *Protoplasma* (75) with permission of A. R. Hardham and Springer-Verlag.

then laid down in random plies, producing the wall structure characteristic of mature cells (123–125, 212).

The ellipsoidal cells of the alga *Oocystis solitaria* supply a striking and particularly well-documented variation upon the theme of morphogenesis with the aid of architectural cellulose microfibrils. In mature cells the fibrils are deposited in successive, regular layers, 25 to 30 layers per cell, like the skins of an onion. Within any one layer the fibrils run parallel to one another, while those in the layers above and below run at right angles to the first. The fibrils wind helically around the cell, converging at the poles.

One can watch the genesis of this remarkable structure as the cell grows. *O. solitaria* reproduces by becoming multinucleate, each nucleus then giving rise to an intracellular autospore. The autospores, naked to begin with, lay down one wall layer every hour or so; whenever they start a new layer the direction of the helix changes by 90°, producing the alternating plies (Fig. 25). The alternation of helix orientation depends upon microtubules: if colchicine is present, cellulose microfibrils continue to be deposited in parallel arrays, but the change in orientation fails to occur. The fact that autospores developing in the presence of colchicine are spherical rather than ellipsoidal testifies to the morphogenetic significance of the alternating plies. As in *Nitella* cells, only the innermost lamella is affected by colchicine; upon removal of the inhibitor, formation of alternating lamellae resumes (220, 232, 238).

Cellulose synthase is represented by an elongated terminal complex in the outer leaflet of the plasma membrane, which marks the end of each cellulose microfibril. The complex is subtended by a granule band of uncertain nature and then, deeper in the cortex, by a microtubule. Microtubules always run parallel to one of the two microfibril directions, usually to that of the innermost layer, and extensive evidence supports the thesis that the microtubule direction specifies that of the cellulose fibrils. Apparently the cellulase synthase complexes are inserted pairwise into the membrane; they move apart when synthesis begins, reversing direction at the pole, laying down parallel cellulose fibrils like so many paving machines. How the direction is switched upon the completion of each layer is not too clear; it has been proposed that a new set of microtubules and cellulose synthases is manufactured and inserted into the plasma membrane at right angles to the old set (192, 218, 219).

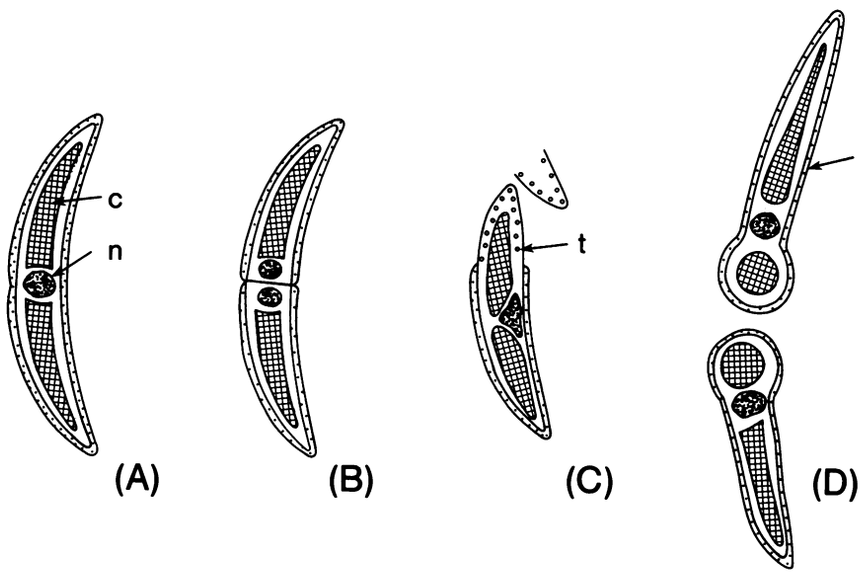


FIG. 23. Aspects of division in *Closterium* cells. (A) Symmetrical interphase cell with central nucleus (n) and large chloroplast (c). (B) Cell after the nucleus (n) has divided and the septum closed. (C) Daughter cell with semicell expansion under way; note the transverse microtubules (t). (D) Dividing cell incubated in colchicine for 1 day. Panels A to C reprinted from reference 212 with permission from Sinauer Associates. Panel D based on artwork in reference 125.

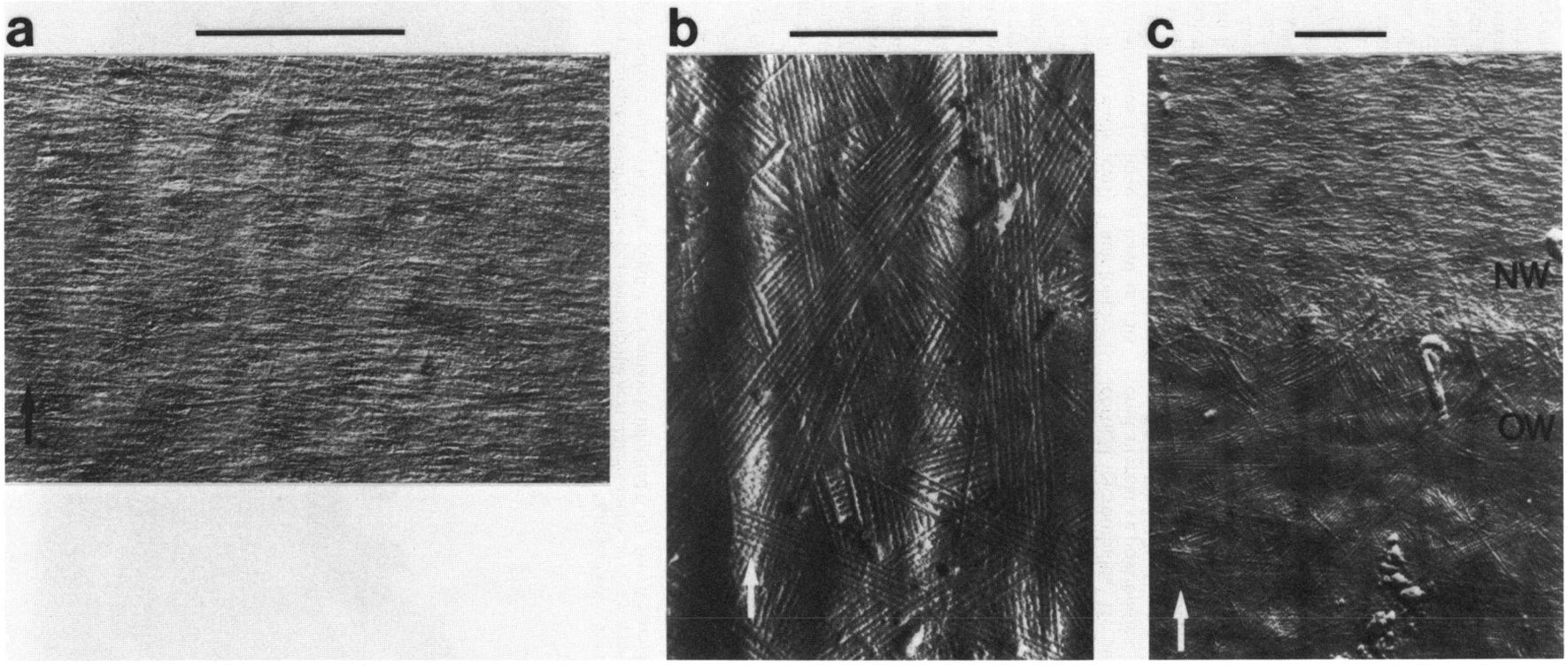


FIG. 24. Arrangement of microfibrils on the inner surface of the cell wall of *Closterium acerosum*. (A) Microfibrils in new wall, 2 h after septum formation; the fibrils lie transverse to the axis of the cell. (B) Microfibrils in old wall, 2 h after septum formation; the wall consists of multiple lamellae, without preferred orientation. (C) The boundary between new and old wall. Bar, 1 μm; the arrows mark the axis of the cell. Reprinted from *Planta* (124) with permission of the publisher.

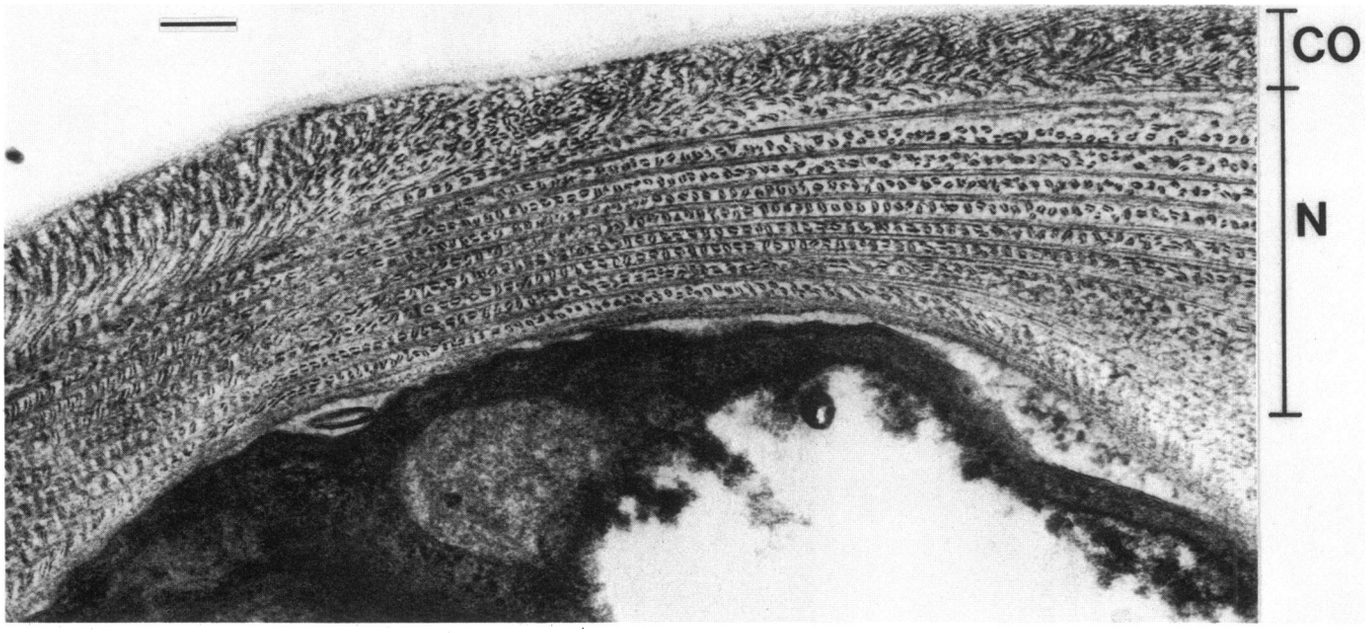


FIG. 25. Correspondence of microtubules and wall microfibrils in *Oocystis* cells. As described in the text, wall microfibrils lie parallel to one another, but their direction alternates by 90° from one lamella to the next. Microtubules always run parallel to one of these directions (N). When microtubules are depolymerized with colchicine, regular alternation is inhibited (CO). Bar, 0.1 μm. Photomicrograph courtesy of D. G. Robinson.

The ellipsoidal cells of the alga *Glaucocystis nostchinearum* resemble those of *O. solitaria*, although the two are thought to be unrelated. The wall consists of cellulose microfibrils embedded in an amorphous matrix; as many as 12 bands of alternating right-handed and left-handed helices are wound about the cell. The configuration of the poles is especially remarkable. Individual microfibrils do not terminate at the poles but continue, looping around and between a trio of rotation centers. Willison and Brown (301, 302) proposed a model to explain how the wall is deposited: whenever a synthase complex executes this turnabout, it proceeds on its way with changed handedness and at approximately right angles to its previous direction, laying down the next band (actually, a front of synthases travels more or less in register). Successive bands are continuous; were it possible to unravel the wall it would consist, like a silk cocoon, of continuous cellulose microfibrils each nearly 1 mm long.

One would expect microtubules to have a hand in this weaving, but that has become unlikely. In *Glaucocystis* cells, broad flat vesicular shields underlie the plasma membrane. Cortical microtubules are present, but so distant from the plasma membrane that it is hard to envisage how spatial coherence between the microtubules and wall microfibrils could be maintained. Colchicine, even at high concentrations, has no effect on wall organization.

Questions of causality. The preceding discussion bears upon two levels of spatial order, the role of wall microfibrils in shaping the cell as a whole, and the role of microtubules in orienting the deposition of microfibrils. This chain of cause and effect is presently one of the most explicit models for the generation of order at the cellular level, and its links deserve respectful but critical scrutiny.

There is little doubt that for the organisms considered in this section, the deposition of wall microfibrils in the proper order is a necessary condition for normal morphogenesis. Cylindrical cells require reinforcement by transverse hoops, and ellipsoidal ones depend on the helically crossed lamellae (although in the latter instance it is not so obvious how the configuration of the microfibrils generates the form of the cell). To clinch the case it will be necessary to devise a method to change the pattern of microfibril deposition from one orderly mode to another. However, it is also clear that we do not yet have the whole story. Transverse microfibrils are necessary, but cannot be sufficient, to shape the gently curved wing of an expanding *Closterium* semicell; what additional constraints must be applied is not known.

The case that cortical microtubules orient the deposition of wall microfibrils is less compelling, if only because it is not clear how they might do so. Heath (112), who first formulated an explicit proposal, suggested that the movement of cellulose synthase in the plasma membrane is driven by force-generating proteins that interact with the subjacent cortical microtubules. He and others subsequently considered alternative possibilities, which were reviewed by Heath and Seagull (117). Recent studies agree that there is no unique one-to-one correspondence between microtubules and wall microfibrils, not even perfectly parallel alignment (77, 219). This strongly suggests that coupling must be loose: for instance, microtubules may define plasma membrane channels within which cellulose-synthesizing complexes are free to wander or to be swept along by membrane flow.

The evidence for correlated orientation of cortical microtubules and of wall microfibrils is very strong. Some of the best evidence that the former precedes and predicts the latter comes from higher plants (reviewed in references 97, 174, and 175; see also reference 186). Contradictory examples such as *Glaucocystis* spp. may be true exceptions that test the rule or may merely underscore the limited information presently available. What is at issue is whether the orientation of cortical microtubules determines that of the microfibrils, and this thesis rests to an uncomfortable degree on the effects of inhibitors, chiefly colchicine, which may

have other targets besides microtubules. It would be reassuring if mutants, resistant to colchicine or other antitubulins by virtue of a lesion in the structure of tubulin (or of a microtubule-associated protein), were found to be resistant to the morphological effects as well. Until the incontrovertible experiment is reported, we should lend an ear to skeptics such as Preston (216), himself a pioneer in the study of wall microfibrils and a leading contributor for 40 years. Preston gives reasons to doubt that microtubules guide microfibril deposition, suggesting instead that both are oriented by a common agency, possibly an electric field.

Searching for a Paradigm

Eucaryotic microorganisms have explored myriad solutions to the problem of controlled surface enlargement; depending upon one's attitude to historical contingency as a driving force for evolutionary change, their extravagant variety can be either delightful or exasperating. As far as I can judge, only two common themes underlie the diversity of forms and functions: localized compliance with hydrostatic pressure, and localized exocytosis of secretory vesicles.

The central place of hydrostatic pressure as the driving force for growth, and by implication for morphogenesis, is quite generally accepted by plant physiologists, thanks to the pioneering researches of Green and co-workers with *Nitella* spp. (90, 91, 225) and to much contemporary work on wall mechanics in higher plants (52, 268). Plant cells enlarge by imbibing water. This flows passively into the cell down the gradient of water potential, which is the result of the accumulation of ions and metabolites in the cytoplasm. Water influx is restrained by the wall, which is consequently subjected to considerable hydrostatic pressure; net influx ceases when the two forces balance. Enlargement depends, then, on controlled expansion of the surface; and the spatial constraints upon this process mold the form.

Walled microorganisms are also commonly, perhaps universally, subject to turgor, and their forms must at least be such as to withstand the stress. Turgor is commonly required for cell growth, since the addition of osmolites to the external medium halts growth at least temporarily. However, it must be said that there is no direct evidence that turgor provides the driving force for surface expansion, rather than serving only to appress the plasma membrane to the wall. The study of turgor—its magnitude, genesis, and regulation—is hampered by the lack of convenient and direct methods to measure the hydrostatic pressure in live microbial cells. Experimental manipulation of the pressure, mechanically or perhaps by the use of mutants that accumulate osmoprotectants constitutively, would help to clarify the physiological functions of cell turgor. One wonders particularly about the many unicellular organisms that are equipped with a contractile vacuole and can thus regulate turgor independently of the osmotic gradient, e.g., *Chlamydomonas*, *Glaucocystis*, and the scale-forming marine algae. It is not clear to me whether these have significant turgor pressure at all (although *Ochromonas* cells, which have a contractile vacuole but no cell wall, do maintain turgor [148]). Does the form of the alga *Chlamydomonas*, whose wall is composed of self-assembling subunits (1), owe anything to hydrostatic pressure? Clearly, the thesis that morphogenesis in walled eucaryotic microorganisms revolves around the theme of global force and local compliance proclaims no established truth; on the contrary, it is intended precisely to encourage reflection and to provoke challenge.

Whether or not surface expansion is in all cases driven by hydrostatic pressure, wall deposition must reflect the manner in which the exocytosis of secretory vesicles is organized in time and in space. This general constraint leaves ample room for diversity, and there is no fixed relationship between the architecture of the secretory apparatus and the visible form. In some organisms, the vectorial character of the secretory pathway is apparent in its ultrastructure. Fucoid zygotes are a case in point, and the scale-forming algae are an even more spectacular one (34, 35); yet the former make an apex, whereas the latter come out nearly spherical, probably because the protoplast rotates during secretion. Fungal hyphae and internodal cells both make cylinders, but by entirely different mechanisms. In neither case is there any obvious relationship between the placement of Golgi units and the loci of secretion; localization must reflect either the pathway of vesicle transport or control of the exocytosis site.

In this context, it is noteworthy that polarized secretion plays a role even in unwalled fibroblasts and amoebae, which renew their surface as they crawl along, targeting vesicles to the front with the aid of a highly structured cytoskeleton. If it is true, as Oster and Perelson (208) have recently argued, that motive power is supplied by a gradient of hydrostatic pressure, the gulf that apparently separates morphogenesis in walled and unwalled cells will narrow still further.

How can one experimentally address morphogenesis as an integrated process, over and above the contributions of its individual strands? Of the techniques now becoming available, I have been most impressed by the power of computer simulation, especially when it is grounded in explicit and physiologically meaningful assumptions. That a given set of premises generates a realistic shape is, of course, no guarantee that the hypothesis is correct; but those that fail the test are surely suspect. When the computational techniques that simulate the elementary shapes or bacteria and fungal hyphae have been extended to *Closterium* spp. and, better yet, to *Micrasterias* spp., we shall be entitled to feel that morphogenesis is becoming understood.

SELF-ORGANIZATION

One seeks to understand morphogenesis on two levels. The upper level centers on the mechanics of shape generation; this has been a productive line of research, and in the preceding sections we surveyed what has been learned regarding the forces that mold unicellular organisms and the physiological mechanisms by which cells comply in a controlled, localized manner. We turn now to the deeper level, pattern formation. Here we ask not what cells do, but how they direct what is done, in space and in time; the central issue is localization. Progress along this line has been slow: morphogenesis remains baffling precisely because we have no satisfying hypothesis to account for pattern formation.

A pattern, according to Webster, is simply "a coherent structure or design." Spatial patterns among single-celled organisms vary enormously, from the plain but regular shapes of bacteria and hyphae to the elaborate architecture of ciliate surfaces. Pattern formation, then, lumps together a variety of processes that generate spatial design—elementary shapes no less than baroque ensembles, the transformation of an existent pattern into a new one as well as the emergence of order from an undifferentiated, homogeneous prior state.

The appearance of organized structure where there was none before is a quintessential characteristic of life. At the turn of the century the German embryologist Hans Driesch,

despairing of the hope that physics and chemistry could in principle explain the self-organization of an embryo, invoked a nonmaterial agency called entelechy to call forth order out of chaos. Students of microorganisms face analogous problems, simpler perhaps, but scarcely less mystifying. The germination of a spore, the outgrowth of a fucoid zygote, the sculpting of a desmid semicell in the course of division, all entail the spontaneous emergence of a regular spatial pattern. How this comes about still largely passes understanding, but in one crucial respect we are better off than Driesch was: we know now that the spontaneous emergence of order is quite compatible with the laws of physics and chemistry. However long and stony the road may be, we can be confident that biological pattern formation is not an ineffable mystery but a legitimate province of natural science.

Morphogens and Fields

Kinetic basis of pattern formation. With the benefit of hindsight, the transformation of self-organization from a mystery into a problem can be credited to a single person: the British mathematician A. M. Turing (1912–1954). In a paper boldly entitled *The Chemical Basis of Morphogenesis*, Turing (287) made two important points. One was the proposition that developmental events are called forth by specialized informational molecules, the morphogens, whose distribution in space supplies a prepattern for the subsequent genesis of biological structures. In contemporary idiom, the graded distribution of morphogens constitutes a field of positional information, a kind of map, that instructs a population of cells concerning the course of their future development. The other was the wholly novel insight that spatial patterns of concentration can arise spontaneously when two substances that react with one another diffuse at different rates. Contrary to intuition, which associates diffusion with smoothing out concentration differences, in systems that obey particular kinetic constraints, random fluctuations arising within a homogeneous region will be spontaneously amplified, generating stable local maxima and minima of reagent concentration. It was this demonstration that spatial order can in principle arise ex nihilo that brought morphogenesis within the purview of physical science. In the present context, it is important to note that Turing's principle can be applied as readily to single cells as to populations (67, 68).

Reaction-diffusion theory, as this field of inquiry is called, has been of more interest to theoreticians than to experimentalists. Part of the reason is, no doubt, the advanced mathematical skills required to pursue such work; but I concur with Harrison (105) that what so many biologists find uncongenial is the kinetic preconception: those who are convinced that cells and organisms are shaped by the specific association of macromolecules (as viruses are) will see little need for mathematical subtleties. This is a pity, for in truth only kinetic theories can account for the spontaneous generation of spatial order on the scale of cells and above. Reviews by Meinhardt (185), Peacocke (210), and Harrison (105) are relatively accessible to unsophisticated readers (including this reviewer) and convey the flavor of the field. Briefly, these investigators and others (notably I. A. Prigogine and the Brussels school) have devised several reaction schemes which, when fitted with judiciously chosen rate constants and run on a computer, simulate the spontaneous generation of spatial patterns; some bear an uncanny resemblance to biological ones. An exceedingly important feature is that spatial patterns can arise only so long as the state of the system is far from thermodynamic equilibrium. These are "dissipative structures" (198, 210), which are dependent on a flux of energy, and they represent the conversion of energy into organization.

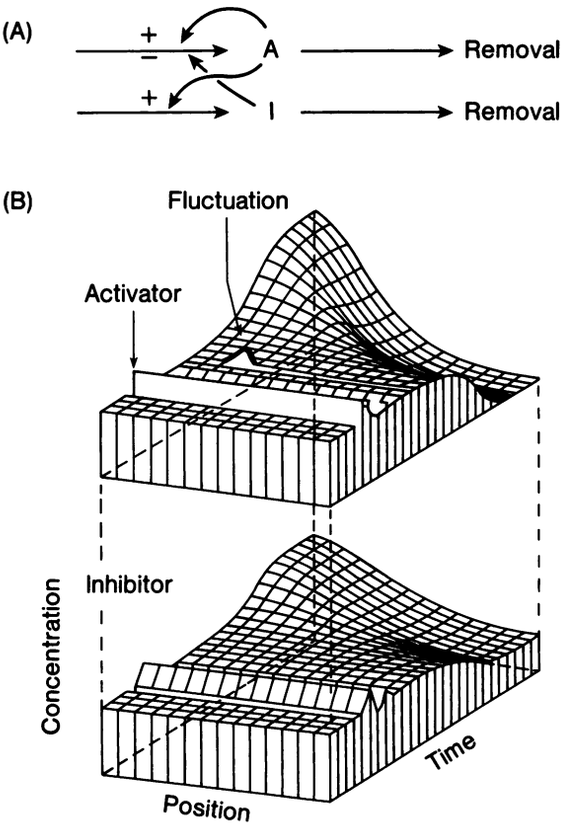


FIG. 26. Development of a spatial pattern of morphogen concentration. (A) Interaction of the activator, A, and the inhibitor, I. (B) Progressive development of a polar pattern. The activator and inhibitor concentrations in a linear array of cells are plotted as a function of position and of time. A uniform increase in the activator concentration (arrow) disappears following a compensating increase in the inhibitor concentration. However, a small local activator increase, even a random fluctuation, is amplified and generates pronounced localized maxima of both substances. Panel A is based on the scheme in reference 254; panel B is modified and reprinted from reference 185 with permission from Academic Press, Inc.

One version whose behavior can be appreciated intuitively has been intensively developed by Meinhardt (185). Spatial patterns can arise in a system containing two morphogens, one autocatalytic and slow to diffuse, the other inhibitory and capable of rapid diffusion (Fig. 26). Imagine a substance, A, an activator that stimulates its own production and also elicits the production of an inhibitor, I, that inhibits the formation of A. Both substances are assumed to diffuse, I more rapidly than A, and each is removed at a rate that is proportional to its concentration. In an extended field, a homogeneous distribution of A and I will be unstable since any local elevation in the concentration of A, perhaps as a result of random fluctuation, will be amplified by autocatalysis. The increase in the concentration of A will also stimulate the production of I. Since I rapidly diffuses away, it does not prevent the local increase of activator concentration, but suppresses its production in the surrounding region. In consequence, the local concentration of A increases spontaneously, the maximum sharpening until some limiting

factor comes into play (for instance, the rate of diffusion or degradation of A rising to equal its production). A stable profile can thus arise, whose form depends on factors such as the stability and diffusion constants of A and I (Fig. 26). Monotonic gradients, periodic patterns, even oscillating ones, can be generated and can exhibit properties such as regeneration of a maximum following its removal and independence of the pattern from the overall size of the field.

Reaction-diffusion theory can, in principle, account for the specification of most (possibly of all) biological patterns, although the mathematical obstacles are often formidable. In the present context, the most pertinent efforts are those of Lacalli (170) and of Harrison and Kolar (106) to stimulate pattern generation during the development of desmid algae, and Harrison's studies on *Acetabularia* development. Stems of that giant unicellular marine alga (Fig. 1E) extend by tip growth. From time to time a tip halts, throws off a whorl of hairs evenly spaced around its circumference, and then resumes extending. Periodic patterns of this kind lend themselves to simulation (Fig. 27). The diagram depicts, not the shapes of tip and hairs, but the calculated distribution of a hypothetical morphogen (104). In a subsequent paper, Harrison et al. showed that the distribution of membrane-associated calcium corresponds to the predicted morphogen pattern (107), one of many hints that calcium ions play a central role in pattern formation (see below). Results of this kind are at once impressive and frustrating, as their authors are well aware: "The fact that a particular reaction-diffusion mechanism can generate patterns resembling those seen in a biological example is not necessarily of great significance, since a variety of quite different mechanisms could give the same or very similar behavior. Without a means to identify or test the possible alternatives systematically, the worth of any one model either in itself or as a representative of a particular class of related mechanisms is difficult to assess." (170).

Nearly 40 years have passed since Turing's leap of the imagination, and with time the meaning of the word morphogen has become more diffuse. Regrettable as it may be (105), biologists now use it to designate any substance that conveys information by virtue of being distributed in a graded or patterned manner. The simplest spatial pattern is a diffusion gradient that stretches from a source of that substance to a sink where it is removed. Embryologists have suspected for nearly a century that simple linear gradients of diffusible molecules may be involved in cell specification (171, 254). During the past decade at least three molecules have been identified as morphogens in that sense: certain peptides in the regeneration of *Hydra* cells (105), retinoic acid in vertebrate limb development (27), and now the protein encoded by the *bicoid* gene of *Drosophila* embryos (61). In all these cases, a primary asymmetry is present beforehand; the molecules in question do not break the symmetry of the system, and they are not morphogens in Turing's original sense. However, one can scarcely doubt that their discovery constitutes a giant advance in our understanding of the steps that intervene between genes and organismic forms. Whether there are diffusible molecules that play a corresponding role at the level of single cells remains to be seen.

All manner of fields. Morphogens illustrate what developmental biologists mean by a field. The *Oxford English Dictionary* cites the definition given by Huxley and de Baer, who introduced the concept 50 years ago: a region of space over which some agency acts in a coordinated manner. More generally, a field is any domain of relational order (83–85, 206). Gradations within a field are likely to be smooth, but the mathematical description may allow for discontinuities. Morphogenetic fields have special spatial and temporal features, notably a characteristic distance over which order holds (a "wavelength" in the range of micrometers, not nanometers) and a time frame of minutes to hours. The spatial distribution of signaling molecules could in principle constitute a field that guides growth and development at the cellular level, but this hypothesis is open to the commonsense objection that the cytoplasm of many cells streams vigorously. In any event, there is no reason to believe that diffusible molecules are uniquely suited to pattern formation, and several alternative proposals are under discussion.

The most familiar alternative is that proposed by Jaffe, who has long championed the role of endogenous electric fields in cellular self-organization (138–141). His proposal stems from the discovery that fucoid embryos, and indeed almost all eucaryotic cells and organisms, drive electric currents through themselves. The electric current bespeaks a flow of ions into some regions and out of others; currents are spatially correlated with anatomy and function, and they sometimes precede and predict developmental events (140, 201, 202). A transcellular current, more precisely the electric field generated by that current, could serve as a localizing mechanism by the electrophoresis of organelles or membrane proteins (140, 215). Recent research from this reviewer's and other laboratories indicates that transcellular ionic currents are commonly a manifestation of cell polarity rather than its cause and tends to discount the localizing function of the electric field (101, 246). However, generalizations about developmental mechanisms are notoriously risky, and electric fields remain in contention, especially at the multicellular level (202).

A more appealing version of the idea, that ionic currents localize morphogenetic events, identifies the spatial signal with a localized flux of a particular ion, especially calcium. This hypothesis also owes much to Jaffe's advocacy, and it is probably fair to say that it is presently the dominant paradigm in cellular pattern formation (101, 119, 140, 213, 245). It is well known that calcium ions regulate numerous physiological processes including contraction, exocytosis, cytoskeleton dynamics, and diverse enzyme activities. Insofar as these very processes are the physical basis of cell shaping, calcium ions (and, to a lesser degree, protons) are plausible candidates for the role of cellular morphogens. The hypothesis to be developed and tested by application to particular cases states that a localized flux of Ca^{2+}, and/or a spatial steady-state gradient of cytosolic free Ca^{2+}, plays a causal role in localizing morphogenetic events.

Endogenous mechanical forces may seem a prosaic solution to so profound a riddle as self-organization, but this has recently become an especially promising avenue of inquiry. It should be recalled that the plasma membrane of eucaryotic cells is subtended by a continuous actin-rich cortex. Although the architecture of the cortex is not well understood (and will in any event vary from one organism to another), some insight into its behavior can be obtained by treating the cell cortex as a special kind of ionic gel that exhibits viscoelastic behavior (85, 206–208). The strength and direction of forces exerted upon any particle within the gel will be the result of several interacting influences that are more or less quantifiable. The tendency of the gel to undergo osmotic swelling is balanced by its elasticity; both are influenced by the length of the actin filaments and by the degree of cross-linking, which will be modulated by the pH and other ionic variables. Calcium ions play an especially prominent role in cortical mechanics. As a rule of thumb, a local rise in

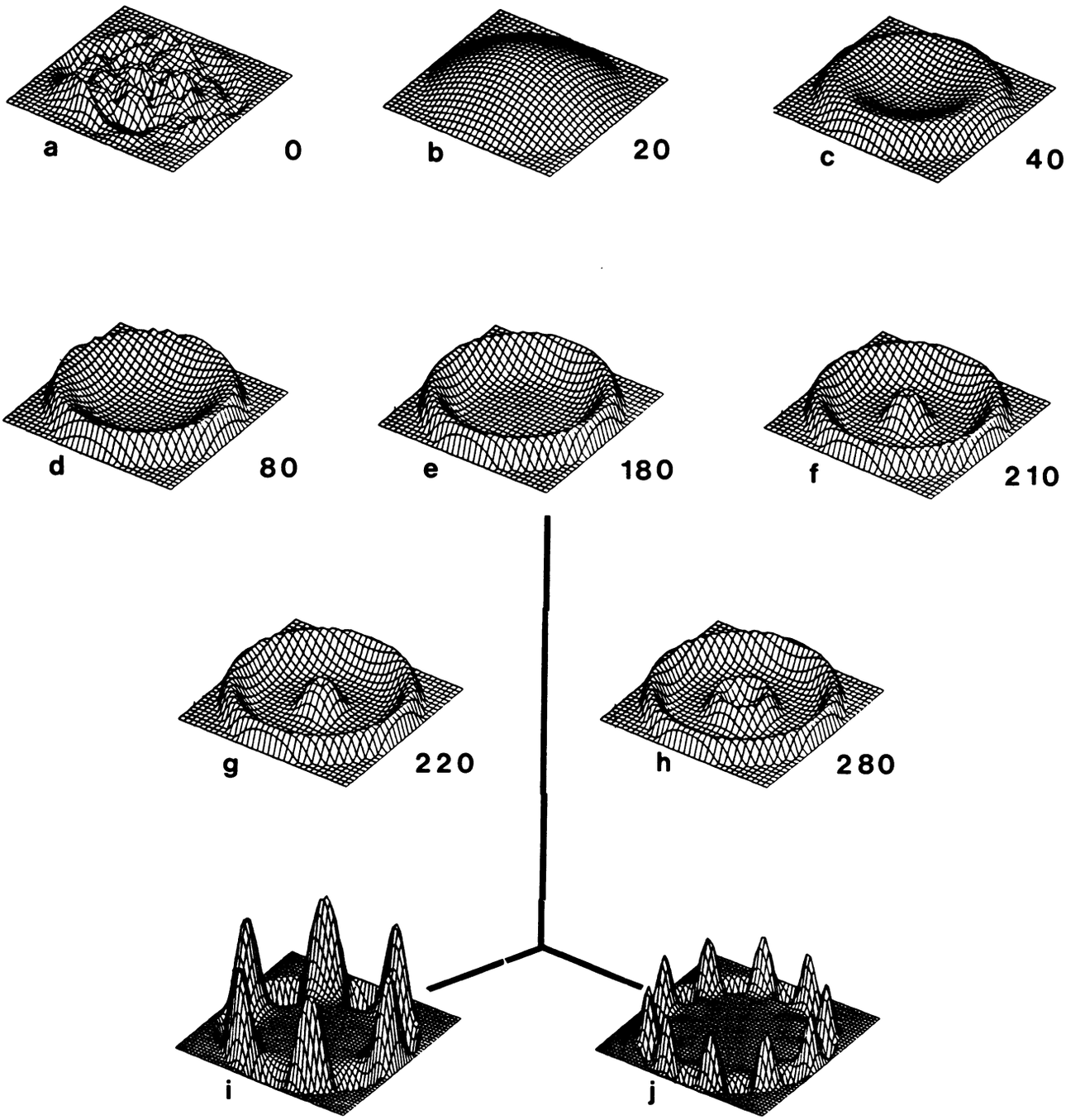


FIG. 27. Computer simulation of morphogen waves that may underlie whorl formation in an *Acetabularia* growing tip. An initial random input (a) changes into a shape resembling that of the extending tip (b) and then to a crater whose rim could represent tip flattening (c). Growth then resumes at the center (f), and the sequence repeats (g and h). When certain parameters are altered at stage e, the crater rim sprouts periodic maxima that mimic the distribution of hairs (i and j). Photograph courtesy of L. G. Harrison. Reprinted from references 104 and 108 with permission of Springer-Verlag.

the calcium concentration should favor solation of the gel by activating proteins of the gelsolin type, resulting in a tendency of the gel to swell. A local rise in calcium concentration will also stimulate contraction that depends on myosin. The two effects interact: partial solation is a prerequisite for gel contraction, but excessive solation would dissolve the cohesive framework and prevent contraction. One can thus begin to appreciate intuitively that determination of the mechanical properties of the cytogel, supplemented with information about the local concentration of effector molecules such as Ca^{2+} and H^+, may allow deduction of the forces such a gel should exert and even the form it should assume.

By applying the methods of continuum mechanics, Oster has been able to model amoeboid motility (207) and the morphogenesis of epithelial sheets. Extension of this approach to walled cells requires the additional assumption that cortical strain elicits corresponding changes in contigu-

ous portions of the wall. For example, if cortical strain were to activate proton pumps, local acidification could induce local loosening of the wall, followed by bulging or buckling under pressure. Such considerations underlie a set of interesting but (to me) impenetrable papers that attempt to model tip growth in *Acetabularia* cells on mechanochemical principles (28, 87). Still, even those lacking mathematical sophistication will appreciate the conceptual merits of identifying an intangible morphogenetic field with the dynamics of the cell cortex (see especially references 26, 86, 206, and 208). A pattern of mechanical stress and strain will not be dissipated by subcortical cytoplasmic streaming, and it evades the awkward dichotomy between a field of spatial information and its implementation by the physiological machinery. Indeed, these ideas minimize the need for detailed prepatterns by putting the burden of self-organization on physical forces generated by the cell itself. They have, to my ears, the ring of truth.

Before I consider the pertinence of chemical morphogens and physical fields to actual organisms, let me underscore what seems to be an emerging consensus. Biological self-organization is the gradual, cumulative epigenetic process whereby the linear information encoded in the genome takes on three-dimensional form. Small initial differences (sometimes random or nonspecific, sometimes environmental cues) are progressively amplified, generating spatial fields of one kind or another; the nature of these fields may vary from one case to the next. These fields direct the localization of molecules and forces that actually shape the visible structure, and therefore serve as obligatory intermediates in all developmental pathways (except for those that are wholly explicable as the result of molecular self-assembly). The identification of spatial fields, their genesis, and their mechanisms of action therefore becomes the chief objective of research on biological self-organization.

Polarization of the Fucoid Embryo

If a single organism supplies a paradigm for cellular self-organization, it is the embryo of the marine brown algae *Fucus* and *Pelvetia* spp. As discussed in the preceding section, the fertilized egg quickly surrounds itself with a wall and accumulates salt. It remains spherically symmetrical for several hours but then begins to bulge slightly; the protuberance signals the future rhizoid. By this stage, about 13 h after fertilization in *Fucus* embryos, the axis of polarity has been established. This imaginary line, approximately corresponding to the embryo's axis of symmetry, defines the vector along which structure and function become organized. Mitochondria and several kinds of vesicles accumulate in the rhizoidal half of the cell, mitochondria and endoplasmic reticulum become aligned with the axis, and Golgi apparatuses reorient to face the prospective rhizoid; eventually, secretion becomes localized to the site of rhizoid outgrowth (Fig. 21). Treatments that prevent germination, such as 0.4 M sucrose (222) or cytochalasin D (165), block physiological polarization as well.

It is essential to understand what the process of self-organization in fucoid embryos is about. New proteins are synthesized during polarization and outgrowth, but their structure is specified by stored mRNA. There is no evidence for stage-specific gene transcription, and the requisite proteins are synthesized hours before the morphogenetic events. Macromolecule production and morphogenesis can be uncoupled: cytochalasins and sucrose prevent outgrowth without affecting protein synthesis, whereas growth in the dark delays protein production but does not prevent polarization (167). Evidently, self-organization is not primarily a matter of generating new and specialized macromolecules or even new organelles. It revolves around the establishment of a particular set of spatial relationships that confer direction upon the physiology of the embryo. Polarity is not, and cannot be, ordained by the genome.

The orientation of the polar axis, i.e., the locus of rhizoid outgrowth, is not random but is determined by spatial cues from the environment. A cluster of zygotes germinates such that the rhizoids point toward the center; what the zygotes sense is not certain, possibly CO_2 or metabolic acid. In the laboratory it is convenient to orient germination with unilateral light; rhizoids emerge from the shaded half. However, there is nothing unique about light. Outgrowth begins on the acid side of a pH gradient, on the high side of a potassium gradient, and on the low side of an osmotic gradient and preferentially faces the cathode in an electric field; in the absence of any other cues, the site of sperm entry appears to determine the locus of outgrowth (138). It should be emphasized that the stimulus truly induces the axis, rather than selecting one of many preformed potential axes, for plane-polarized light induces bipolar embryos (138). We can therefore sharpen the focus of inquiry: we are asking how the embryo registers directional cues and uses their vector to confer polarity upon its structure and function.

Over the years, thanks especially to the work of L. F. Jaffe and his associates, students of this system have become persuaded that external vectors exert their primary effect upon the plasma membrane (Fig. 28). The initial asymmetry may be a localized change in ion permeability, possibly involving the preferential accumulation of calcium channels in the region of the prospective rhizoid. The localized calcium flux would be amplified progressively, generating a frank transcellular ionic current with attendant electric field and a gradient of calcium concentration across the cytoplasm. These gradients, in turn, would be instrumental in directing the development of the secretory pathway toward the prospective rhizoid. The Golgi vesicles are presumed to carry calcium channels, so that their exocytosis at the site of outgrowth ensures continued amplification of polarized secretion (24, 138, 141).

Considerable experimental work has been done with this system, most of which is broadly consistent with the model. The evidence is worth recapitulating in a little detail, for it has shaped most contemporary thinking about the mechanism of pattern formation at the cellular level. (i) Transcellular electric current begins to flow as early as 30 min after fertilization, preceding any visible signs of polarized outgrowth such as local cortical clearing. The pattern is unstable at first but becomes progressively better defined over the next 12 h; the site of stable current entry, which reaches about 1 $\mu A/cm^2$, predicts the future locus of outgrowth with high accuracy. Experimental manipulations that alter the current pattern, such as shining a second beam of light onto the egg from another direction, alter the locus of outgrowth in a corresponding manner. The finding that the current precedes outgrowth and predicts its locus supports the hypothesis that the current is part of the mechanism of polarization (140, 141, 200).

(ii) During the earliest stages of self-polarization, a fraction of the transcellular electric current is carried by calcium ions. Ingenious tracer flux measurements carried out at 6 to 8 h clearly showed that the amount of Ca^{2+} entering the prospective rhizoid pole was four- to fivefold greater than that entering the prospective thallus, whereas Ca^{2+} efflux

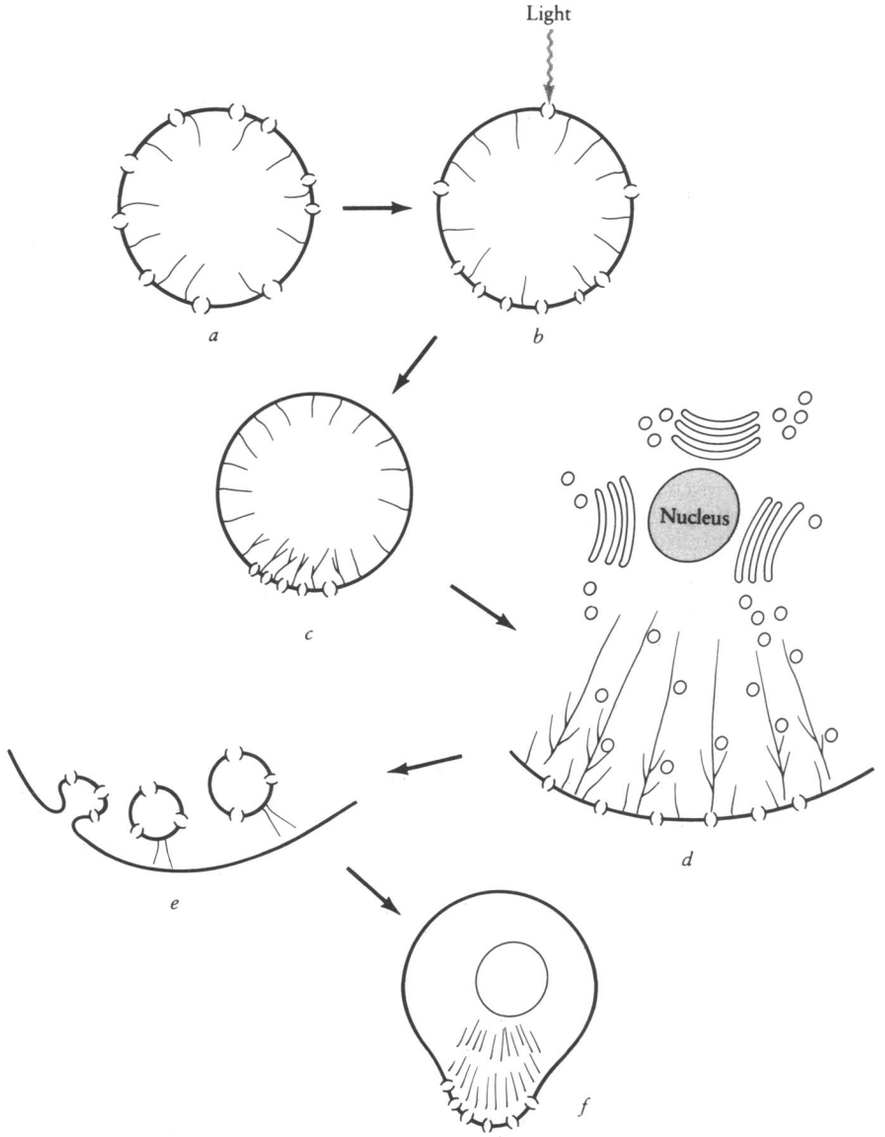


FIG. 28. Polarization of the *Fucus* zygote; a hypothesis. (*a*) In the fertilized egg, Ca^{2+} channels are uniformly distributed. (*b*) After illumination, membrane patches bearing Ca^{2+} channels migrate into the shaded half by a cytochalasin-sensitive process. (*c*) Ca^{2+} channels have clustered at the presumptive rhizoid pole and initiate growth of a network of actin filaments. (*d*) Golgi vesicles bearing Ca^{2+} channels (among other things) are guided to the presumptive rhizoid pole, where they fuse with the plasma membrane (*e*). (*f*) Well-developed actin cables extending to the nucleus; rhizoid outgrowth has begun. Modified and reprinted from reference 24 with permission of the publisher.

occurred preferentially from the thallus end (234). The calcium current at this stage was estimated at 2 pA per egg, perhaps 5% of the total transcellular electric current. (The original estimate, that most or all of the transcellular current was carried by Ca^{2+} ions, was revised in subsequent publications [140, 200].) Preferential calcium influx into the rhizoid declined progressively as the time of germination approached (10 to 11 h).

(iii) The most direct link between localized calcium influx and polarization was forged by Robinson and Cone (233). When *Pelvetia* zygotes were germinated in gradients of the calcium ionophore A23187, rhizoids formed predominantly on the high side of the gradient, presumably the region of maximal calcium influx.

(iv) Whether endogenous calcium gradients arise in the course of normal development is very much under investigation. Brownlee and Wood (38) documented a pronounced maximum of free cytosolic calcium just behind the tip. This, however, was seen only in germinated embryos whose rhizoids were actively extending. Subsequent work with more advanced technology (37; see also reference 169) confirmed the existence of an overt calcium gradient in some growing rhizoids, although not in all, and leaves the calcium distribution at earlier stages unresolved. On the other hand, Speksnuder et al. (263) found that certain calcium buffers of the 1,2-bis(*o*-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) type inhibit self-polarization of *Pelvetia* embryos; they argue persuasively that these buffers act by dissipating a spatial gradient of calcium concentration immediately subjacent to the plasma membrane.

Do these observations establish that calcium influx localizes the site of germination? The evidence is suggestive but far from compelling. Even the finding that a gradient of A23187 imposes its own polarity upon germination of the

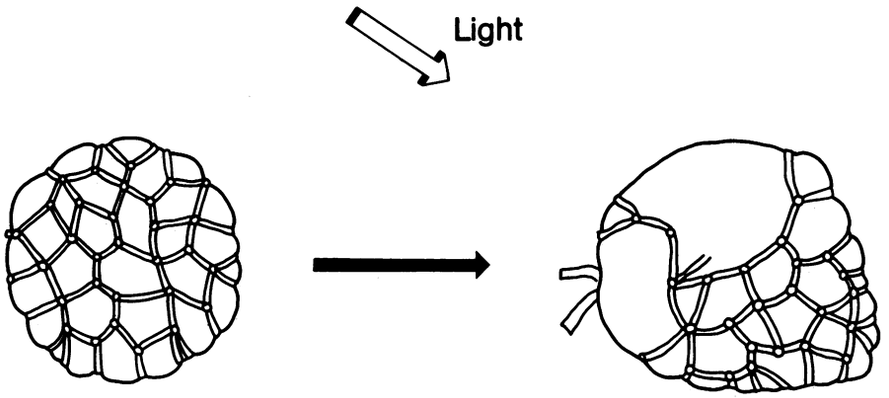


FIG. 29. Mechanical analogy for changes in cortical structure during polarization of the fucoid zygote. The spherical zygote on the left is enclosed in a network of elastic threads. Cutting some of the threads causes the remainder to migrate and form a cap. Based on a sketch by D. Bray (J. Cell Sci. Suppl. 4:71–88, 1985); redrawn with permission of The Company of Biologists.

zygote (233) is weakened by the fact that many other gradients do the same. An outright challenge to the proposition that a calcium current plays an obligatory role in the polarization of fucoid embryos was issued by Kropf and Quatrano (169). By use of the fluorescent probe chlorotetracycline, which is thought to mark membrane-bound calcium, these investigators readily confirmed that calcium ions enter the rhizoid of germinated embryos. Indeed, calcium entry proved essential for the polarized extension of the rhizoid tip. However, they were unable to detect a calcium gradient prior to germination, while the embryo was developing a polar axis. To be sure, the force of this conclusion is diminished by the fact that chlorotetracycline is a somewhat unsatisfactory probe, which probably displays calcium-binding vesicles rather than cytosolic free calcium; but it is nevertheless a cause for concern. Experiments designed to manipulate the flux of calcium ions across the plasma membrane reinforce these misgivings. Reduction of the extracellular calcium concentration from 9 mM (artificial seawater) to 10 μM all but abolished germination of the embryos and elongation of the rhizoid, yet formation and fixation of the polar axis in response to unilateral light took place normally down to 0.1 nM Ca^{2+} (10^{-10} M). Reagents that block calcium uptake, especially D-600 (methoxyverapamil) and lanthanum ions (100 μM), had similar effects: they stopped polarized extensions of the rhizoid tip, but did not prevent formation and fixation of the polar axis. The authors conclude that calcium influx into the rhizoid tip is required for extension, presumably because Ca^{2+} ions control the exocytosis of precursor vesicles, but that a localized calcium current is not required for polarization of the embryo (169).

Biological hypotheses are not easily falsified, and the proposal that a calcium current localizes outgrowth still stands. However, it may turn out that a calcium current is optional rather than mandatory. Perhaps we should learn to regard self-polarization, not as a single chain of sequential events but as a net of parallel processes, with no one single strand being indispensable as long as others are in place.

What other strands might there be? About the time that transcellular electric currents were discovered, both Quatrano and Jaffe noted that germination is blocked by cytochalasin D, a well-known inhibitor of actin polymerization (222). Subsequent research revealed that a spectacular actin cap forms over the outgrowth early in germination; cytochalasin prevents that (24, 165). Building upon the extensive literature on the role of cortical actin in cytokinesis and animal cell motility (26), I would suggest that redistribution of cortical actin may in fact be the primary event in polarization (Fig. 29). Suppose that the fucoid zygote is bounded by a cortical actin meshwork, subjacent to the plasma membrane; that this cortex is under tension; and that the primary effect of unilateral light is to sever actin filaments in the illuminated half. Thanks to tensile forces, the remaining actin cortex would collect in the shaded half, pulling along membrane proteins linked to the cytoskeleton; calcium channels may be among those, but so may be proton pumps or other enzymes that can initiate the loosening of the cell wall and localize the bulge.

At this juncture, we do not understand how the fucoid embryo localizes outgrowth; calcium gradients, electric fields, actin networks, and mechanical tension all have their advocates. It is time to try to disentangle the web of causality by simple manipulative experiments. Could one, for instance, elicit germination under conditions that do not allow a transcellular electric current to develop? Could one localize outgrowth by manipulating mechanical stress in the cortex or by injecting calcium ions? What would be the effect of injecting pH buffers? I would not be surprised to learn that the *Fucus* embryo is more nimble than we, and can use more than one kind of field to mark its polar axis.

Localizing Tips, Branches, and Buds

Hyphae of the oomycetes *Achlya* and *Saprolegnia* (Fig. 1B), like other tip-growing organisms, display characteristic linear gradients of structure, physiology, and behavior extending behind the tip. Synthesis of new wall is maximal at the apex and falls off sharply behind. Membrane vesicles are most abundant in the tip; other organelles are excluded but stratified in the extension zone and along the trunk. The membrane potential is lowest at the apex, probably because proton influx is maximal there. Microfilaments, generally oriented axially, form a conspicuous apical cap, and the wall is likely to be more plastic at the apex than along the trunk (113, 115, 164, 183, 247).

As a hypha extends, it continuously reincarnates, at successive locations, the same form and gradients it had displayed earlier. How is the polarized organization perpetuated? Most of the observable gradients are probably consequences of the basic phenomenon, which is that secretion of new wall occurs predominantly at the tip. However, at

least one directional or localized process must feed back upon the extending tip, if only to maintain localized secretion! Such generative or recursive loops would effectively serve as morphogens; they may or may not be the same loops that established polarity in the first place during spore germination or branch initiation. Analogous issues arise for yeasts. How does the mother cell select the site of bud emergence and then control the spatial distribution of secretory activity? We know very little about the emergence of pattern during fungal morphogenesis, but the terms of discussion are familiar: electric currents, calcium, actin, and mechanochemical fields.

Ionic signals. I was drawn into fungal morphogenesis by the prospect that transcellular electric currents may help confer polarity upon the apex, either by establishing an electric field across the cytoplasm or by generating local concentrations of Ca^{2+} ions or protons (140, 141). *Achlya bisexualis* hyphae do drive electric current through themselves, such that positive charges usually flow into the hyphal anterior and exit distally. The current apparently represents a spatially extended chemiosmotic proton circulation: protons are expelled by H^+-ATPase molecules, located predominantly behind the tip, and return via amino acid/proton symporters which favor the apical region (166). Such hyphae also generate an electric field along the apical cytoplasm, tip positive (about 20 mV over the apical 100 μm [164]). Nevertheless, closer examination has convinced me that the electric current is a manifestation of polarity rather than its cause: under appropriate nutritional conditions, hyphae generate no current or, indeed, a reversed one, yet they continue to extend in the usual apical manner (246; C.-W. Cho, W. J. A. Schreurs, and F. M. Harold, submitted for publication). Analogous results were obtained with *Neurospora* hyphae (181, 269) and with *Allomyces* (305).

The proposition that influx of calcium ions into the tip is part of a recursive loop that localizes the apex is deservedly popular. Calcium ions are essential nutrients for fungal growth and are known to be required for exocytosis. If secretory vesicles carry calcium channels to the tip, as Brawley and Robinson (24) proposed, a simple mechanism of tip perpetuation would be at hand (Fig. 28). The model is supported by the studies on fucoid zygotes reviewed in the preceding section and by the extensive researches on pollen tubes conducted by Picton and Steer (213, 214) and by Reiss et al. (228, 229). In *Achlya* and *Neurospora* cells, as in pollen tubes, chlorotetracycline staining displays fluorescent granules that probably contain calcium, arranged in a gradient with its maximum at the apex (244); and many investigators have observed the perturbation of fungal polarity by calcium ionophores (103, 230, 236). The discovery that *CDC24* codes for a calcium-binding protein involved in the division of yeast cells (205) is another straw in that wind. However, it must be pointed out that *Achlya* and *Saprolegnia* hyphae, at least, continue to extend for a time in the virtual absence of extracellular Ca^{2+} ions (0.1 μM) (134, 246). Evidently, hyphal extension requires Ca^{2+} ions in the long term, but there is no obligatory coupling between calcium influx into the tip and localized secretion. A cytoplasmic calcium gradient could still serve as a morphogen if it stems from internal stores, such as the granules stained with chlorotetracycline; however, the vigorous streaming of apical cytoplasm in *Achlya* hyphae makes this, too, a questionable proposition. The involvement of a cytoplasmic pH gradient, apex acidic (see reference 286 and references cited therein), is also subjected to doubt on those and other grounds.

Spatial memory and the cytoskeleton. Recursive loops that perpetuate the hyphal tip need not, of course, involve an ionic signal at all; the cytoskeleton or the cell wall could supply the requisite spatial memory. Microtubules are prominent in animal cells, and their involvement in polarized motility is well documented (253). They are present in hyphal tips, although not especially abundant, and are commonly thought to furnish tracks for the transport of vesicles to the apex (see, e.g., references 129 and 130). In yeast cells, microtubules extending from the spindle-pole body on the nuclear membrane into the developing bud have long been assigned a role in selecting the locus of budding (2, 44). Given this presumption of prominence, it is surprising that much of the recent evidence indicates no fundamental role for microtubules in pattern formation: both tip extension and bud emergence proceed in mutants deficient in tubulin synthesis (132, 204) and in the presence of microtubule-depolymerizing drugs (110, 122, 137, 224). It may be recalled that the same appears to be true in *Fucus* cells. Microtubules are strongly implicated in the transport of nuclei and other organelles, as well as in spindle formation, but they seem to be dispensable for patterning.

By contrast, I have been all the more impressed by the regular association of fungal pattern formation with microfilaments and actin. Structures rich in actin are characteristically found in hyphal tips and yeast buds, in association with septa, at the sites of germination, outgrowth, and extension (4, 110, 113, 114, 122, 146, 150, 153, 237, 239, 284). Mutants deficient in the synthesis of actin or of actin-binding proteins exhibit abnormal bud formation (62, 199). Finally, cytochalasins typically prevent or perturb germination, pattern formation, and polarized extension (95, 102, 103, 122, 153, 247, 284). In fungi, and also in *Fucus* spp. and in several other algae, microfilaments seem to play a central role in localized wall synthesis; and that, in walled organisms, is what morphogenesis is all about.

It is presently not clear whether the assembly and modulation of actin-based structures are aspects of pattern formation or merely track the instructions supplied by another morphogen. Resolution of this uncertainty will require better understanding of both the architecture and the functions of microfilaments in fungi. Kilmartin and Adams (150) originally described two chief configurations in yeasts: filaments coursing through the cytoplasm and spots or plaques at the cell periphery. They, as well as subsequent investigators, noted that the spots are particularly abundant at sites of wall deposition (2, 4, 153, 284). The spots may represent organelles called filasomes (129) or else adhesion plaques where the cytoskeleton attaches to the wall. Subsequently, Heath (113) noted an additional configuration, a cap of cortical actin confined to the apex; thus far, this has been clearly seen only in oomycetes.

The functions of actin in fungi are as uncertain as its arrangement, and possibly as diverse. Microfilament bundles furnish tracks for vesicle translocation in certain algae, and they may serve the same role in fungi (but see reference 114). If the conspicuous spots correspond to adhesion plaques, they may also report sites where mechanical tension is applied. I am also intrigued by the proposal (26, 266) that a fountain flow of actin underlies the motility of amoebae and animal cells: cortical actin flows toward the rear, exerting traction that pushes the cell forward, while a stream of actin through the interior cytoplasm maintains the circulation. Extrapolated to hyphae, the model helps explain cytoplasmic migration and emphasizes that the tip is the focus not only of wall deposition, but also of cytoskeleton dynamics and of mechanical work.

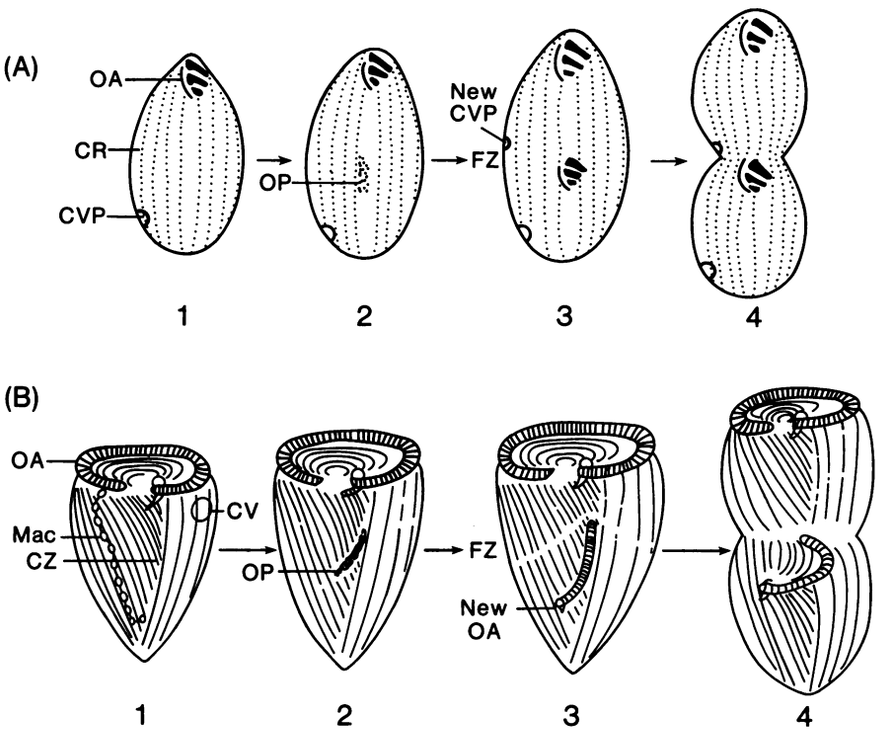


FIG. 30. Schematic diagrams of growth and division in the ciliates *Tetrahymena* (A) and *Stentor* (B). The four stages represent the mature cell (stage 1), initiation of the new oral apparatus (stage 2), development of the fission zone (stage 3), and cytokinesis (stage 4). Symbols: OA, oral apparatus, CR, ciliary row; CVP, contractile vacuole pore; FZ, fission zone; OP, oral primordium; Mac, macronucleus; CZ, contrast zone. Modified and reprinted from *Microbiological Reviews* (6) with permission.

So where do we now stand regarding the cycle that perpetuates the polarized order of the tip? It will be plain that I have developed reservations concerning the role of ionic signals; that hypothesis has not been disproven, but it seems considerably less attractive than it once did. Let me offer three alternatives that presently seem promising and in which microfilament dynamics may find a logical place.

(i) Assuming that a hyphal apex is defined by a restricted zone of vesicle fusion, one must explain how such a zone is continuously generated anew. Many years ago, Mullins and co-workers put forward the idea that secretion occurs only where the wall is soft or distensible (193, 275); so long as a hypha extends, it would tend to perpetuate an apical fusion zone. A variation of this idea calls for a sheath of cortical actin that prevents vesicle exocytosis everywhere except where the sheath is absent, at the very tip. So long as vesicle fusion extends the tip, the apical fusion site would also be perpetuated. A branch, or a bud, would begin with a local weakening of the actin sheath; the effects of cytochalasin find a plausible interpretation on this view.

(ii) If hyphal form is spewed forth from a moving vesicle supply center, as Bartnicki-Garcia and his colleagues maintain (11, 12), it is necessary only to ensure that the center continue to move in its original direction and that vesicle production be coupled to translation of the center (were there no such feedback, the tip must bulge whenever it stops extending, which is not usually the case). Emergence of a branch would require the formation of a new vesicle supply center at an early stage of the proceedings.

(iii) Finally, we may think of the apex as a kind of amoeba, dwelling in a tube of its own making (see reference 183 and references therein). One can imagine the pathway of exocytosis to be coupled to the circulation of cortical actin, much as it must be in amoebae (26, 266). I hasten to add that this speculation does not solve the problem, but merely connects one mystery to another.

Patterns and Fields in the Cortex of Ciliates

When applied to surface organization in ciliates, the term pattern obviously points beyond the gross form of the cell to the spatial relationships between its parts. I have argued above that this holds for cells generally (vectorial physiology), but in ciliates morphological patterns are expressed in two dimensions on the surface and can be displayed for all to see by staining the cells. Partly for that reason, pattern formation in ciliates has been more successfully studied than that in other organisms, and the involvement of morphogenetic fields has been documented in thoughtful articles by Frankel and co-workers (6, 67–69). The only justification for including a cursory third-hand summary in the present article is the expectation that what is true of ciliates may also apply with qualifications to other eukaryotic cells.

The *Tetrahymena* cell illustrates ciliate surface organization at its simplest (Fig. 30A). The cells are pear shaped and are ornamented with gently spiraling lengthwise rows of cilia that are responsible for motility. At the anterior end is the oral apparatus, equipped with specialized ciliary arrays, whose beating drives food particles into the gullet; posteriorly is a contractile vacuole, opening to the exterior through a specialized pore. The relative position of mouth, ciliary rows, and contractile vacuole are the landmarks that define the cortical pattern. Beneath the cortex lie a vegetative macronucleus and the germinal micronucleus; these also occupy regular positions, but will not be considered here. At the other extreme is the *Stentor* cell (Fig. 30B), up to 1 mm

in length, which lends itself to microsurgical procedures that have proved especially informative. The *Stentor* cell, like the *Tetrahymena* cell, can be viewed as a modified cylinder with the mouth at the anterior, a holdfast at the posterior, and longitudinal rows of cilia. Conspicuous features are the pigmented stripes wrapped diagonally around the cell, alternating with the ciliary rows, such that the narrowest stripes abut upon the widest along a particular line. These are of the utmost importance, for the locus of stripe contrast marks a zone of special morphogenetic significance. There is reason to believe that analogous sites exist in other ciliates, but only in *Stentor* cells is one visible.

Both organisms grow by extending along their polar axis to generate two tandem segments, each of which duplicates the standard pattern (Fig. 30). Therefore, before the *Tetrahymena* cell divides at its waist, it constructs a new contractile vacuole for the anterior daughter and a new mouth for the posterior one. Growth of the clone thus involves two general requirements: longitudinal extension of the ciliary rows, followed by the formation of specialized organelles at sites remote from the original ones.

How do the cells place each organelle in its proper location? The first insight pertained to the patterning of ciliary rows and grew out of the experiments of Beisson and Sonneborn (18) on the propagation of inverted rows. It will be recalled (see the section on structural inheritance in ciliates, above) that a row consists of a linear array of ciliary units, whose geometry is schematically depicted in Fig. 5. At the center is a basal body from which the cilium springs; a striated rootlet and accessory bands of microtubules extend toward the anterior right of the cell. Each ciliary unit is thus intrinsically asymmetric, and the beating pattern is determined by its geometry. Rows propagate by the intercalation of new basal bodies anterior to existing ones in a definite geometric relationship; they acquire accessory fibers in conformity with those already present and finally sprout cilia that beat in unison with others in the same row. In an inverted ciliary unit, all the elements have been rotated by 180°. Inverted rows propagate just as normal ones do; they maintain their intrinsic polarity, but new ciliary units are now inserted on the side facing the posterior of the cell and all accessory structures are likewise rotated. "The site of initiation of basal body assembly, its path of migration to the surface of the cell, and the organization of associated structures around it are [all] determined by the molecular geography within the unit territory and not by any outside influence, either nuclear or cellular." (261).

The molecular geography extends its influence beyond each ciliary row to cellular elements unconnected with motility. For example, contractile vacuole pores in *Tetrahymena* cells are found posteriorly and to the right of particular ciliary rows. If those rows are inverted, the pores appear on the left; presumably, the contractile vacuole is linked by cross-bridges to the ciliature. The same is true for mitochondria: they occur in the cortex in a patterned distribution that is linked to ciliary rows and undergoes inversion together with those rows.

Sonneborn (261) coined the term cytotaxis to designate the "ordering and arranging of new cell structure under the influence of preexisting cell structure." Subsequent work in his and other laboratories revealed many additional instances in which the existing order constrains the future order, both locally and globally. A recent example is the perpetuation of the handedness of surface structures in *Tetrahymena* cells (195). However, the classical and also the most spectacular illustration is the propagation of doublet cells which have fused back to back as a result of abortive division or of surgical intervention (Fig. 6). Doublet cells can propagate indefinitely as doublets, by an unknown mechanism that depends on the continuity of surface organization from one generation to the next. What maintains the doublet organization is probably something other than the visible ciliature. This is most convincingly inferred from experiments with another ciliate, *Oxytricha fallax*, that forms cysts when starved. Neither cilia nor basal bodies or other microtubular structures can be seen in cysts by electron microscopy; nevertheless, doublets emerge from their cysts as doublets and singlets emerge as singlets. The emerging cells even remember their original polarity (6, 92). It follows that some other aspect of cortical organization persists through the cyst stage.

Can all instances of cytotaxis be attributed to local interactions, reminiscent of self-assembly at the protein level, that explain the propagation of ciliary rows? The answer is no. Perhaps the two most important conclusions from two decades of research on ciliate morphogenesis are that global patterns are independent of, and superposed upon, local structural guidance, and that whatever system generates global patterns has properties traditionally associated with embryonic fields. The positioning of organelles is relational rather than fixed, the pattern is the same regardless of size, and part of the pattern can reconstitute the whole. The evidence has been marshaled by Frankel (67–69) in several closely reasoned articles and cannot be recapitulated here. Suffice it to indicate in a general way what distinguishes global patterning from the local variety.

There are now several instances in which the local pattern is reversed while the global one is normal. Rotation of a ciliary row inverts not only the orientation of new ciliary units but also the placement of the contractile vacuole pore with respect to that row. However, the pore continues to form in the posterior half, and selection of the particular row that bears the pore is a matter for the global system, which is unaffected by rotation of the row. Conversely, the global system can be altered without affecting local patterns. A spectacular case in point comes from certain doublets: the two halves are related as mirror images, yet the ciliary rows are superimposable! It appears that only a single isomer of the ciliary unit can exist, the one diagrammed in Fig. 5. By contrast, the global axis can flip over, resulting in a mirror-image configuration.

That the global system has the organizational character of a field is demonstrated chiefly by studies of *Stentor* cells, whose capacity to tolerate physical insults is almost beyond belief. In classical researches conducted 3 decades ago, Tartar (271) showed that starving *Stentor* cells undergo successive reorganization, each resulting in a normal but smaller cell. The parts, e.g., the mouth, become smaller, but the pattern (the spatial relationship between the parts) is preserved down to the lower limit beyond which a viable cell can no longer be generated. Tartar also discovered the legendary capacity of cell fragments, some as small as 1/100 of the normal cell size, to reconstitute a normal albeit smaller *Stentor* cell. Regeneration requires that the fragment include a part of the macronucleus and of the cortex, specifically a region in which broad pigment stripes abut upon narrow ones. A locus of stripe contrast is necessary to organize the oral apparatus: whenever such a zone is created, whether naturally or by surgical procedures, an oral apparatus is produced. It follows that the surface of the cell is essentially totipotent, requiring only an organizing region to direct assembly of an oral primordium.

Uhlig, who repeated and extended many of Tartar's observations, put forward a more illuminating interpretation of the role of the contrast zone. Instead of viewing it as an essentially local landmark, he considered it to represent the termini of a circular gradient wrapped around the cell (289; a summary of this somewhat inaccessible work will be found in references 6, 67, and 69). Positional information along the equatorial meridians would then be read out along the gradient displayed by the pigment stripes. To account for the placement of organelles, Uhlig found it necessary to invoke a second gradient, orthogonal to the first. The circular gradient locates the oral apparatus and the contractile vacuole pore, while the anteroposterior one positions, inter alia, the region known as the oral spiral.

The upshot is that the positioning of surface organelles in ciliates is controlled by at least two hierarchically superposed systems. One is local and associated with the geometry of ciliary units. The other is global and largely independent of the ciliary units. The global system(s) behaves as though it embodied gradients of positional information laid out along orthogonal axes; reference points are located at the anterior and posterior ends and at special reference meridians which can sometimes be recognized as regions of structural discontinuity. Such global fields presumably underlie Goodwin's remarkable proposition (83) that the diversity of ciliate shapes can be encompassed by a single mathematical formula, Laplace's general field equation. Each organism corresponds to a particular set of boundary conditions, but all conform to the same rules of order. Fields of positional information are associated with the cell cortex, not the nucleus, but we know nothing of their physical nature. Would a research program centered on cortical mechanics be illuminating? Frankel's comment in 1982 (68) remains apt: "This problem still stands where the problem of inheritance stood before the rediscovery of Mendel. . . . What is needed is a more explicit and mechanistic field theory, or a wholly new concept that provides a superior understanding of the same underlying phenomena, much as the concept of oxidation replaced the phlogiston theory in accounting for the facts of chemical combustion."

DEVELOPMENTAL DYNAMICS OF MICROBIAL CELLS

General and abstract matters, such as the principles of cell polarization or pattern formation, can be addressed only obliquely by research. The concrete objective of any research project must be to understand how this or that particular creature shapes itself throughout its growth and development. For none is this objective within reach; the rags and patches of information presently at hand afford no more than glimpses of the developmental dynamics (296) of microbial cells in general. Like archaeologists hoping to reconstruct from broken crockery and charred bricks a vanished society whose organization and purposes they but dimly perceive, we must make do with the fragments that have come to light. By way of a summary and prospect, I have collected in this final section what I have learned from this long dig. It goes without saying that what follows are opinions on which honest persons can differ and which will not be universally shared. Some may prove to be mistaken, but, to quote Sir Peter Medawar once more, "Humility is not a frame of mind conducive to the advancement of science."

(i) Morphogenesis lies somewhere between the poles of biochemical unity and biological diversity. Organisms differ enormously with respect to all operational details; common principles are more likely to be found on the organizational level than the mechanistic one. By all means, seek the general in the welter of particulars, but never lose sight of what Stephen Jay Gould called the quirkiness of evolution.

(ii) Cells shape themselves by local compliance with applied force. All objects are shaped by one force or another. Self-assembly of viruses and organelles results from intermolecular forces. Cells generate longer-range forces from within and yield to these forces in a controlled, localized manner. The argument was developed in some detail for walled organisms, in which the driving force for morphogenesis is supplied by hydrostatic pressure. In unwalled cells, amoebae and fibroblasts, for example, contractile forces seem to predominate.

(iii) Morphology is the outward expression of dynamic functional organization. This is especially plain in polarized walled cells, such as the fungi and algae that are the principal subjects of this article. Secretion, cytoplasmic transport of vesicles, and motility illustrate functional organization on a cellular scale: vectorial physiology. Transcellular electric currents, which report the spatial organization of nutrient transport, are another illustration of large-scale physiological order. Incidentally, this is surely what polarity is about: not just that the two ends of an organism differ, but that function, behavior, and also form have a directional character.

(iv) Growth and morphogenesis can be decoupled. As a rule, the forms of cells arise in the course of growth, pari passu with the accretion of new matter. However, biosynthesis and morphogenesis need not be concurrent; transcription and translation are often completed in advance of the shape changes. Germination of the *Blastocladiella* zoospore supplies an extreme instance of pattern formation without biosynthesis: spores encyst, retract their flagella, and put forth germ tube initials, all in the absence of any new macromolecule synthesis (259). The converse, growth without morphogenesis, is induced by certain inhibitors (cytochalasins especially) and also by some gene mutations. Dissociation of morphogenesis from growth correlates with failure of the organism to establish the requisite vectorial organization.

(v) Genetic and spatial memory are complementary. A growing or developing cell models itself upon itself with the aid of a hierarchical set of organizing procedures. Its molecular constituents are determined by the genome, and self-assembly plays a large role in specifying the form and function of supramolecular structures, to the level of membranes and organelles. At the cellular level, structures formed during earlier stages often help new gene products to find their proper place and orientation. Such spatial memory is relatively short-lived, is ancillary to the genetic memory, and takes a variety of forms. Examples include the persistence of ciliary units and of microtubule-organizing centers in general.

(vi) Self-organization is the heart of the matter. Even in the apparent absence of structural memory, cells have an astonishing capacity to organize their physiology into a spatial pattern, commonly a polarized one. Fucoid embryos, which draw upon spatial cues from the environment, supply the best example but are certainly not unique; fungal spores and branching hyphae do likewise. The regeneration of protoplasts, which apparently hinges on the reconstitution of an organized cytoskeleton, may prove to be well suited to systematic analysis (75, 76, 153).

(vii) The cytoskeleton integrates cellular space. The intimate relationship between cellular forms and the cytoskele-

ton has been recognized for 30 years, but continues to elude precise and succinct description. Microtubules and microfilaments transmit mechanical force, provide support, and serve as tracks for the transport of vesicles and organelles. They are invariably part of the mechanism by which a eucaryotic cell shapes itself. The metaphor of spatial integration, borrowed from Elias Lazarides, evokes the multifarious functions of the cytoskeleton. It is often said, or at least implied, that the cytoskeleton shapes the cell, but it is equally true that the cell molds its cytoskeleton. To what extent cytoskeletal elements participate in the establishment of primary patterns is one of the central questions in morphogenesis.

(viii) Self-organization is gradual, progressive, and hierarchical. No matrix or template has ever been observed. Instead, the process begins with a rudimentary prepattern and builds up higher levels of order stepwise. At successive stages, different molecular and physiological mechanisms come into play. Self-organization is apparently not a linear sequence of causes and effects, but a braided network of alternative routes.

(ix) A role for ion currents and electric fields? Eucaryotic cells and organisms commonly drive electric currents through themselves: charges enter in one region and exit from another. These currents represent the differential localization of transport systems responsible for the uptake of organic and inorganic nutrients. In my view, transcellular electric currents (and the cytoplasmic electric fields that they engender) are manifestations of the spatial organization of the organisms rather than its cause. A stronger case can be made for the proposition that a localized flux of some particular ion, especially Ca^{2+}, helps to localize growth or development. Self-polarization of fucoid embryos and the extension of fungal hyphae serve to illustrate the promise and pitfalls of this line of inquiry. No one doubts that an appropriate level of cytosolic calcium ions is required for normal growth and development. The question is whether a flux of calcium ions into the cell at a particular locus, or a gradient of calcium concentration across cytoplasmic space, plays a causal role in localization.

(x) The prevalence of actin. I have been much impressed by the regular association of early developmental events with actin. Actin-rich structures are characteristically found in hyphal tips and yeast buds, in forming septa, and at sites of germination, outgrowth, and extension. Mutants deficient in the synthesis of actin or actin-binding proteins have abnormalities in bud formation, and cytochalasins commonly prevent or perturb germination, pattern formation, and polarized extension. The association of polarization with actin gains strength from the common observation that microtubules are dispensable or play only a marginal role in the generation of spatial order. I take the observations as a hint that actin flow and cortical mechanics play a larger role in the localization of growth than we have been accustomed to believe.

(xi) Genes neither encode morphology nor direct its inheritance. The relationship of genes to form is paradoxical. Forms are quite strictly inherited, and all heritable information is encoded in the genomes (nuclear plus organellar); yet the evidence does not support the widespread belief in an explicit genetic program of morphogenesis. Cellular continuity does play a role in the inheritance of form, but it is plainly an ancillary one; there is no salvation in a hypothetical cytoplasmic code. I am compelled to conclude that form arises epigenetically, as a cumulative symphonic implication of the molecular instructions that the genomes do encode. The processes that link linear genes to three-dimensional form belong in the pigeonhole labeled self-organization. It is all but empty, an abiding challenge to those who seek to understand how microorganisms shape themselves.

(xii) Fields implement the generation of pattern. A recurrent theme in the literature of spatial organization is the field: a region of space over which some agency is at work in a coordinated manner. Morphogenetic fields—be they chemical, electrical, or mechanical—seem to be obligatory intermediates in the strategy of the genes. Their physical nature, genesis, and functions are too important to be left to theoreticians alone.

(xiii) Do molecules make cells, or cells their molecules? An organism is constructed of and by molecules, and any given cell is the creature of its biochemistry. A significant molecular defect implies an impaired cell, if not a dead one. However, natural selection acts not upon molecules, but upon organisms. Thanks to the interplay of chance and necessity, organisms come to possess molecules to fit their needs as judged by the performance of the whole ensemble. It follows that if we wish to understand such global features as form, we must look beyond the molecular level and learn anew to think of organisms as integrated, functional systems. To do this, we shall need both advanced computer programs and comprehensible metaphors.

(xiv) Are cells dissipative structures? Cells and organisms convert energy into organization. The pathways by which molecular order is produced are the stuff of biochemistry and physiology and are largely known to us. However, the recent excitement in thermodynamics suggests the extrapolation that the very forms of cells are called forth by the flux of energy, with genes and gene products selecting from among the forms compatible with a given thermodynamic regimen the one that is brought into existence. At present this is rank speculation, nearly devoid of observational or even theoretical content. But it may not always be so: the writings of Prigogine, Oster, Goodwin, and Peacocke indicate a path of sorts, however stony. Don't bite my finger; look where I am pointing.

ACKNOWLEDGMENTS

This article was written at Colorado State University, but all the preparatory work was completed at the National Jewish Center for Immunology and Respiratory Medicine, while I was a member of the Department of Molecular and Cellular Biology (now defunct). I thank that institution for dealing responsibly with the individual consequences of global policy decisions. My reflections have been sharpened by innumerable discussions with colleagues, especially with members of my own laboratory: Ruth Harold, Darryl Kropf, Nicholas Money, and Willie Schreurs. I am indebted to them, and also to Arthur Koch and A. Young Woody for suggestions concerning the manuscript, to Shirley Downs and Carol Martin for typing it, and to Joyce Patterson for preparing the illustrations.

Research in this laboratory has been supported in part by grant DCB 8996200 from the National Science Foundation and by Public Health Service grant AI-03568 from the National Institutes of Health.

LITERATURE CITED

1. **Adair, W. S., S. A. Steinmetz, D. M. Mattson, U. W. Goodenough, and J. E. Heuser.** 1987. Nucleated assembly of *Chlamydomonas* and *Volvox* cell walls. J. Cell Biol. **105**:2373–2382.
2. **Adams, A. M., and J. R. Pringle.** 1984. Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. **98**:934–945.
3. **Adams, T. H., M. T. Boylan, and W. E. Timberlake.** 1988. brlA

is necessary and sufficient to direct conidiophore development in *Aspergillus nidulans*. Cell **54**:353–362.
4. **Anderson, J. M., and D. R. Soll.** 1986. Differences in actin localization during bud and hypha formation in the yeast *Candida albicans*. J. Gen. Microbiol. **132**:2035–2047.
5. **Anderson, R. G. W.** 1977. The biogenesis of cell structures and the expression of assembly information. J. Theor. Biol. **67**: 535–548.
6. **Aufderheide, K. J., J. Frankel, and N. E. Williams.** 1980. Formation and positioning of surface-related structures in protozoa. Microbiol. Rev. **44**:252–302.
7. **Baba, M., N. Baba, Y. Ohsumi, K. Kanaya, and M. Osumi.** 1989. Three-dimensional analysis of morphogenesis induced by mating pheromone α factor in *Saccharomyces cerevisiae*. J. Cell Sci. **94**:207–216.
8. **Barker, D. G., A. L. Johnson, and L. H. Johnson.** 1985. An improved assay for DNA ligase reveals temperature-sensitive activity in *cdc9* mutants of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **200**:458–462.
9. **Bartnicki-Garcia, S.** 1973. Fundamental aspects of hyphal morphogenesis. Symp. Soc. Gen. Microbiol. **23**:245–267.
10. **Bartnicki-Garcia, S., C. E. Bracker, and J. Ruiz-Herrera.** 1984. Chitosomes from the wall-less "slime" mutant of *Neurospora crassa*. Arch. Microbiol. **139**:105–112.
11. **Bartnicki-Garcia, S., F. Hergert, and G. Gierz.** 1989. A novel computer model for generating cell shape: application to fungal morphogenesis, p. 43–60. *In* P. J. Kuhn, A. P. J. Trinci, M. J. Jung, M. W. Goosey, and L. G. Copping (ed.), Biochemistry of cell walls and membranes of fungi. Springer-Verlag KG, Berlin.
12. **Bartnicki-Garcia, S., F. Hergert, and G. Gierz.** 1989. Computer simulation of fungal morphogenesis and the mathematical basis of hyphal (tip) growth. Protoplasma **153**:46–57.
13. **Bartnicki-Garcia, S., and F. Lippman.** 1969. Fungal morphogenesis: cell wall construction in *Mucor rouxii*. Science **165**: 302–308.
14. **Bartnik, E., and A. Sievers.** 1988. *In vivo* observations of a spherical aggregate of endoplasmic reticulum and of Golgi vesicles in the tip of fast-growing *Chara* rhizoids. Planta **176**:1–9.
15. **Baum, P., C. Furlong, and B. Byers.** 1986. Yeast gene required for spindle pole body duplication: homology of its product with Ca^{2+}-binding proteins. Proc. Natl. Acad. Sci. USA **83**:5512–5516.
16. **Begg, K. J., and W. D. Donachie.** 1985. Cell shape and division in *Escherichia coli*: experiments with shape and division mutants. J. Bacteriol. **163**:615–622.
17. **Beisson, J.** 1977. Non-nucleic acid inheritance and epigenetic phenomena, p. 375–421. *In* L. Goldstein and D. Prescott (ed.), Cell biology, vol. 1. Academic Press, Inc., New York.
18. **Beisson, J., and T. M. Sonneborn.** 1965. Cytoplasmic inheritance of the organization of the cell cortex in *Paramecium aurelia*. Proc. Natl. Acad. Sci. USA **53**:275–282.
19. **Bouck, G. B., and D. L. Brown.** 1973. Microtubule biogenesis and cell shape in *Ochromonas*. I. The distribution of cytoplasmic and mitotic microtubules. J. Cell Biol. **56**:340–359.
20. **Bouck, G. B., and D. L. Brown.** 1976. Self-assembly in development. Annu. Rev. Plant Physiol. **27**:71–94.
21. **Bracker, C. E., J. Ruiz-Herrera, and S. Bartnicki-Garcia.** 1976. Structure and transformation of chitin synthetase particles (chitosomes) during microfibril synthesis *in vitro*. Proc. Natl. Acad. Sci. USA **73**:4570–4574.
22. **Brada, D., and R. Schekman.** 1988. Coincident localization of secretory and plasma membrane proteins in organelles of the yeast secretory pathway. J. Bacteriol. **170**:2775–2783.
23. **Brawley, S. H., and R. S. Quatrano.** 1979. Sulfation of fucoidin in *Fucus* embryos. IV. Autoradiographic investigations of fucoidin sulfation and secretion during differentiation and the effect of cytochalasin treatment. Dev. Biol. **79**:193–205.
24. **Brawley, S. H., and K. R. Robinson.** 1985. Cytochalasin treatment disrupts the endogenous currents associated with cell polarization in fucoid zygotes: studies of the role of F-actin in embryogenesis. J. Cell Biol. **100**:1173–1184.
25. **Brawley, S. H., R. Wetherbee, and R. S. Quatrano.** 1976. Fine-structural studies of the gametes and embryo of *Fucus vesiculosus*. I. Fertilization and pronuclear fusion. J. Cell Sci. **20**:233–254.
26. **Bray, D., and J. G. White.** 1988. Cortical flow in animal cells. Science **239**:883–888.
27. **Brickell, P. M., and C. Tickle.** 1989. Morphogens in chick limb development. BioEssays **11**:145–149.
28. **Briere, C., and B. C. Goodwin.** 1988. Dynamics of tip morphogenesis in *Acetabularia*. J. Theor. Biol. **131**:461–475.
29. **Brinkley, B. R.** 1985. Microtubule organizing centers. Annu. Rev. Cell Biol. **1**:145–172.
30. **Brody, S.** 1973. Metabolism, cell walls and morphogenesis, p. 107–154. *In* S. J. Coward (ed.), Developmental regulation: aspects of cell differentiation. Academic Press, Inc., New York.
31. **Brody, S., and E. L. Tatum.** 1966. The primary biochemical effect of a morphological mutation in *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **56**:1290–1297.
32. **Brody, S., and E. L. Tatum.** 1967. Phosphoglucomutase mutants and morphological changes in *Neurospora crassa*. Proc. Natl. Acad. Sci. USA **58**:423–430.
33. **Brown, D. L., and G. B. Bouck.** 1974. Microtubule biogenesis and cell shape in *Ochromonas*. II. Effects of the herbicidal mitotic inhibitor isopropyl N-phenylcarbamate on shape and flagellar regeneration. J. Cell Biol. **61**:514–536.
34. **Brown, R. M.** 1969. Observations on the relationship of the Golgi apparatus to wall formation in the marine chrysophycean alga *Pleurochrysis scherfelii*. J. Cell Biol. **41**:109–123.
35. **Brown, R. M., W. W. Franke, H. Kleinig, H. Falk, and P. Sitte.** 1970. Scale formation in chrysophycean algae. I. Cellulosic and noncellulosic wall components made by the Golgi apparatus. J. Cell Biol. **45**:246–271.
36. **Brown, R. M., Jr.** 1985. Cellulose microfibril assembly and orientation: recent developments. J. Cell Sci. Suppl. **2**:13–32.
37. **Brownlee, C. L., and A. L. Pulsford.** 1988. Visualization of the cytoplasmic Ca^{2+} gradient in *Fucus serratus* rhizoids: correlation with cell ultrastructure and polarity. J. Cell Sci. **91**:249–256.
38. **Brownlee, C. L., and J. W. Wood.** 1986. A gradient of cytoplasmic free calcium in growing rhizoid cells of *Fucus serratus*. Nature (London) **320**:624–626.
39. **Burdett, I. D. J.** 1988. Structure, growth and division of the *Bacillus subtilis* surface. Can. J. Microbiol. **34**:373–380.
40. **Burdett, I. D. J., and A. L. Koch.** 1984. Shape of nascent and complete poles of *Bacillus subtilis*. J. Gen. Microbiol. **130**: 1711–1722.
41. **Burman, L., J. Reichler, and J. T. Park.** 1983. Evidence for diffuse growth of the cylindrical portion of *Escherichia coli* murein sacculus. J. Bacteriol. **155**:983–988.
42. **Burman, L. G., and J. T. Park.** 1984. Molecular model for elongation of the murein sacculus of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:1844–1848.
43. **Byers, B.** 1981. Cytology of the yeast life cycle, p. 59–96. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
44. **Byers, B., and L. Goetsch.** 1975. Behavior of spindles and spindle plaques in the cell cycle and conjugation of *Saccharomyces cerevisiae*. J. Bacteriol. **124**:511–523.
45. **Byers, B., and L. Goetsch.** 1976. A highly ordered ring of membrane-associated filaments in budding yeast. J. Cell Biol. **69**:717–721.
46. **Cabib, E., and B. Bowers.** 1975. Timing and function of chitin synthesis in yeast. J. Bacteriol. **124**:1586–1593.
47. **Cabib, E., R. Roberts, and B. Bowers.** 1982. Synthesis of the yeast cell wall and its regulation. Annu. Rev. Biochem. **51**:763–793.
48. **Casperson, G. F., N. Walker, and H. R. Bourne.** 1985. Isolation of the gene encoding adenylate cyclase in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **82**:5060–5063.
49. **Cole, G. T.** 1986. Models of cell differentiation in conidial

fungi. Microbiol. Rev. **50**:95–132.
50. **Collinge, A. J., M. H. Fletcher, and A. P. J. Trinci.** 1978. Physiology and cytology of septation and branching in a temperature sensitive colonial mutant (cot 1) of *Neurospora crassa*. Trans. Br. Mycol. Soc. **71**:107–120.
51. **Cook, W. R., T. J. MacAlister, and L. I. Rothfield.** 1986. Compartmentalization of the periplasmic space at division sites in gram-negative bacteria. J. Bacteriol. **168**:1430–1438.
52. **Cosgrove, D.** 1986. Biophysical control of plant cell growth. Annu. Rev. Plant Physiol. **37**:377–405.
53. **Daneo-Moore, L., and G. D. Shockman.** 1977. The bacterial cell surface in growth and division, p. 597–715. *In* G. Poste and G. L. Nicolson (ed.), The synthesis, assembly and turnover of cell surface components. Elsevier/North-Holland Publishing Co., Amsterdam.
54. **DeBoer, P. A. J., R. E. Crossley, and L. I. Rothfield.** 1989. A division inhibitor and a topological specificity factor coded by the minicell locus determines proper placement of the division septum in *E. coli*. Cell **56**:641–649.
55. **De Jonge, B. L. M., F. B. Wientjes, I. Jurida, F. Driehuis, J. T. Wouters, and N. Nanninga.** 1989. Peptidoglycan synthesis during the cell cycle of *E. coli*: composition and mode of insertion. J. Bacteriol. **171**:5783–5794.
56. **Delmer, D. P.** 1987. Cellulose biosynthesis. Annu. Rev. Plant Physiol. **38**:259–290.
57. **Dickinson, J. R., and A. S. Williams.** 1987. The *cdc30* mutation in *Saccharomyces cerevisiae* results in a temperature-sensitive isoenzyme of phosphoglucose isomerase. J. Gen. Microbiol. **133**:135–140.
58. **Donachie, W. D., K. J. Begg, and N. F. Sullivan.** 1984. Morphogenes of *Escherichia coli*, p. 27–62. *In* R. Losick and L. Shapiro (ed.), Microbial development. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
59. **Doyle, R. J., and A. L. Koch.** 1987. The functions of autolysins in the growth and division of *Bacillus subtilis*. Crit. Rev. Microbiol. **15**:169–222.
60. **Driehuis, F., and J. T. M. Wouters.** 1987. Effect of growth rate and cell shape on the peptidoglycan composition in *E. coli*. J. Bacteriol. **169**:97–101.
61. **Driever, W., and C. Nüsslein-Volhard.** 1988. A gradient of *bicoid* protein in *Drosophila* embryos. Cell **54**:83–93.
62. **Drubin, D. G., K. G. Miller, and D. Botstein.** 1988. Yeast actin-binding proteins: evidence for a role in morphogenesis. J. Cell Biol. **107**:2551–2561.
63. **Edelman, G. M.** 1988. Topobiology: an introduction to molecular embryology. Basic Books, Inc., New York.
64. **Elorza, M. V., A. Murgui, H. Rico, F. Miragall, and R. Sentandreu.** 1987. Formation of a new cell wall by protoplasts of *Candida albicans*: effects of papulacandin B, tunicamycin and nikkomycin. J. Gen. Microbiol. **133**:2315–2325.
65. **Farkas, V., J. Kovarik, A. Kosinova, and S. Bauer.** 1974. Autoradiographic study of mannan incorporation into the growing cell walls of *Saccharomyces cerevisiae*. J. Bacteriol. **117**:265–269.
66. **Field, C., and R. Schekman.** 1980. Localized secretion of acid phosphatase reflects the pattern of cell surface growth. J. Cell Biol. **86**:123–128.
67. **Frankel, J.** 1974. Positional information in unicellular organisms. J. Theor. Biol. **47**:439–481.
68. **Frankel, J.** 1982. Global patterning in single cells. J. Theor. Biol. **99**:119–134.
69. **Frankel, J.** 1984. Pattern formation in ciliated protozoa, p. 163–196. *In* G. M. Malacinski and S. V. Bryant (ed.), Pattern formation. Macmillan Publishing Co., New York.
70. **Frankel, J., and E. M. Nelsen.** 1981. Discontinuities and overlaps in patterning within single cells. Philos. Trans. R. Soc. London Ser. B **295**:525–538.
71. **Frankel, J., and E. M. Nelsen.** 1987. Positional reorganization in compound *janus* cells of *Tetrahymena thermophila*. Development **99**:51–68.
72. **Frankel, J., E. M. Nelsen, and L. M. Jenkins.** 1987. Intracellular pattern reversal in *Tetrahymena thermophila*: *janus* mutants and their geometrical phenocopies, p. 219–244. *In* W. F. Loomis (ed.), Genetic regulation of development. Alan R. Liss, Inc., New York.
73. **Fulton, A. B.** 1981. How do eucaryotic cells construct their cytoarchitecture? Cell **24**:4–5.
74. **Fulton, C., and C. Walsh.** 1980. Cell differentiation and flagellar elongation in *Naegleria gruberi*: dependence on transcription and translation. J. Cell Biol. **85**:346–360.
75. **Galway, M. E., and A. R. Hardham.** 1986. Microtubule reorganization, cell wall synthesis and establishment of the axis of elongation in regenerating protoplasts of the alga *Mougeotia*. Protoplasma **135**:130–143.
76. **Gambino, J., L. C. Bergen, and N. R. Morris.** 1984. Effects of mitotic and tubulin mutations on microtubule architecture in actively growing protoplasts of *Aspergillus nidulans*. J. Cell Biol. **99**:830–838.
77. **Giddings, T. H., and L. A. Staehelin.** 1988. Spatial relationship between microtubules and plasma-membrane rosettes during the deposition of primary wall microfibrils in *Closterium* sp. Planta **173**:22–30.
78. **Girbardt, M.** 1969. Die Ultrastruktur der Apikalregion von Pilzhyphen. Protoplasma **67**:413–441.
79. **Goebl, M. G., J. Yochem, S. Jentsch, J. P. McGrath, A. Varshavsky, and B. Byers.** 1988. The yeast cell cycle gene *cdc34* encodes a ubiquitin-conjugating enzyme. Science **241**: 1331–1335.
80. **Gooday, G. W.** 1971. An autoradiographic study of hyphal growth of some fungi. J. Gen. Microbiol. **67**:125–133.
81. **Gooday, G. W.** 1983. The hyphal tip, p. 315–356. *In* J. E. Smith (ed.), Fungal differentiation. Marcel Dekker, Inc., New York.
82. **Gooday, G. W., and A. P. J. Trinci.** 1980. Wall structure and biosynthesis in fungi. Symp. Soc. Gen. Microbiol. **30**:207–251.
83. **Goodwin, B. C.** 1980. Pattern formation and its regeneration in the protozoa. Symp. Soc. Gen. Microbiol. **30**:377–404.
84. **Goodwin, B. C.** 1986. What are the causes of morphogenesis? BioEssays **3**:32–36.
85. **Goodwin, B. C.** 1988. Problems and prospects in morphogenesis. Experientia **44**:633–637.
86. **Goodwin, B. C., C. Briere, and P. S. O'Shea.** 1987. Mechanisms underlying the formation of spatial structures in cells, p. 1–9. *In* R. K. Poole and A. P. J. Trinci (ed.), Spatial organization in eukaryotic microbes. IRL Press, Oxford.
87. **Goodwin, B. C., and L. E. H. Trainor.** 1985. Tip and whorl morphogenesis in *Acetabularia* by calcium-regulated strain fields. J. Theor. Biol. **117**:79–106.
88. **Gozalbo, D., F. Dubon, J. Schwencke, and R. Sentandreu.** 1987. Characterization of chitosomes in *Candida albicans* protoplasts. Exp. Mycol. **11**:331–338.
89. **Grain, J.** 1986. The cytoskeleton in protists: nature, structure and functions. Int. Rev. Cytol. **104**:153–249.
90. **Green, P. B.** 1969. Cell morphogenesis. Annu. Rev. Plant Physiol. **20**:365–394.
91. **Green, P. B., R. O. Erickson, and P. A. Richmond.** 1970. The physical basis of cell morphogenesis. Ann. N.Y. Acad. Sci. **175**:712–731.
92. **Grimes, G. W.** 1982. Pattern determination in hypotrich ciliates. Am. Zool. **22**:35–46.
93. **Grove, S. N.** 1978. The cytology of hyphal tip growth, p. 28–50. *In* J. E. Smith and R. D. Berry (ed.), The filamentous fungi, vol. III. John Wiley & Sons, Inc., New York.
94. **Grove, S. N., and C. E. Bracker.** 1970. Protoplasmic organization of hyphal tips among fungi: vesicles and Spitzenkörper. J. Bacteriol. **104**:989–1009.
95. **Grove, S. N., and J. A. Sweigard.** 1980. Cytochalasin A inhibits spore germination and hyphal tip growth in *Gilbertella persicara*. Exp. Mycol. **4**:234–250.
96. **Gunderson, J. H., H. Elwood, A. Ingold, K. Kindle, and M. Sogin.** 1987. Phylogenetic relationships between chlorophytes, chrysophytes and oomycetes. Proc. Natl. Acad. Sci. USA **84**:5823–5827.
97. **Gunning, B. E. S., and A. R. Hardham.** 1982. Microtubules. Annu. Rev. Plant Physiol. **33**:561–698.
98. **Hall, J. L., Z. Ramanis, and D. J. L. Luck.** 1989. Basal body/centriolar DNA: molecular genetic studies in *Chlam-*

ydomonas. Cell **59**:121–132.
99. **Hämmerling, J.** 1953. Nucleo-cytoplasmic relationships in the development of *Acetabularia*. Int. Rev. Cytol. **2**:475–498.
100. **Hanes, S. D., R. Koren, and K. A. Bostian.** 1986. Control of cell growth and division in *Saccharomyces cerevisiae*. Crit. Rev. Biochem. **21**:153–223.
101. **Harold, F. M., and J. H. Caldwell.** 1990. Tips and currents: electrobiology of apical growth, p. 59–90. *In* I. B. Heath (ed.), Tip growth in plant and fungal cells. Academic Press, Inc., Orlando, Fla.
102. **Harold, R. L., and F. M. Harold.** 1980. Oriented growth of *Blastocladiella emersonii* in gradients of ionophores and inhibitors. J. Bacteriol. **144**:1159–1167.
103. **Harold, R. L., and F. M. Harold.** 1986. Ionophores and cytochalasins modulate branching in *Achlya bisexualis*. J. Gen. Microbiol. **132**:213–219.
104. **Harrison, L. G.** 1982. An overview of kinetic theory in developmental modeling, p. 3–33. *In* S. Subtelny and P. B. Green (ed.), Developmental order, its origin and regulation. Alan R. Liss, Inc., New York.
105. **Harrison, L. G.** 1987. What is the status of reaction-diffusion theory thirty-four years after Turing? J. Theor. Biol. **125**:369–384.
106. **Harrison, L. G., and M. Kolar.** 1988. Coupling between reaction-diffusion prepattern and expressed morphogenesis, applied to desmids and dasyclads. J. Theor. Biol. **130**:493–515.
107. **Harrison, L. G., K. T. Graham, and B. C. Lakowski.** 1988. Calcium localization during *Acetabularia* whorl formation: evidence supporting a two-stage hierarchical mechanism. Development **104**:255–262.
108. **Harrison, L. G., J. Snell, R. Verdi, D. E. Vogt, G. D. Zeiss, and B. R. Green.** 1981. Hair morphogenesis in *Acetabularia mediterranea*: temperature-dependent spacing and models of morphogen waves. Protoplasma **106**:211–221.
109. **Hartwell, L. H.** 1974. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. **38**:164–196.
110. **Hašek, J., I. Rupes, J. Svobodova, and E. Streiblova.** 1987. Tubulin and actin topology during zygote formation of *Saccharomyces cerevisiae*. J. Gen. Microbiol. **133**:3355–3363.
111. **Hayles, J., and P. Nurse.** 1986. Cell cycle regulation in yeast. J. Cell Sci. Suppl. **4**:155–170.
112. **Heath, I. B.** 1974. A unified hypothesis for the role of membrane-bound enzyme complexes and microtubules in plant cell wall synthesis. J. Theor. Biol. **48**:445–449.
113. **Heath, I. B.** 1987. Preservation of a labile cortical array of actin filaments in growing hyphal tips of the fungus *Saprolegnia ferax*. Eur. J. Cell Biol. **44**:10–16.
114. **Heath, I. B.** 1988. Evidence against a direct role for cortical actin arrays in saltatory organelle motility in hyphae of the fungus *Saprolegnia ferax*. J. Cell Sci. **91**:41–47.
115. **Heath, I. B., and S. G. W. Kaminskyi.** 1989. The organization of tip-growth-related organelles and microtubules revealed by quantitative analysis of freeze-substituted oomycete hyphae. J. Cell Sci. **93**:41–52.
116. **Heath, I. B., K. Rethoret, A. L. Arsenault, and F. P. Ottensmeyer.** 1985. Improved preservation of the form and contents of wall vesicles and the Golgi apparatus in freeze substituted hyphae of *Saprolegnia*. Protoplasma **128**:81–93.
117. **Heath, I. B., and R. W. Seagull.** 1982. Oriented cellulose fibrils and the cytoskeleton: a critical comparison of models, p. 162–182. *In* C. W. Lloyd (ed.), The cytoskeleton in plant growth and development. Academic Press, Inc. (London), Ltd., London.
118. **Henning, U.** 1975. Determination of cell shape in bacteria. Annu. Rev. Microbiol. **29**:45–60.
119. **Hepler, P. K., and R. O. Wayne.** 1985. Calcium and plant development. Annu. Rev. Plant Physiol. **36**:397–439.
120. **Higgins, M. L., and G. D. Shockman.** 1976. Study of a cycle of cell wall assembly in *Streptococcus faecalis* by three-dimensional reconstruction of thin sections of cells. J. Bacteriol. **127**:1346–1358.
121. **Hightower, M. J., J. Bruno, and J. J. Lucas.** 1983. Identification of key regulated events early in the life of hybrid animal cells constructed by nuclear transplantation. Proc. Natl. Acad. Sci. USA **80**:5310–5314.
122. **Hoch, H. C., B. E. Tucker, and R. C. Staples.** 1988. An intact microtubule cytoskeleton is necessary for mediation of the signal for cell differentiation in *Uromyces*. Eur. J. Cell Biol. **45**:209–218.
123. **Hogetsu, T., and Y. Oshima.** 1985. Immunofluorescence microscopy of microtubule arrangement in *Closterium acerosum*. Planta **166**:169–175.
124. **Hogetsu, T., and M. Shibaoka.** 1978. The change of pattern in microfibril arrangement on the inner surface of the cell wall of *Closterium acerosum* during cell growth. Planta **140**:7–14.
125. **Hogetsu, T., and M. Shibaoka.** 1978. Effects of colchicine on cell shape and on microfibril arrangement in the cell wall of *Closterium acerosum*. Planta **140**:15–18.
126. **Holcomb, C. L., W. J. Hansen, T. Etcheverry, and R. Schekman.** 1988. Secretory vesicles externalize the major plasma membrane ATPase in yeast. J. Cell Biol. **106**:641–648.
127. **Holland, I. B.** 1987. Genetic analysis of the *E. coli* division clock. Cell **48**:351–362.
128. **Holmes, J. A., and S. K. Dutcher.** 1989. Cellular asymmetry in *Chlamydomonas reinhardtii*. J. Cell Sci. **94**:273–285.
129. **Howard, R. J.** 1981. Ultrastructural analysis of hyphal tip cell growth in fungi: Spitzenkörper, cytoskeleton and endomembranes after freeze-substitution. J. Cell Sci. **48**:89–103.
130. **Howard, R. J., and J. R. Aist.** 1980. Cytoplasmic microtubules and fungal morphogenesis: ultrastructural effects of methyl benzimidazole-2-yl carbamate determined by freeze-substitution of hyphal tip cells. J. Cell Biol. **87**:55–64.
131. **Huffaker, T. C., M. A. Hoyt, and D. Botstein.** 1987. Genetic analysis of the yeast cytoskeleton. Annu. Rev. Genet. **21**:259–284.
132. **Huffaker, T. C., J. H. Thomas, and D. Botstein.** 1988. Adverse effects of β-tubulin mutations on microtubule formation and function. J. Cell Biol. **106**:1977–2010.
133. **Ingham, P. W.** 1988. The molecular genetics of embryonic pattern formation in *Drosophila*. Nature (London) **335**:25–34.
134. **Jackson, S. L., and I. B. Heath.** 1989. Effects of exogenous calcium ions on tip growth, intracellular Ca^{2+} concentration and actin arrays in hyphae of the fungus *Saprolegnia ferax*. Exp. Mycol. **13**:1–12.
135. **Jacob, F.** 1973. The logic of life: a history of heredity. Pantheon Books, New York.
136. **Jacob, F., S. Brenner, and F. Cuzin.** 1963. On the regulation of DNA replication in bacteria. Cold Spring Harbor Symp. Quant. Biol. **28**:329–348.
137. **Jacobs, C. W., A. E. M. Adams, P. J. Szaniszlo, and J. R. Pringle.** 1988. Functions of microtubules in the cell cycle. J. Cell Biol. **107**:1409–1426.
138. **Jaffe, L. F.** 1968. Localization in the developing *Fucus* egg and the general role of localizing currents. Adv. Morphog. **7**:295–328.
139. **Jaffe, L. F.** 1981. The role of ionic currents in establishing developmental pattern. Philos. Trans. R. Soc. London Ser. B **295**:553–566.
140. **Jaffe, L. F., and R. Nuccitelli.** 1977. Electrical controls of development. Annu. Rev. Biophys. Bioeng. **6**:445–476.
141. **Jaffe, L. F., K. R. Robinson, and R. Nuccitelli.** 1974. Local cation entry and self-electrophoresis as an intracellular localization mechanism. Ann. N.Y. Acad. Sci. **238**:372–389.
142. **Jerka-Dziadosz, M., and J. Frankel.** 1979. A mutant of *Tetrahymena thermophila* with a partial mirror-image duplication of cell surface pattern. J. Embryol. Exp. Morphol. **49**:167–202.
143. **Johnson, B. F., and E. J. Gibson.** 1966. Autoradiographic analysis of regional cell wall growth of yeasts. Exp. Cell Res. **41**:580–591.
144. **Jong, A. Y. S., C. L. Kuo, and J. L. Campbell.** 1984. The CDC8 gene of yeast encodes thymidylate kinase. J. Biol. Chem. **259**:11052–11059.
145. **Kallio, P., and J. Lehtonen.** 1981. Nuclear control of morphogenesis in *Micrasterias*, p. 191–213. *In* O. Kiermayer (ed.), Cytomorphogenesis in plants. Springer-Verlag, New York.
146. **Kanbe, T., I. Kobayashi, and K. Tanaka.** 1989. Dynamics of

cytoplasmic organelles in the cell cycle of the fission yeast *Schizosaccharomyces pombe*: three-dimensional reconstruction from serial sections. J. Cell Sci. **94**:647–656.
147. **Katz, D., and R. F. Rosenberger.** 1970. A mutation in *Aspergillus nidulans* producing hyphal walls which lack chitin. Biochim. Biophys. Acta **208**:452–460.
148. **Kauss, H.** 1978. Osmotic regulation in algae. Progr. Phytochem. **5**:1–27.
149. **Kiermayer, O.** 1981. Cytoplasmic basis of morphogenesis in *Micrasterias*, p. 147–189. *In* O. Kiermayer (ed.), Cytomorphogenesis in plants. Springer-Verlag, New York.
150. **Kilmartin, J. V., and A. E. M. Adams.** 1984. Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. **98**:922–933.
151. **King, J.** 1980. Regulation of structural protein interactions as revealed in phage morphogenesis, p. 101–132. *In* R. F. Goldberger (ed.), Biological regulation and development, vol. 2. Plenum Publishing Corp., New York.
152. **Kirchner, M., and T. Mitchison.** 1986. Beyond self-assembly: from microtubules to morphogenesis. Cell **45**:329–342.
153. **Kobori, H., N. Yamada, A. Taki, and M. Osumi.** 1989. Actin is associated with the formation of the cell wall in reverting protoplasts of the fission yeast *Schizosaccharomyces pombe*. J. Cell Sci. **94**:635–646.
154. **Koch, A. L.** 1982. The shape of the hyphal tips of fungi. J. Gen. Microbiol. **128**:947–951.
155. **Koch, A. L.** 1983. The surface stress theory of microbial morphogenesis. Adv. Microb. Physiol. **24**:301–366.
156. **Koch, A. L.** 1985. How bacteria grow and divide in spite of internal hydrostatic pressure. Can. J. Microbiol. **31**:1071–1083.
157. **Koch, A. L.** 1988. Biophysics of bacterial walls viewed as stress-bearing fabric. Microbiol. Rev. **52**:337–353.
158. **Koch, A. L.** 1988. Speculations on the growth strategy of prosthecate bacteria. Can. J. Microbiol. **34**:390–394.
159. **Koch, A. L., and I. D. J. Burdett.** 1984. The variable T model for gram-negative morphology. J. Gen. Microbiol. **130**:2325–2338.
160. **Koch, A. L., and I. D. J. Burdett.** 1986. Normal pole formation during total inhibition of wall synthesis. J. Gen. Microbiol. **132**:3441–3449.
161. **Koch, A. L., M. L. Higgins, and R. J. Doyle.** 1981. Surface tension-like forces determine bacterial shapes. J. Gen. Microbiol. **123**:151–161.
162. **Koch, A. L., M. L. Higgins, and R. J. Doyle.** 1982. The role of surface stress in the morphology of microbes. J. Gen. Microbiol. **128**:927–945.
163. **Koch, A. L., H. L. T. Mobley, R. J. Doyle, and U. N. Streips.** 1981. The coupling of wall growth and chromosome replication in gram-positive rods. FEMS Microbiol. Lett. **12**:201–208.
164. **Kropf, D. L.** 1986. Electrophysiological properties of *Achlya* hyphae: ionic currents studied by intracellular recording. J. Cell Biol. **102**:1209–1216.
165. **Kropf, D. L., S. K. Berge, and R. S. Quatrano.** 1989. Actin localization during *Fucus* embryogenesis. Plant Cell **1**:191–200.
166. **Kropf, D. L., J. M. Caldwell, N. A. R. Gow, and F. M. Harold.** 1984. Transcellular ion currents in the water mold *Achlya*: amino acid/proton symport as a mechanism of current entry. J. Cell Biol. **99**:486–496.
167. **Kropf, D. L., R. Hopkins, and R. S. Quatrano.** 1989. Protein synthesis and morphogenesis are not tightly linked during embryogenesis in *Fucus*. Dev. Biol. **134**:452–461.
168. **Kropf, D. L., B. Kloareg, and R. S. Quatrano.** 1988. Cell wall is required for fixation of the embryonic axis in *Fucus* zygotes. Science **239**:187–190.
169. **Kropf, D. L., and R. S. Quatrano.** 1987. Localization of membrane-associated calcium during development of fucoid algae using chlorotetracycline. Planta **171**:158–170.
170. **Lacalli, T. C.** 1981. Dissipative structures and morphogenetic pattern in unicellular algae. Philos. Trans. R. Soc. London Ser. B **294**:547–588.
171. **Lawrence, P. A.** 1988. Background to *bicoid*. Cell **54**:1–2.
172. **Linnemans, W. A. M., P. Boer, and P. F. Elbers.** 1977. Localization of acid phosphatase in *Saccharomyces cerevisiae*: a clue to cell wall formation. J. Bacteriol. **131**:638–644.
173. **Lisziewicz, J., A. Godany, M.-H. Förster, and H. Küntzel.** 1987. Isolation and nucleotide sequence of a *Saccharomyces cerevisiae* protein kinase gene suppressing the cell cycle start mutation *cdc25*. J. Biol. Chem. **262**:2549–2553.
174. **Lloyd, C. W.** 1984. Toward a dynamic helical model for the influence of microtubules on wall patterns in plants. Int. Rev. Cytol. **86**:1–51.
175. **Lloyd, C. W.** 1986. Microtubules and the cellular morphogenesis of plants, p. 31–56. *In* L. W. Browder (ed.), Developmental biology, a comprehensive synthesis, vol. 2. Plenum Publishing Corp., New York.
176. **MacAlister, T. J., W. R. Cook, R. Weigand, and L. I. Rothfield.** 1987. Membrane-murein attachment at the leading edge of the division septum: a second membrane-murein structure associated with morphogenesis of the gram-negative bacterial division septum. J. Bacteriol. **169**:3945–3951.
177. **Marchant, H. J., and E. Hines.** 1979. Role of microtubules and cell wall deposition in elongating and regenerating protoplasts of *Mougeotia*. Planta **146**:41–48.
178. **Margulis, L., and K. V. Schwartz.** 1982. Five kingdoms: an illustrated guide to the phyla of life on earth. W. H. Freeman & Co., San Francisco.
179. **Matile, P., M. Cortat, A. Wiemken, and A. Frey-Wissling.** 1971. Isolation of glucanase-containing particles from budding *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **68**:636–640.
180. **Matile, P., H. Moor, and C. F. Robinow.** 1969. *In* A. H. Rose and J. S. Harrison (ed.), The yeasts, p. 219–302. Academic Press, Inc. (London), Ltd., London.
181. **McGillivray, A. M., and N. A. R. Gow.** 1987. The transhyphal electric current of *Neurospora crassa* is carried principally by protons. J. Gen. Microbiol. **133**:2875–2881.
182. **McIntosh, E. M., R. W. Ord, and R. K. Storms.** 1988. Transcriptional regulation of the cell-cycle-dependent thymidylate synthase gene of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **8**:4616–4624.
183. **McKerracher, L. J., and I. B. Heath.** 1987. Cytoplasmic migration and intracellular organelle movements during tip growth of fungal hyphae. Exp. Mycol. **11**:79–100.
184. **Meindl, U.** 1985. Experimental and ultrastructural studies on cell shape formation in the defect mutant cell *Micrasterias thomasiana f. uniradiata*. Protoplasma **129**:74–87.
185. **Meinhardt, H.** 1982. Models of biological pattern formation. Academic Press, Inc. (London), Ltd., London.
186. **Melan, M. A.** 1990. Taxol maintains organized microtubule patterns in protoplasts which lead to the resynthesis of organized cell wall microfibrils. Protoplasma **153**:169–177.
187. **Merad, T., A. R. Archibald, I. C. Hancock, C. R. Harwood, and J. A. Hobot.** 1989. Cell wall assembly in *B. subtilis*: visualization of old and new wall material by electron microscopic examination of samples stained selectively for teichoic acid and teichuronic acid. J. Gen. Microbiol. **135**:645–655.
188. **Metraux, J.-P., P. A. Richmond, and L. Taiz.** 1980. Control of cell elongation in *Nitella* by endogenous cell wall pH gradients. Plant Physiol. **65**:204–210.
189. **Mishra, N. C., and E. L. Tatum.** 1970. Phosphoglucomutase mutants of *Neurospora sitophila* and their relation to morphology. Proc. Natl. Acad. Sci. USA **66**:638–645.
190. **Mitchell, P.** 1962. Molecule, group and electron translocation through natural membranes. Biochem. Soc. Symp. **22**:142–169.
191. **Monod, J.** 1971. Chance and necessity. Alfred A. Knopf, Inc., New York.
192. **Montezinos, D.** 1982. The role of the plasma membrane in cellulose microfibril assembly, p. 147–162. *In* C. W. Lloyd (ed.), The cytoskeleton in plant growth and development. Academic Press, Inc. (London), Ltd., London.
193. **Mullins, J. T., and E. A. Ellis.** 1974. Sexual morphogenesis in *Achlya*: ultrastructural basis for the hormonal induction of antheridial hyphae. Proc. Natl. Acad. Sci. USA **71**:1347–1350.
194. **Nanney, D. L.** 1977. Molecules and morphologies: the perpetuation of pattern in the ciliated protozoa. J. Protozool. **24**:27–35.

195. **Nelsen, E. M., J. Frankel, and L. M. Jenkins.** 1989. Non-genic inheritance of cellular handedness. Development **105**:447–456.
196. **Newport, J.** 1987. Nuclear reconstitution *in vitro*: stages of assembly around protein-free DNA. Cell **48**:205–217.
197. **Ng, S. F., and J. Frankel.** 1977. 180° rotation of ciliary rows and its morphogenetic implications in *Tetrahymena pyriformis*. Proc. Natl. Acad. Sci. USA **74**:1115–1119.
198. **Nicolis, G., and I. Prigogine.** 1989. Exploring complexity. W. H. Freeman & Co., New York.
199. **Novick, P., and D. Botstein.** 1985. Phenotypic analysis of temperature-sensitive yeast actin mutants. Cell **40**:405–416.
200. **Nuccitelli, R.** 1978. Öoplasmic segregation and secretion in the *Pelvetia* egg is accompanied by a membrane-generated electric current. Dev. Biol. **62**:13–33.
201. **Nuccitelli, R.** 1983. Transcellular ion currents: signals and effectors of cell polarity. Mod. Cell Biol. **2**:451–481.
202. **Nuccitelli, R.** 1988. Ionic currents in morphogenesis. Experientia **44**:657–666.
203. **Oakley, B. R., and N. R. Morris.** 1980. Nuclear movement is β-tubulin dependent in *Aspergillus nidulans*. Cell **19**:255–262.
204. **Oakley, B. R., and J. E. Rinehart.** 1985. Mitochondria and nuclei move by different mechanisms in *Aspergillus nidulans*. J. Cell Biol. **101**:2392–2397.
205. **Ohya, Y., S. Miyamoto, Y. Ohsumi, and Y. Anraku.** 1986. Calcium-sensitive *cls4* mutant of *Saccharomyces cerevisiae* with a defect in bud formation. J. Bacteriol. **165**:28–33.
206. **O'Shea, P. S.** 1988. Physical fields and cellular organisation: field-dependent mechanisms of morphogenesis. Experientia **44**:684–694.
207. **Oster, G. F.** 1984. On the crawling of cells. J. Embryol. Exp. Morphol. **83**(Suppl.):329–364.
208. **Oster, G. F., and A. S. Perelson.** 1987. The physics of cell motility. J. Cell Sci. Suppl. **8**:35–54.
209. **Pall, M. L., J. M. Trevillyan, and N. Hinman.** 1981. Deficient cyclic adenosine 3′,5′-monophosphate control in mutants of two genes of *Neurospora crassa*. Mol. Cell. Biol. **1**:1–8.
210. **Peacocke, A. R.** 1983. An introduction to the physical chemistry of biological organization. Clarendon Press, Oxford.
211. **Peng, B., and L. F. Jaffe.** 1976. Cell-wall formation in *Pelvetia* embryos. A freeze-fracture study. Planta **133**:57–71.
212. **Pickett-Heaps, J. D.** 1975. Green algae: structure, reproduction and evolution in selected genera. Sinauer Associates, Sunderland, Mass.
213. **Picton, J. M., and M. W. Steer.** 1982. A model for the mechanism of tip extension in pollen tubes. J. Theor. Biol. **98**:15–20.
214. **Picton, J. M., and M. W. Steer.** 1985. The effects of ruthenium red, lanthanum, fluorescein isothiocyanate and trifluoperazine on vesicle transport, vesicle fusion and tip extension in pollen tubes. Planta **163**:20–26.
215. **Poo, M.-M.** 1981. *In situ* electrophoresis of membrane components. Annu. Rev. Biophys. Bioeng. **10**:245–276.
216. **Preston, R. D.** 1988. Cellulose-microfibril-orienting mechanisms in plant cell walls. Planta **174**:67–74.
217. **Pringle, J. R., and L. H. Hartwell.** 1981. The *Saccharomyces cerevisiae* cell cycle, p. 97–142. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), The molecular biology of the yeast *Saccharomyces*: life cycle and inheritance. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
218. **Quader, H.** 1983. Morphology and movement of cellulose synthesizing (terminal) complexes in *Oocystis solitaria*: evidence that microfibril assembly is the motive force. Eur. J. Cell Biol. **32**:174–177.
219. **Quader, H.** 1986. Cellulose microfibril orientation in *Oocystis solitaria*: proof that microtubules control the alignment of the terminal complexes. J. Cell Sci. **83**:223–234.
220. **Quader, H., and D. G. Robinson.** 1981. *Oocystis solitaria*: a model organism for understanding the organization of cellulose synthesis. Ber. Dtsch. Bot. Ges. **94**:75–84.
221. **Quatrano, R. S.** 1972. An ultrastructural study of the determined site of rhizoid formation in *Fucus* zygotes. Exp. Cell Res. **70**:1–12.
222. **Quatrano, R. S.** 1978. Development of cell polarity. Annu. Rev. Plant Physiol. **29**:487–510.
223. **Quatrano, R. S., L. R. Griffing, V. Huber-Walchli, and R. S. Doubet.** 1985. Cytological and biochemical requirements for the establishment of a polar cell. J. Cell Sci. Suppl. **2**:129–141.
224. **Quinlan, R. A., C. I. Pogson, and K. Gull.** 1980. The influence of the microtubule inhibitor methyl benzimidazole-2-yl-carbamate (MCB) on nuclear division and the cell cycle in *Saccharomyces cerevisiae*. J. Cell Sci. **46**:341–352.
225. **Ray, P. M., P. B. Green, and R. Cleland.** 1972. Role of turgor in plant cell growth. Nature (London) **239**:163–164.
226. **Reed, S. I., J. A. Madwiger, and A. T. Lorincz.** 1985. Protein kinase activity associated with the product of the yeast cell division cycle gene CDC28. Proc. Natl. Acad. Sci. USA **82**:4055–4059.
227. **Reinhardt, M. O.** 1892. Das Wachstum der Pilzhyphen. Ein Beitrag zur Kenntnis des Flächenwachstums vegetalischer Zellmembranen. Jahrb. Wiss. Bot. **23**:479–566.
228. **Reiss, H.-D., and W. Herth.** 1985. Nifedipine-sensitive calcium channels are involved in polar growth of lily-pollen tubes. J. Cell Sci. **76**:247–254.
229. **Reiss, H.-D., W. Herth, and R. Nobiling.** 1985. Development of membrane and calcium gradients during pollen germination in *Lilium longiflorum*. Planta **163**:84–90.
230. **Reissig, J. L., and S. G. Kinney.** 1983. Calcium as a branching signal in *Neurospora crassa*. J. Bacteriol. **154**:1397–1402.
231. **Roberson, R. W., and M. S. Fuller.** 1988. Ultrastructural aspects of the hyphal tip of *Sclerotium rolfsii* preserved by freeze substitution. Protoplasma **146**:143–149.
232. **Robinson, D. G., and H. Quader.** 1982. The microtubule-microfibril syndrome, p. 110–126. *In* C. W. Lloyd (ed.), The cytoskeleton in plant growth and development. Academic Press, Inc. (London), Ltd., London.
233. **Robinson, K. R., and R. Cone.** 1980. Polarization of fucoid eggs by a calcium ionophore gradient. Science **207**:77–78.
234. **Robinson, K. R., and L. F. Jaffe.** 1975. Polarizing fucoid eggs drive a calcium current through themselves. Science **187**:70–72.
235. **Rogers, H. J., H. R. Perkins, and J. B. Ward.** 1980. Microbial cell walls and membranes. Chapman & Hall, Ltd., London.
236. **Ruiz-Herrera, J., C. Valenzuela, G. Martinez-Cadena, and A. Obregon.** 1989. Alterations in the vesicular pattern and wall growth in *Phycomyces* induced by the calcium ionophore A23187. Protoplasma **148**:15–25.
237. **Runeberg, P., M. Raudaskoski, and I. Virtanen.** 1986. Cytoskeletal elements in the hyphae of the homobasidiomycete *Schizophyllum commune* visualized with indirect immunofluorescence and NBD-phallacidin. Eur. J. Cell Biol. **41**:25–32.
238. **Sachs, H., I. Grimm, and D. G. Robinson.** 1976. Structure, synthesis and orientation of microfibrils. I. Architecture and development of the wall of *Oocystis solitaria*. Cytobiologie **14**:49–60.
239. **Salo, V., S. S. Niini, I. Virtanen, and M. Raudaskoski.** 1989. Comparative immunocytochemistry of the cytoskeleton in filamentous fungi with dikaryotic and multinucleate hyphae. J. Cell Sci. **94**:11–24.
240. **Santos, T., F. del Rey, J. Conde, J. R. Villanueva, and C. Nombela.** 1979. *Saccharomyces cerevisiae* mutant defective in exo-1,3-β-glucanase production. J. Bacteriol. **139**:333–338.
241. **Saunders, P. T., and A. P. J. Trinci.** 1979. Determination of tip shape in fungal hyphae. J. Gen. Microbiol. **110**:469–473.
242. **Sburlati, A., and E. Cabib.** 1986. Chitin synthetase 2, a presumptive participant in septum formation in *Saccharomyces cerevisiae*. J. Biol. Chem. **261**:15147–15152.
243. **Schekman, R.** 1985. Protein localization and membrane traffic in yeast. Annu. Rev. Cell Biol. **1**:115–143.
244. **Schmid, J., and F. M. Harold.** 1988. Dual roles for calcium ions in apical growth of *Neurospora crassa*. J. Gen. Microbiol. **134**:2623–2631.
245. **Schnepf, E.** 1986. Cellular polarity. Annu. Rev. Plant Physiol. **37**:23–47.
246. **Schreurs, W. J. A., and F. M. Harold.** 1988. Transcellular proton current in *Achlya bisexualis* hyphae: relationship to polarized growth. Proc. Natl. Acad. Sci. USA **85**:1534–1538.

247. **Schreurs, W. J. A., R. L. Harold, and F. M. Harold.** 1989. Chemotropism and branching as alternative responses of *Achlya bisexualis* to amino acids. J. Gen. Microbiol. **135**:2519–2528.
248. **Sclafani, R. A., and W. L. Fangman.** 1984. Yeast gene CDC8 encodes thymidylate kinase and is complemented by herpes thymidylate kinase gene TK. Proc. Natl. Acad. Sci. USA **81**:5821–5825.
249. **Shockman, G. D., L. Daneo-Moore, and M. L. Higgins.** 1974. Problems of cell wall and membrane growth, enlargement and division. Ann. N.Y. Acad. Sci. **235**:161–196.
250. **Sietsma, J. H., A. M. S. Sonnenberg, and J. A. H. Wessels.** 1985. Localization by autoradiography of synthesis of (1→3)-β and (1→6)-β linkages in a wall glucan during hyphal growth of *Schizophyllum commune*. J. Gen. Microbiol. **131**:1331–1337.
251. **Sievers, A., and E. Schnepf.** 1981. Morphogenesis and polarity of tubular cells with tip growth, p. 265–299. *In* D. Kiermayer (ed.), Cytomorphogenesis in plants. Springer-Verlag, Vienna.
252. **Silverman, S. J., A. Sburlati, M. L. Slater, and E. Cabib.** 1988. Chitin synthase 2 is essential for septum formation and cell division in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **85**:4735–4739.
253. **Singer, S. J., and A. Kupfer.** 1986. The directed migration of eukaryotic cells. Annu. Rev. Cell Biol. **2**:337–365.
254. **Slack, J. M. W.** 1983. From egg to embryo. Cambridge University Press, London.
255. **Sloat, B. F., A. Adams, and J. R. Pringle.** 1981. Roles of the CDC24 gene product in cellular morphogenesis during the *Saccharomyces cerevisiae* cell cycle. J. Cell Biol. **89**:395–405.
256. **Sogin, M. L., J. H. Gunderson, H. J. Elwood, R. A. Alonso, and D. A. Peattie.** 1989. Phylogenetic meaning of the kingdom concept: an unusual ribosomal RNA from *Giardia lamblia*. Science **243**:75–77.
257. **Soll, D. R., M. A. Herman, and M. A. Staebell.** 1985. The involvement of cell wall expansion in the two modes of mycelium formation of *Candida albicans*. J. Gen. Microbiol. **131**:2367–2375.
258. **Soll, D. R., and L. H. Mitchell.** 1983. Filament ring formation in the dimorphic yeast *Candida albicans*. J. Cell Biol. **96**:486–493.
259. **Soll, D. R., and D. Sonnenborn.** 1971. Zoospore germination in *Blastocladiella emersonii*: cell differentiation without protein synthesis. Proc. Natl. Acad. Sci. USA **68**:459–463.
260. **Sonneborn, T. M.** 1963. Does preformed cell structure play an essential role in cell heredity? *In* J. M. Allen (ed.), The nature of biological diversity. McGraw-Hill Book Co., New York.
261. **Sonneborn, T. M.** 1970. Gene action in development. Proc. R. Soc. London Ser. B **176**:347–366.
262. **Sonnenfeld, E. M., A. L. Koch, and R. J. Doyle.** 1985. Cellular location of origin and terminus of replication in *Bacillus subtilis*. J. Bacteriol. **163**:895–899.
263. **Speksnuder, J. E., A. E. Miller, M. H. Weisenseel, T. H. Chen, and L. F. Jaffe.** 1989. Calcium buffer injections block fucoid egg development by facilitating calcium diffusion. Proc. Natl. Acad. Sci. USA **86**:6607–6611.
264. **Staebell, M., and D. R. Soll.** 1985. Temporal and spatial differences in cell wall expansion during bud and mycelium formation in *Candida albicans*. J. Gen. Microbiol. **131**:1467–1480.
265. **Stent, G. S.** 1981. Strength and weakness of the genetic approach to the development of the nervous system. Annu. Rev. Neurosci. **4**:163–194.
266. **Stockem, W., and W. Klopocka.** 1988. Amoeboid movement and related phenomena. Int. Rev. Cytol. **112**:137–183.
267. **Stubblefield, E.** 1986. A theory for developmental control by a program encoded in the genome. J. Theor. Biol. **118**:129–143.
268. **Taiz, L.** 1984. Plant cell expansion: regulation of cell wall mechanical properties. Annu. Rev. Plant Physiol. **35**:585–657.
269. **Takeuchi, Y., J. Schmid, J. H. Caldwell, and F. M. Harold.** 1988. Transcellular ion currents and extension of *Neurospora crassa* hyphae. J. Membr. Biol. **101**:33–41.
270. **Tanford, C.** 1978. The hydrophobic effect. Science **200**:1012–1018.
271. **Tartar, V.** 1961. The biology of *Stentor*. Pergamon Press, London.
272. **Taschner, P. E. M., N. Ypenburg, B. G. Pratt, and C. L. Woldringh.** 1988. An amino acid substitution in penicillin-binding protein 3 creates pointed polar caps in *Escherichia coli*. J. Bacteriol. **170**:4828–4837.
273. **Terasaki, M., L. B. Chen, and K. Fujiwara.** 1986. Microtubules and the endoplasmic reticulum are highly interdependent structures. J. Cell Biol. **103**:1557–1568.
274. **Terenzi, M. F., M. M. Flawia, M. T. Tellez-Inon, and M. N. Torres.** 1976. Control of *Neurospora crassa* morphology by cyclic adenosine 3′,5′-monophosphate and dibutyryl cyclic adenosine 3′,5′-monophosphate. J. Bacteriol. **126**:91–99.
275. **Thomas, D. S., and J. T. Mullins.** 1967. Role of enzymatic wall softening in plant morphogenesis: hormonal induction in *Achlya*. Science **156**:84–85.
276. **Thompson, D. W.** 1961. On growth and form. Abridged, J. T. Bonner (ed.). Cambridge University Press, London.
277. **Thyberg, J., and S. Moskalewski.** 1985. Microtubules and the organization of the Golgi complex. Exp. Cell Res. **159**:1–16.
278. **Tipper, D. J., and A. Wright.** 1979. The structure and biosynthesis of bacterial cell walls, p. 291–426. *In* J. R. Sokatch and L. N. Ornston (ed.), The bacteria, vol. 7. Academic Press, Inc., New York.
279. **Tkacz, J. S., and J. O. Lampen.** 1972. Wall replication in *Saccharomyces* species: use of fluorescein-conjugated concanavalin A to reveal the site of mannan insertion. J. Gen. Microbiol. **72**:243–247.
280. **Trinci, A. P. J.** 1978. Wall and hyphal growth. Sci. Prog. **65**:75–99.
281. **Trinci, A. P. J., and N. R. Morris.** 1979. Morphology and growth of a temperature-sensitive mutant of *Aspergillus nidulans* which forms aseptate mycelia at non-permissive temperatures. J. Gen. Microbiol. **114**:53–54.
282. **Trinci, A. P. J., and P. T. Saunders.** 1977. Tip growth of fungal hyphae. J. Gen. Microbiol. **103**:243–248.
283. **Tschopp, J., P. C. Esmon, and R. Schekman.** 1984. Defective plasma membrane assembly in yeast secretory mutants. J. Bacteriol. **160**:966–970.
284. **Tucker, B. E., H. C. Hoch, and R. C. Staples.** 1986. The involvement of F-actin in *Uromyces* differentiation: the effects of cytochalasin E and phalloidin. Protoplasma **135**:88–101.
285. **Tucker, J. B.** 1984. Spatial organization of microtubule-organizing centers and microtubules. J. Cell Biol. **99**:55S–62S.
286. **Turian, G., T. C. Ton-That, and R. O. Perez.** 1985. Acid tip linear growth in fungi: requirements for H^+/Ca^{2+} inverse gradients and cytoskeleton integrity. Bot. Helv. **95**:311–322.
287. **Turing, A. M.** 1952. The chemical basis of morphogenesis. Philos. Trans. R. Soc. London Ser. B **237**:37–72.
288. **Ueda, K., and T. Noguchi.** 1988. Microfilament bundles of F-actin and cytomorphogenesis in the green alga *Micrasterias crux-melitensis*. Eur. J. Cell Biol. **46**:61–67.
289. **Uhlig, G.** 1960. Entwicklungsphysiologische Untersuchungen zur Morphogenese von *Stentor coeruleus*. Arch. Protistenkd. **105**:1–109.
290. **Vale, R. D.** 1987. Intracellular transport using microtubule-based motors. Annu. Rev. Cell Biol. **3**:347–378.
291. **Vermeulen, C. A., and J. G. H. Wessels.** 1984. Ultrastructural differences between wall apices of growing and non-growing hyphae of *Schizophyllum commune*. Protoplasma **120**:123–131.
292. **Vreeland, V., and W. M. Laetsch.** 1988. Role of alginate self-associating subunits in the assembly of *Fucus* embryo cell walls, p. 77–96. *In* J. E. Varner (ed.), Self-assembling architecture. Alan R. Liss, Inc., New York.
293. **Waddington, C. H.** 1957. The strategy of the genes. Allen & Unwin, Publishers Ltd., London.
294. **Walsby, A. E.** 1980. A square bacterium. Nature (London) **283**:69–71.
295. **Walworth, N. C., and P. N. Novick.** 1987. Purification and characterization of secretory vesicles from yeast. J. Cell Biol. **105**:163–174.
296. **Webster, G., and B. Goodwin.** 1981. History and structure in biology. Perspect. Biol. Med. **25**:39–62.
297. **Weidel, W., and H. Pelzer.** 1964. Bag-shaped macromol-

ecules—a new outlook on bacterial cell walls. Adv. Enzymol. **26**:193–232.
298. **Wessels, J. G. H.** 1986. Cell wall synthesis in apical hyphal growth. Int. Rev. Cytol. **104**:37–79.
299. **Wheals, A. E.** 1987. Biology of the cell cycle in yeasts, p. 283–340. *In* A. M. Rose and J. S. Harrison (ed.), The yeasts, vol. 1. Academic Press, Inc., New York.
300. **Willison, J. H. M.** 1981. Secretion of cell wall material in higher plants. Encycl. Plant Physiol. New Ser. **13B**:513–541.
301. **Willison, J. H. M., and R. M. Brown, Jr.** 1978. Cell wall structure and deposition in *Glaucocystis*. J. Cell Biol. **77**:103–119.
302. **Willison, J. H. M., and R. M. Brown, Jr.** 1978. A model for the pattern of deposition of microfibrils in the cell wall of *Glaucocystis*. Planta **141**:51–58.
303. **Woldringh, C. L., P. Huls, E. Pas, and G. J. Brakenhoff.** 1987. Topography of peptidoglycan synthesis during elongation and polar cap formation in a cell division mutant of *Escherichia coli* MC4100. J. Gen. Microbiol. **133**:575–586.
304. **Wood, B.** 1979. Bacteriophage T4 assembly and the morphogenesis of subcellular structure. Harvey Lect. **73**:203–223.
305. **Youatt, J., N. A. R. Gow, and G. W. Gooday.** 1988. Bioelectric and biosynthetic aspects of cell polarity in *Allomyces macrogynus*. Protoplasma **146**:118–126.