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Perspectives

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ROLAND THAXTER's Legacy and the Origins of Multicellular Development

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ROLAND THAXTER published a bombshell in December, 1892. He reported that *Chondromyces crocatus*, before then considered an imperfect fungus because of its complex fruiting body, was actually a bacterium (Figure 1). THAXTER had discovered the unicellular vegetative stage of *C. crocatus*; the cells he found were relatively short and they divided by binary fission. *C. crocatus* was, he concluded, a "communal bacterium." THAXTER described the locomotion, swarming, aggregation and process of fruiting body formation of *C. crocatus* and its relatives, which are collectively called myxobacteria, with an accuracy that has survived 100 years of scrutiny. He recognized the behavioral similarity to the myxomycetes and the cellular slime molds, drawing attention in all three to the transition from single cells to an integrated multicellular state. He described the behavior of myxobacteria in fructification in terms of a "course of development" because it was "a definitely recurring aggregation of individuals capable of concerted action toward a definite end" (THAXTER 1892). This essay will emphasize some implications of THAXTER's demonstrations, often apparently unrecognized.

The striking similarities to cellular slime mold development probably led JOHN TYLER BONNER and KENNETH B. RAPER, 50 years after THAXTER's discovery, to take independent forays into myxobacterial development. RAPER, an eminent mycologist, had in fact discovered *Dictyostelium discoidium*, recognizing it as a superb subject for the study of morphogenesis, cellular differentiation and intercellular communication. BONNER was fascinated by morphogenesis and sought unifying principles behind the bewildering diversity (BONNER 1952, 1974). Both RAPER and BONNER seemed to be intrigued by the unusual example of morphogenetic movements exhibited by the myxobacteria as they formed fruiting bodies. RAPER saw "examples of interdependent cellular behavior that involve purposeful orientation, morphogenetic movements, intercellular integration and finally coordinated differentiation that are in some ways comparable to higher forms" (QUINLAN and RAPER 1965). Both BONNER and RAPER sought the factors in *C. crocatus* that coordinated and guided the morphogenetic cell movements, noting that individual myxobacterial swarm cells retained their physical individuality throughout the process of cooperative morphogenesis, and in that respect differed from the myxomycetes but resembled the cellular slime molds.

FIGURE 1.—*Chondromyces crocatus* fruiting body. Photograph by HANS REICHENBACH, GBF, Braunschweig.

Their search was extended by HANS KUHLWEIN and his students, particularly REICHENBACH, who prepared a series of time-lapse films of the behavior of different types of myxobacteria (REICHENBACH, HEUNERT and KUCZKA 1965a,b,c,d; REICHENBACH, GALLE and HEUNERT 1976). Using time-lapse photography to condense the roughly day-long process of fruiting body development into a few minutes of running time, as BONNER had done for *D. discoideum*, brought the morphogenesis into a time scale more suggestive and intriguing to the human psyche. The myxobacterial movies showed the simplest fruiting bodies to be mounds of myxospores covered by slime, while the more complex fruiting body structures enclosed myxospores within acellular skins of slime that either rested directly on the substratum or were raised on slime stalks. REICHENBACH (1962) concluded that the formation of fruiting bodies generally passed through several stages: vegetative growth of a multicellular swarm, induction by starvation to begin development, cell accumulation, rearrangement of cells within the originally undifferentiated mass (including production of slime stalks or sporangiole walls) and, finally, myxospore formation. REICHENBACH (1965) also discovered that the ripples noted in myxobacterial swarms were traveling waves generated by many myxobacteria during the aggregation phase.

Work with dispersed (*i.e.*, non-clumping) strains of *Myxococcus xanthus* enabled DWORKIN, ROSENBERG and their students to study this bacterium's nutrition and metabolism, prerequisites for understanding the role of starvation in the induction phase of fruiting body development (summarized by DWORKIN 1984). Genetic studies of mutants defective in fruiting body development became possible through the isolation of transducing myxophages (CAMPOS and ZUSMAN 1975; MARTIN *et al.* 1978), the introduction of transposons from *Escherichia coli* into *M. xanthus* (KUNER and KAISER 1981) and the infusion of gene cloning techniques (GILL and SHIMKETS 1993).

THAXTER's discovery called attention to the transition from single cells to an integrated multicellular unit. There is general agreement that this step has been taken many times in the course of organic evolution. For example, the sponges probably arose from solitary cells separately from all other animals, and the seed plants, the fungi, and the algae all gained their multicellular condition independently (WHITTAKER 1969). Comparing these independent experiments of nature should provide insight into the general attributes of multicellular life.

Myxobacteria, which belong to the δ subgroup of purple bacteria, are a well defined and unique experiment in multicellularity. All myxobacteria construct multicellular fruiting bodies (LUDWIG *et al.* 1983), which is to say that no aerobic gliding bacterial species are known which form spores, have a high G·C content in their DNA, but do not build fruiting bodies, even though gliding bacteria have been systematically examined (REICHENBACH *et al.* 1988). That all the myxobacteria arose from the same ancestor within the δ subgroup of purple bacteria is supported by an extensive set of characters they hold in common: swarming behavior, closely related 16S ribosomal RNA sequences (LUDWIG *et al.* 1983; WOESE 1987; SHIMKETS 1993), high (66–72) mole % G·C content in their DNA (MANDEL and LEADBETTER 1965; MCCURDY and WOLF 1967; BEHRENS, FLOSSDORF and REICHENBACH 1976) and a set of notable chemosystematic markers (REICHENBACH and DWORKIN 1981). None of the other members of the δ subgroup of purple bacteria form fruiting bodies or spores; in addition to the myxobacteria, this phylogenetic subgroup includes the bdellovibrios and the mesophilic sulfate-reducing bacteria (WOESE 1987; STACKEBRANDT 1992; WIDDEL and BAK 1992).

In contrast to the monophyletic origin of myxobacteria, cell aggregation of eukaryotes leading to fruiting bodies appears, on cytostructural grounds, to have evolved several times among the cellular slime molds (OLIVE 1975). BONNER (1982) has argued that there is likely to have been an independent origin from single-celled amoebae for each group because of unique aggregation attractants; he distinguishes at least eight different attractants.

No matter how many independent events there were among the cellular slime molds, it is clear that the changes from single to multicellular organism were taken independently from the myxobacteria. To date there has been no report of lateral gene transfer between myxobacteria and the slime molds. The cell biology of the slime molds and the myxobacteria are very different, as THAXTER first discovered. The former are eukaryotic amoebae with a flexible cell membrane and a well defined cytoskeleton. We now recognize that the latter are rigid-walled, rod-shaped, Gram-negative procaryotic cells that lack the structural anatomic features of a cytoskeleton. Molecular studies of small ribosomal rRNA sequences as well as physiological and morphological studies show that the myxobacteria arose among the purple sulfur eubacteria, while the cellular slime molds arose among the protists. The two groups are thus separated by a wide evolutionary gap (Figure 2) (WOESE 1987). Features common to these two types of microorganisms promise insight into basic biological attributes of multicellular development. The width of the phylogenetic gap decreases the effects of chance evolutionary "tinkering" (JACOB 1982), the accidents of mutational history. The point is that features shared by different organisms will be the more robust and functionally informative the less the organisms share a common descent.

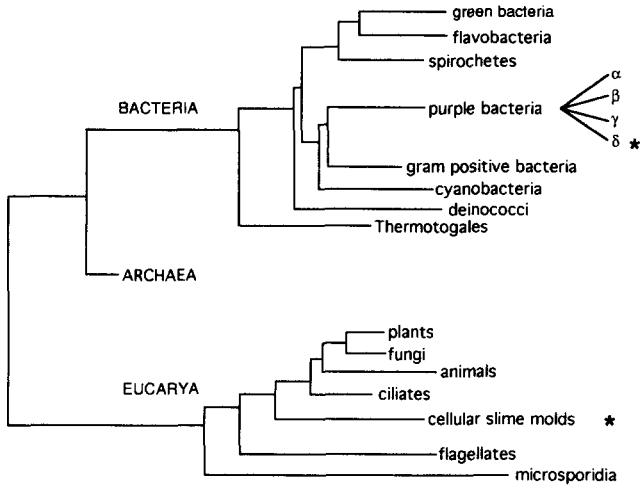

FIGURE 2.—Phylogeny of myxobacteria (* in δ purple bacteria) and the cellular slime molds (* in Eucarya). Adapted from WOESE (1992) and STACKEBRANDT (1992).

Common qualities: As understood today, cellular slime molds (exemplified by *D. discoideum*) and myxobacteria have these similarities:

• Fruiting body development is asexual. The growing cells are haploid and the fruiting bodies are filled with haploid spores. As expected, the genome sizes are different. The sizes of several myxobacterial genomes have been determined by pulsed-field gel electrophoresis and include the closed circular genome of *M. xanthus* at 9.4 Mb (CHEN *et al.* 1991), *Stigmatella aurantiaca* at 9.2–9.9 Mb and *Stigmatella erecta* at 9.7–10 Mb (NEUMAN, POSPIECH and SCHAIRER 1992). The genome of *D. discoideum* consists of six (possibly seven) (DARCY *et al.* 1993) linkage groups that total 40 Mb of DNA (KUSPA *et al.* 1992).

• Both move on surfaces, neither can swim. Slime mold cells translocate by amoeboid movement that involves dynamic changes in their cytoskeleton. Myxobacteria move by gliding on surfaces without apparent rotation or change in cell shape; they lack flagella or any other obvious organelles of movement. The mechanism of gliding is currently unexplained even though many bacteria can do so (MCBRIDE, HARTZELL and ZUSMAN 1993).

• Cell division is separate from development. Cells grow and divide when food is abundant. Starvation stops growth and induces development. Amino acid starvation appears to be a prime factor in the induction of development. Addition of a complete set of the amino acids required for *D. discoideum* growth delays the initiation of development (MARIN 1976). In *M. xanthus*, limitation for any of the amino acids induces fruiting body development (MANOIL and KAISER 1980).

• The program of morphological change and development begins with recognition of starvation, aggregation of preexisting cells, arrangement of cells within the aggregate in a species-specific pattern, then differentiation of individual cells into spores.

• Both pass several chemical signals between their cells. In *D. discoideum*, the signals include cAMP and DIF (WILLIAMS and JERMYN 1991). cAMP seems not to be significant in myxobacteria in the way it is in *D. discoideum*. Instead, in *M. xanthus* a mixture of eight amino acids (called A-factor) is a signal early in development, and a 17-kDa surface protein known as C-factor is a signal later during the aggregation and sporulation phases (KIM, KAISER and KUSPA 1992; KAISER and KROOS 1993).

• The cells respond to signal reception by expressing new batteries of genes. In *D. discoideum* there are cAMP-dependent and DIF-dependent genes (DEVREOTES 1989; WILLIAMS and JERMYN 1991). In *M. xanthus* there are A-factor-dependent and C-factor-dependent genes (reviewed in KROOS, KUSPA and KAISER 1986; KAISER and KROOS 1993).

• During aggregation, cells are swept into a fruiting body from neighboring regions. The mechanisms of sweeping are similar in several ways, including the generation of traveling waves. In *D. discoideum*, the traveling waves are generated by pulses of cAMP that emanate from an aggregation center (TOMCHIK and DEVREOTES 1981), while in *M. xanthus* the traveling waves are local accumulations of cells, which depend for their formation on C-factor (SHIMKETS and KAISER 1982).

• Stalk formation differs. In *C. crocatus*, cells migrate upward inside a tube of "slime" (apparently mostly polysaccharide), depositing more slime at the top and elongating the stalk as they pass into the cell mass resting on the top (QUINLAN and RAPER 1965; REICHENBACH, HEUNERT and KUCZKA 1965b; THAXTER 1892). In *D. discoideum*, prestalk cells move up the outside of the preexisting cellulose tube (RAPER and FENNELL 1952). As these cells migrate over the lip of the tube, they deposit more cellulose, elongating the tube (WILLIAMS and JERMYN 1991). Though Dictyostelium stalks contain specialized, differentiated stalk cells, the stalk of another dictyostelid, Acytostelium, is acellular like those of the myxobacteria (RAPER 1984).

• The fruiting bodies of the cellular slime molds and the myxobacteria cover the same morphological range of spheres and cylinders ordered and combined in various ways (REICHENBACH and DWORKIN 1981; BONNER 1982; RAPER 1984). The fruiting bodies of *D. discoideum* and *M. xanthus* each contain up to 100,000 cells.

Selective forces: Slime molds and myxobacteria are found in the same habitats, and are often isolated from the same soil samples by enrichment culture (SINGH 1947). Both feed on bacteria in the soil. However, the slime molds ingest bacteria by endocytosis

while the myxobacteria secrete their digestive enzymes, then take up the products of extracellular digestion (SUDO and DWORKIN 1972; LOOMIS 1975). The observed evolutionary convergence of these two disparate groups is presumably a consequence of natural selection in a common habitat. Both organisms are less insulated from their environment than flowering plants or higher animals and are haploid, so that natural selection can constantly play a role in shaping their development. What might the selective forces have been? Several have been suggested:

Social feeding: The selective advantage for the evolution of multicellularity in myxobacteria is likely to have been cooperative feeding. Myxobacteria feed on particulate organic matter in the soil by means of extracellular bacteriolytic, proteolytic, cellulolytic and other digestive enzymes (REICHENBACH 1984). Based on their secretion of lytic enzymes, DWORKIN (1973) proposed that myxobacteria feed like "packs of microbial wolves." ROSENBERG, KELLER and DWORKIN (1977) measured the growth rate when the only source of carbon and nitrogen for *M. xanthus* cells in liquid culture was the polymeric substrate casein, so that proteolysis was required for growth. The growth rate increased twofold as the cell density was raised above 10^4 cells/ml. When intact casein was replaced with enzymatically hydrolyzed casein, the cells grew at the more rapid rate independently of cell density. Evidently, extracellular digestion of protein is enhanced by cooperation between cells.

A swarm may be the unit of efficient cooperative feeding. REICHENBACH has shown that a single germinating sporangiole of a *Chondromyces apiculatus* fruiting body forms an active swarm that behaves much like a swarm of bees (BONNER 1952; QUINLAN and RAPER 1965; KUHLWEIN and REICHENBACH 1968). Forming a multicellular fruiting body ensures that, when conditions favorable for growth are restored, the myxospores can germinate and the new phase of growth can start as a preformed community of efficiently feeding cells. The success of the myxobacterial design is evident in their distribution; they are common inhabitants of soils drawn from all over the world regardless of climate (REICHENBACH 1984).

Dispersal: BONNER (1982) has suggested that the cellular slime molds evolved from solitary soil amoebae to multicellular forms under selection for an efficient means of dispersal. He argues that " . . . selection pressure for fruiting bodies in small organisms, be they amoebae, hyphae, plasmodia, swarms of bacteria, or even ciliate protozoa (Olive, 1978), must be enormous, and the scale of convergent evolution vast" (BONNER 1982). Rain water, wind, or movement of small soil invertebrates could disperse fruiting bodies. Stalks, multiple sorogens and sporangioles might be explained this way. Mites have been observed to carry myxobacterial fruiting bodies (REICHENBACH 1984).

Survival under marginal conditions in a fluctuating environment: STEPHEN BARCLAY (University of Wisconsin) has pointed out to me that soil amoebae often find themselves in nutritional conditions that are marginal, neither rich enough for rapid growth nor poor enough to trigger efficiently the encystment of individual cells. Marginal conditions may encourage slow growth that would leave cells incapable of completing a final mitotic cycle, with death as the consequence. One strategy to cope with marginal conditions may be to aggregate and construct a fruiting body, withdrawing cells from the ambiguous environment and allowing them to continue starvation-induced development.

Increased reliability of perceiving starvation: The A-factor of *M. xanthus*, which is a mixture of eight amino acids, is a cell-density signal (KUSPA, PLAMANN and KAISER 1992b). The amount of A-factor released is proportional to the number of cells per unit volume, and a certain minimum quantity of A-factor is required to continue development. Thus, the A-signal ensures a cell density sufficient to complete a proper fruiting body (KUSPA, PLAMANN and KAISER 1992a). A-factor, which is released about 2 hr after the beginning of starvation, is also a way for cells to vote their individual assessment of nutritional conditions. Because new proteins must be made during aggregation and sporulation, some protein synthetic capacity must be retained, and the cells must begin to aggregate before they have exhausted all their sources of amino acids and energy. To initiate development or to grow slowly is an important choice on which long-term survival depends. An optimal choice is one that anticipates the future. When this decision is jointly made by a population of cells, it is likely to be more reliable than that made by one cell. A protein, CMF, secreted by starved Dictyostelium cells plays a similar role (JAIN *et al.* 1992).

These forces, and others, can be discriminated by experiment because myxobacteria and cellular slime molds are microbes that can be conveniently handled in large numbers. Moreover, the set of molecular genetic tools currently available in both organisms includes physical/genetic maps, tools for random insertional mutagenesis to identify genes and to provide genetic markers, methods for homologous gene replacements including construction of null alleles, and the capacity to clone genes in *E. coli* or *Saccharomyces cerevisiae* for manipulation before returning them to their proper host for expression; see GILL and SHIMKETS (1993) for myxobacteria and KUSPA and LOOMIS (1992) for Dictyostelium.

The selective forces, whichever may have been effective, will have acted within a set of biological and physical constraints. Unexpected convergence on related body plans and systems for control of multicel-

lular development by cellular slime molds and myxobacteria suggests that the structural differences between eukaryotic and procaryotic cells may in fact be secondary to deeper similarities. We are aware of many metabolic similarities. We are becoming aware that there are also rules for the folding, structuring and assembly of proteins. Perhaps there are also rules about the way development and morphogenesis are regulated for reliability in relatively harsh or changing environments. Use of cellular oscillators revealed by traveling waves and the expression of genes in batteries, triggered by different extracellular signals, are cases in point. Part of ROLAND THAXTER's legacy is the notion that comparisons of eukaryotes and prokaryotes may give insights that would come from neither examined alone.

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