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Recent Advances in the Social and Developmental Biology of the Myxobacteria†

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† This review is dedicated to Richard M. Behmlander, whose premature death terminated his brief love affair with the myxobacteria.

INTRODUCTION

The myxobacteria are a group of gram-negative soil bacteria characterized by social behavior and a complex developmental cycle. They were described in 1892 by Roland Thaxter (246), who was the first to recognize them as bacteria. While many of the myxobacteria have now been microbiologically domesticated (190), most research over the past two decades has focused on *Myxococcus xanthus*, which now can be subjected to an arsenal of molecular manipulations. This has made it possible to examine its social behavior and complex development at a cellular and molecular level (50) that is more convenient than is usually the case with other social organisms.

The myxobacteria have been reviewed frequently (190, 218, 223, 256) and have been the subject of a recent book (50); nevertheless, it is difficult to resist the temptation to display here some of Hans Reichenbach's beautiful photographs of fruiting bodies (Fig. 1). For readers unfamiliar with the group, a cartoon describing their life cycle is presented in Fig. 2.

For a more detailed and graphic description of myxobacterial fruiting bodies, cellular and colonial morphology, ecology, and taxonomy, the reader is referred to the recent chapter by Reichenbach (188).

I hope that this review will be useful to at least two groups of microbiologists. First are those who do not work on the myxobacteria but need to know about them, either for their own amusement or so as to be able to teach the subject more conveniently and effectively. Second, while the book *Myxobacteria II* (50) is an extensive and detailed description of contemporary work on the myxobacteria, those chapters are now 3 years old, and work in this field has been moving rather quickly. Thus, the review is also intended for those myxobacteriologists who need the more recent work sifted, summarized, and evaluated.

MOLECULAR PHYLOGENY

There are 12 genera and approximately 40 species of myxobacteria that have been reliably described (188). It has long been obvious that these myxobacteria could be divided into two groups, distinguished from each other on the basis of morphological and physiological parameters. These were accorded subordinal rank as the *Cystobacterineae* and the *Sorangineae* within the order *Myxobacterales* (now *Myxococcales*) (189). This arrangement was essentially verified by Ludwig et al. (140) on the basis of 16S RNA oligonucleotide catalogs. Shimkets and Woese (227) carried out a more detailed analysis of the primary sequences and secondary structures of the 16S RNAs of 12 different myxobacteria representing all of the cultivated genera. Their results essentially confirmed the prior groupings; however, they found that *Nannocystis exedens* represented a third, distinct, rapidly evolving lineage.

The myxobacteria are a phylogenetically coherent order, allied surprisingly alongside the genera *Bdellovibrio* and *Desulfovibrio* within the δ subdivision of the Proteobacteria. Figure 3 illustrates the phylogenetic tree of the myxobacteria and their relationship to *Bdellovibrio* species and the sulfate-reducing bacteria.

SIGNALING

McVittie et al. (161) found that a pair of developmentally defective mutants of *M. xanthus* could complete development if they were mixed with each other. They recognized that the complementation was not genetic and referred to it as a "synergistic interaction"; they suggested that the material being exchanged was involved in developmental chemotaxis.

Hagen et al. (72) later isolated a large number of developmentally defective mutants and showed that they fell into four sets. The sets were distinguished from each other by the fact that development by members of each set could be extracellularly complemented by admixture either with wild-type cells or with a member of another set. They concluded that wild-type cells exchange at least four developmental signals and that each set of mutants is defective in its ability to produce one of those signals. This paper was an important milestone in the study of myxobacterial development, for it led to the recognition that development depended on the exchange of extracellular signals. We now know that there are at least five such signals, referred to as Asg, Bsg, Csg, Dsg, and Esg (42, 99). Two of these signals, A and C, have been chemically characterized and have been shown to play roles in early aggregation and in fruiting body morphogenesis.

A Signal

Approximately half of the mutants isolated by Hagen et al. (72) are defective in A signaling. Thirteen of these mutations (*asg* mutations) were shown to fall into three loci, *asgA*, *asgB*, and *asgC* (123), which act early in development, at about 1 to 2 h, during the preaggregation stage (152).

Primary A factor (i.e., the A signal itself) is a mixture of amino acids and peptides (124, 125) that is generated in turn by a mixture of proteases (179). Thus, development by the *asg* mutants can be restored by the addition of the appropriate mixtures of amino acids and peptides (125). The substrate for the proteases is unknown.

It now seems clear that starvation is the trigger that sets in motion the events which result in the excretion of the diffusible A signal. The external concentration of A signal then serves as a parameter of cell numbers; this ensures that the subsequent signal to aggregate, as a prelude to fruiting body formation, occurs only when the population has reached a sufficient cell density (99). A signal thus falls into the category of quorum sensing signals, originally described for the luminescent bacteria but now shown to extend to a variety of other organisms (43).

As part of the overall attempt to understand the biosynthesis of A factor and the regulation of A signaling, Plamann et al. (180) have cloned and sequenced the *asgA* gene and found it to contain two domains, one homologous to the transmitter domain of histidine protein kinases and the other homologous to the receiver domain of response regulators. They suggest that the *asgA* gene codes for some aspect of a signal transduction pathway whose function is to sense starvation and to respond by the eventual production of the extracellular A signal.

asgB, on the other hand, codes for a putative DNA-binding protein with a helix-turn-helix motif near the C terminus. Part of the sequence of *asgB* is similar to that segment of major sigma factors that recognizes and interacts with the −35 sequences of promoters. However, *asgB* contains no region similar to the highly conserved region of sigma factors that interact with core RNA polymerase. The authors have concluded that while AsgB is not a sigma factor, it may act as a transcription factor which recognizes a specific sequence of DNA that is part of the region coding for the extracellular protease that generates the A signal (181).

A preliminary report from Plamann's laboratory (34) has shown that the *asgC* gene probably codes for the major sigma factor of *M. xanthus* and is part of a cluster of genes comprising a macromolecular synthesis operon.

Kaplan's laboratory has been studying the mechanism of A signaling by examining the regulation of an A-signal-depen-

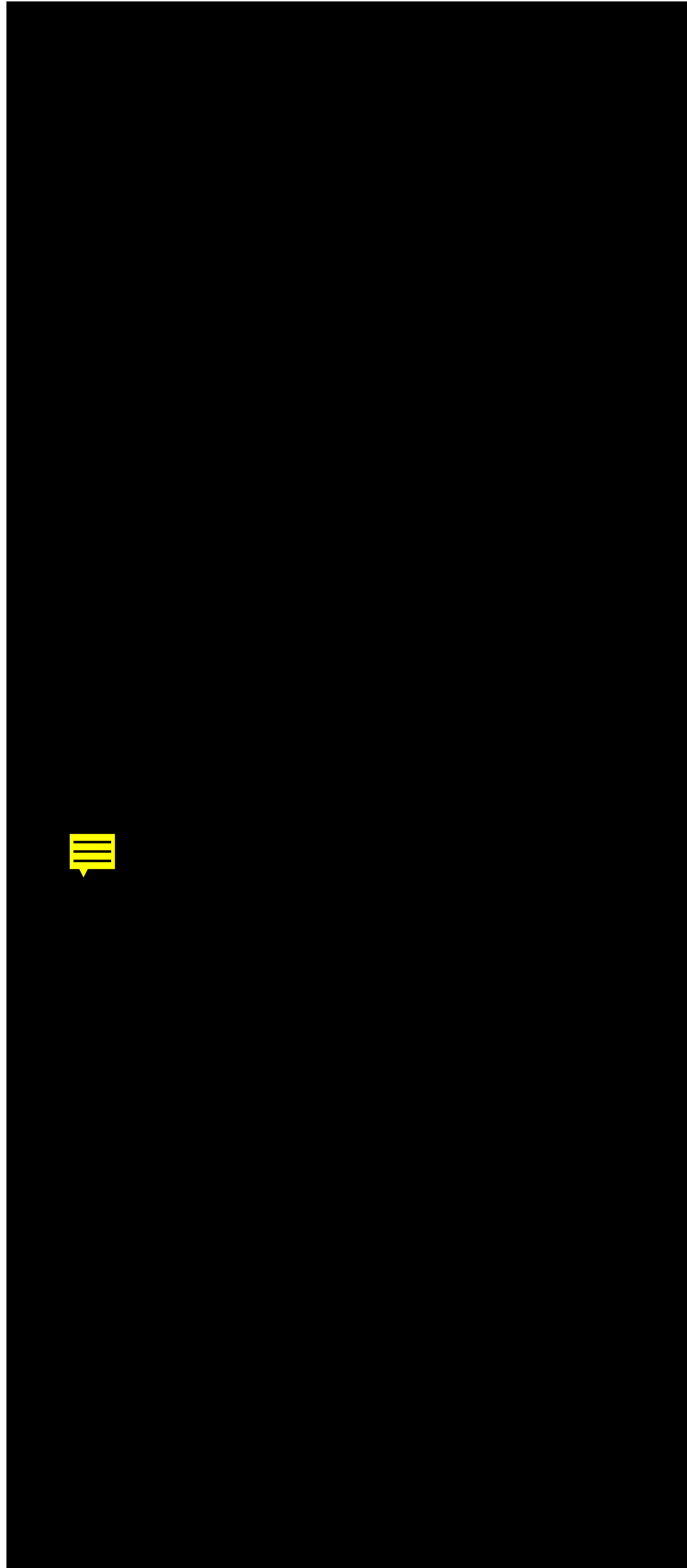

FIG. 1. Fruiting bodies. (A) *M. fulvus*. Phase contrast. Bar, 50 μm. Courtesy of Hans Reichenbach. (B) *S. aurantiaca*. Phase contrast. Fruiting body is about 150 μm tall. Courtesy of Hans Reichenbach. (C) *Chondromyces crocatus*. Slide mount, phase contrast. Bar, 100 μm. Courtesy of Hans Reichenbach.

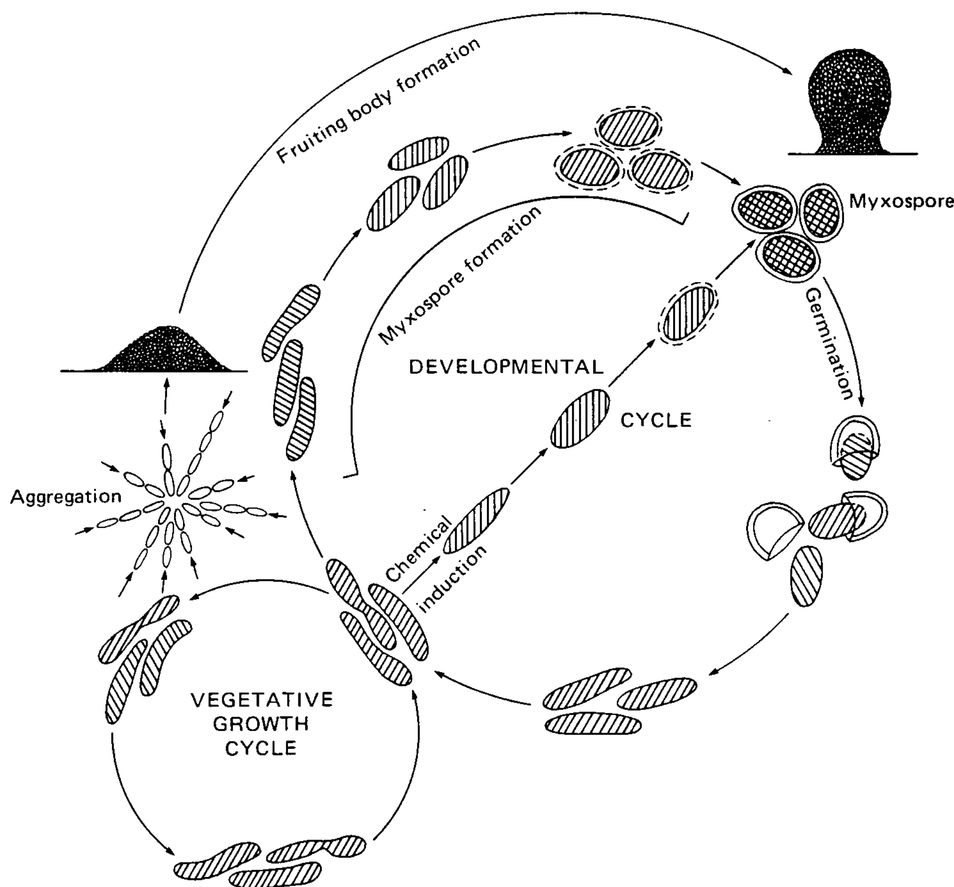

FIG. 2. Diagram of the life cycle of *M. xanthus*. The fruiting body is not drawn to scale but is a few hundredths of a millimeter in diameter. The vegetative cells are about 5 to 7 by 0.7 μm. Reprinted from reference 46 with permission of the publisher.

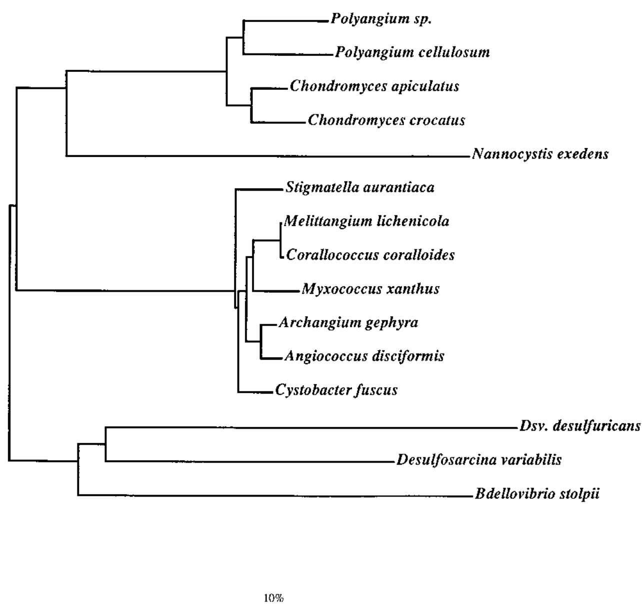

FIG. 3. Phylogenetic tree for the myxobacteria. The tree was derived from evolutionary distances of the 16S rRNA molecules. Dsv., Desulfovibrio. Reprinted from reference 227 with permission of the publisher.

dent gene, *4521* (102). They have isolated a series of second-site suppressors of the *asgB480* mutation which restore the ability to express A-dependent β-galactosidase from the *4521* reporter gene. Expression of gene *4521*, which is only partially dependent on A signal, is regulated by an area upstream of the transcription start site of *4521*, similar in sequence to σ^{54}. This has been confirmed by Keseler and Kaiser (105), who have shown that the start site for *4521* transcription has substantial sequence similarity to the σ^{54} family of promoters. Mutational analysis excluded the possibility of a similarity with the σ^{70}-like promoters. They have further proposed that the σ^{54} requires an upstream activator. This has been confirmed by Gulati et al. (71), who have shown that the regulatory region of *4521* extends 125 to 146 bp upstream of the start site identified by Keseler and Kaiser (105) and that this region of the DNA can be mobility shifted by a crude cell extract. Kaplan et al. (102) propose that there are at least two pathways controlling *4521* gene expression, one of which senses cell density and is A signal dependent, and a second which is an additional checkpoint for nutrient levels and is A signal independent. As has been shown to be the case for sporulation in *Bacillus subtilis*, which must interpret and integrate environmental, nutritional, and cell cycle signals (176), there must be analogous regulatory networks in *M. xanthus* that allow the cell to make the appropriate developmental decisions after integrating a variety of environmental signals. The function of the *4521* gene itself is unknown.

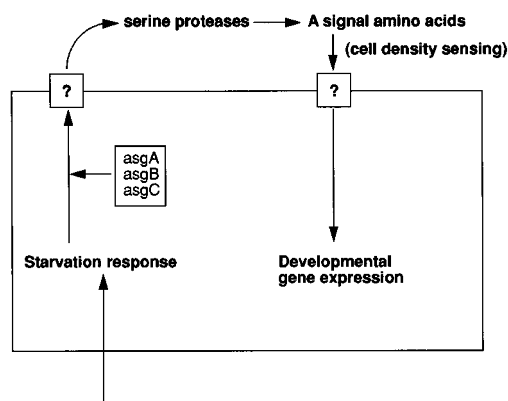

FIG. 4. Diagram of the A signaling circuit. See text for details.

Thus, the model that seems to be emerging for the role of the A signal is as follows (Fig. 4). The starvation signal (see Initiation Signal section) is transduced by the action of AsgA, a response regulator, and histidine kinase, which functions within a phosphorelay system; by AsgB, a DNA-binding protein that acts as a transcription factor; and by AsgC, a major sigma factor. This results in the release of proteases, which generate the A signal (amino acids). The concentration of A signal then acts as a parameter of cell density.

B Signal

A subset of the *bsg* mutations have been mapped, and these fall into a single locus, *bsgA* (65). Expression of nearly all of the developmental markers examined is either reduced or abolished by the *bsgA* mutations, suggesting that, like the A signal, the B signal acts early in development (118).

Gill et al. (64) have cloned and sequenced the *bsgA* gene; it has substantial homology to the *lon* genes of *Escherichia coli* and *Bacillus brevis*. They have partially purified the gene product, a 90.4-kDa protein, which copurifies with an intracellular ATP-dependent protease. They postulate that the *bsgA* phenotype is the result of a defect in intracellular proteolysis and that the activity may function normally to destroy a repressor of development.

Tojo et al. (250) took a different approach to discover the same gene product. They cloned and sequenced a developmental gene mutated by a transposon insertion and found it to correspond to the *lon* gene of *E. coli*, which codes for the 90-kDa protease La. Interestingly, like Kroos and Kaiser previously (118), they were unable to demonstrate extracellular complementation of the *lonD* (*bsgA*) mutant by wild-type cells. However, successful complementation of the *bsgA* mutant obviously depends on the allele and the specific conditions used for complementation.

C Signal

Of all the signals, the C signal has been the most intensively studied. The work stems primarily from two laboratories, those of Dale Kaiser and Larry Shimkets.

All of the *csg* mutations map to one gene, *csgA* (221). The major question is what the specific function of the C signal is. C signal is later-acting than either the B or the A signal, exerting its effect at about 6 to 7 h into development. Under stringent conditions, the *csg* mutants are completely blocked in development and, interestingly, also in rippling.

C factor has been isolated and partially characterized. The gene product of *csgA* has been deduced to be a 17-kDa protein, based on the sequence of the gene (73, 226). There is now some ambivalence about the molecular mass determination; depending on where the start site of the gene is located, the C signal may be as large as 20 kDa (220). Shimkets and Rafiee (226) expressed the *csgA* gene in *E. coli*, developed polyclonal antibodies against it, and showed that those antibodies blocked development unless they were first neutralized with the C factor protein. They also used the antibodies and immunogold labeling to show that most of the *csgA* gene product was located in the extracellular matrix of developing *M. xanthus.*

Kim and Kaiser (107) took a more direct approach and isolated C factor from developing cells of *M. xanthus*. They determined that the partially purified C factor was a dimer of 17-kDa subunits (based on electrophoresis), that it reacted with the Shimkets-Rafiee anti-C factor antibody, and that it could rescue the development of *csgA* cells. They proposed that C factor was acting as a morphogenetic paracrine signal (108).

C factor has been referred to as a membrane-associated protein (99), based solely on the fact that it was found in the same centrifugal fraction as the membrane components (108). However, that fraction also contained components of the extracellular matrix of *M. xanthus*, including the extracellular matrix fibrils (8); furthermore, the immunogold localization studies of Shimkets and Rafiee (226) clearly demonstrated that the C factor is localized in the extracellular matrix. Li and Shimkets (136) examined the possibility that the presence of C factor in the extracellular matrix suggests that C factor is associated with the extracellular fibrils and that the fibrils could thus play some sort of role in the exchange of the C signal. This possibility was bolstered by the earlier finding that complementation of development of the *csgA* mutant by wild-type cells requires close contact between the cells, i.e., complementation is prevented if the cells are separated by a membrane filter (108). Li and Shimkets (136) did a series of complementation experiments which showed that the fibrilless *dsp* mutant is able to complement the *csgA* mutant. They concluded from these experiments that "the fibrils are not necessary for cell-to-cell transmission or perception of CsgA." Their results however, only excluded the possibility that the fibrils were involved in the presentation of the signal. It is possible, and perhaps even likely, that the fibrils play a role in the events leading to the perception of the signal by the recipient cell. For example, the fibrils may be necessary for trapping and therefore accumulating not only the C factor but all of the exchanged extracellular signals. It will thus be interesting to examine whether fibrilless *a*, *b*, *c*, *d*, or *esg* mutants can be complemented.

Understanding of the mechanism of exchange of the C signal was further complicated by the finding that nonmotile cells (i.e., *mgl* mutants) behave as if they are defective in the C signal (120). That is, the nonmotile cells fail to ripple, aggregate, or sporulate, and interference with expression of the C-dependent *lacZ* fusions in *mgl* is the same as in the *csgA* mutants (120). Furthermore, *mgl* mutants are unable to complement *cglA* despite the surprising finding that the *mgl* mutant produces normal amounts of the C signal. This led Kim and Kaiser (109) to suggest that motility by both partners is necessary for the proper exchange of the C signal. (One observation that has not been rationalized is the puzzling fact that the addition of purified C factor restores sporulation and gene expression in the *mgl* mutant despite the fact that *mgl* is able to produce its own C factor [109].)

The requirement for motility implied that it was necessary for the cells to be able to move into close physical contact in order to exchange the C signal effectively. To mimic this ar-

rangement, Kim and Kaiser (110) scratched narrow grooves in an agar surface and deposited *mgl* cells in these grooves. Under these conditions of close cell-to-cell packing, the *mgl* mutant showed normal sporulation and C-dependent gene expression. Recent experiments have revealed that end-to-end collisions between cells initiate C signaling and thus affect the rate of reversal of gliding which plays a role in developmental aggregation (233) and in rippling (210) (see Rippling and Aggregation sections).

There is evidence that C factor may also act as an extracellular developmental timer. Cells with the *csgA* mutation respond differently to different levels of added C factor, i.e., the addition of relatively low levels of C factor induces aggregation and early developmental gene expression, whereas higher levels are required to induce sporulation and the expression of late developmental genes (111). Furthermore, transcription of *csgA*, measured by the expression of a *lacZ* reporter, increases steadily during development (73). Finally, if the regulatory region of *csgA* is mutated by a series of successively larger deletions, there is a corresponding progressive decrease in the levels of C factor produced, which has different effects on the various aspects of development (135). These results have led to the idea that programmed increases in C factor concentration during development may control the sequence of developmental events, i.e., that C-factor coordinates both aggregation and sporulation (111, 135). (See Cell Density section for a more detailed description of these experiments.)

The C signal seems to be the last-acting of the five extracellular signals and eventually results in the expression of more than half of the developmentally regulated genes (118, 135).

Shimkets' laboratory has presented evidence that the *csg* gene product is a short-chain alcohol dehydrogenase (SCAD) which may be involved in generation of the C signal itself. Lee and Shimkets (130) isolated a series of suppressor mutants of *csgA* and showed that these *socA* cells are now able to undergo normal development as well as to carry out extracellular (nongenetic) complementation of myxosporulation by the *csgA* mutant. The *socA* locus contains three protein-coding domains, one of which (SocA1) shows 19 to 39% amino acid identity with the members of the SCAD family. They also demonstrated that the CsgA protein shows this homology (131) and on this basis suggested that both the *csgA* gene coding for the C factor itself and the *socA* suppressor are coding for similar and functionally interchangeable membrane-bound alcohol dehydrogenases. In a similar vein, Baker (6) had earlier shown a substantial sequence similarity between C factor and the catalytic domain of *E. coli* 3-ketoacyl-acyl carrier protein reductase as well as homologs such as human 17β-hydroxysteroid dehydrogenase. However, Baker concluded that the C factor is probably not acting as a SCAD enzyme in view of the fact that its sequence lacks the characteristic binding domain for the nucleotide cofactor. Since then, however, Lee and Shimkets (131) have extended the sequence of CsgA an additional 63 amino acid residues and have shown that it does indeed include the TGG motif near the N terminus of the protein which serves as the SCAD coenzyme-binding site. Recently, Lee et al. (132) have obtained evidence that strongly suggests that the CsgA protein contains an $NAD(P)^+$ binding site and that that binding pocket is necessary for CsgA activity. They have further shown that strains whose *csgA* alleles contain amino acid substitutions in the $NAD(P)^+$ binding pocket fail to develop. The evidence has mounted, in a convincing fashion, that the activity of CsgA as a developmental signal is related in some fashion to its proposed properties as a member of the SCAD family. Finally, while it has not yet been possible to determine a substrate for the dehydrogenase or even to demonstrate biochemically that C factor has actual dehydrogenase activity, Lee et al. (132) have shown that CsgA rescue of development in a *csgA* mutant was improved by the addition of NAD^+ or $NAD(P)^+$ and was inhibited by NADH or NADPH.

C factor, then, may have evolved from a family of eukaryotic enzymes that indirectly regulate hormone-mediated gene transcription by catalyzing the interconversion of active and inactive steroids.

The precise function of C factor as a signal that mediates cell-cell communication is still unclear. Neither the mechanism of transmission of the C signal between cells nor the mechanism of its transduction to a cellular response is understood. Is it indeed a functioning dehydrogenase enzyme, or is it an enzymatic fossil acting now as a paracrine hormone? What is clear, however, is that it is a cell-bound signal that plays a major role in controlling the activities of more than 50 genes involved in rippling, aggregation, sporulation, and motility (99), and it functions not only to regulate the temporal expression of a variety of social and developmental activities but also to monitor the close spatial interactions among the cells.

D Signal

The *dsg* mutants are unlike the other signaling mutants in that their development is only partially disrupted, i.e., sporulation is substantially reduced and aggregation is abnormal and delayed (30).

The *dsg* gene has been cloned and sequenced based on its ability to rescue a *dsg* mutant in partial diploid strains (30) and has been shown to bear 50% sequence homology to the translation initiation factor IF3 of *E. coli* (32). Furthermore, Kalman et al. (101) have shown that the Dsg protein does indeed act in *M. xanthus* analogously to the *E. coli* IF3 protein.

Cheng and Kaiser previously showed (30, 31) that while point mutations in *dsg* generated developmental deficiencies but had no effect on the viability of vegetatively growing cells, Tn*5* insertion mutations were lethal. This reflects the possibility that the point mutations still allow the production of slightly modified proteins which retain some function, while the transposon insertions result in null mutations.

The *dsg* gene product is probably not the signal itself—it is hard to imagine that a translation initiation factor is a signal—but rather signal production may be affected by a translational lesion.

To complicate the matter further, Rosenbluh and Rosenberg (201) have shown that mixtures of fatty acids comprising the autocide AMI rescue fruiting body formation and myxosporulation in the *dsg* mutant.

E Signal

The *esg* mutants were discovered in Downard's laboratory (42) as part of a study on regulation of the *tps* gene, which codes for the abundant spore protein S. Extracellular complementation results in the partial rescue of sporulation and in expression of the *tps* gene but does not rescue normal fruiting body formation. In addition, complementation does not occur when cells are mixed in liquid suspension; it requires cell-cell contact on the agar surface. The mutant is blocked rather early in development, at about 3 to 5 h, indicating that the E signal probably acts before the C signal.

The *esg* gene has been cloned, and an examination of its sequence reveals substantial homology with the E1 decarboxylase of the branched-chain keto-acid dehydrogenase (BCKAD) complex. Furthermore, development of the *esg* mutants could be rescued by the short branched-chain fatty acids isovalerate, methylbutyrate, and isobutyrate (249), the products of the

TABLE 1. The five developmental signals in *M. xanthus*

Signaling group	Signal	Reference(s)	Mutation(s)	Gene product(s)	Demonstrated	Inferred from homology	Reference(s)
A	Mixture of amino acids and peptides generated by a mixture of proteases	121, 124, 125	*asgA*	(i) Transmitter domain of histidine protein kinase		✔	180
				(ii) Receiver domain of response regulator		✔	180
			asgB	DNA-binding transcription factor		✔	181
			asgC	Sigma factor		✔	34
B	Unknown		*bsgA*	90.4-kDa ATP-dependent protease	✔	✔	64, 250
C	Paracrine hormone	108	*csgA*	Dimer of 17-kDa proteins	✔	✔	73, 107, 226
				Short-chain alcohol dehydrogenase		✔	131
D	Fatty acids?	201	*dsg*	Translation initiation	✔	✔	32, 101
E	Fatty acids	39	*esg*	E1 decarboxylase of the branched-chain keto-acid dehydrogenase		✔	249

BCKAD complex. This supports the idea that the *esg* mutants are defective in their ability to metabolize the branched-chain amino acids leucine, isoleucine, and valine via the BCKAD complex. Downard and Toal (39) have proposed that these short branched-chain fatty acids are the precursors in the biosynthesis of the long branched-chain fatty acids (e.g., i-15:O) that are incorporated into membrane phospholipids during vegetative growth and released as the actual E signal. Mueller and Dworkin (163) had earlier proposed that the fatty acids of the autocide AMI, which induce developmental autolysis, are released from phosphatidylethanolamine (AMV) by a developmentally activated phospholipase (see Glucosamine, Phospholipase, Glycerol, and Autocides section). Toal et al. have proposed that a similar developmental increase in membrane phospholipase activity results in the release of the long branched-chain fatty acids that act as the actual developmental signals (249). Bartholomeusz and Downard have, in fact, recently shown that i-15:O is effective in rescuing development and expression of the *tps* gene of the *esg* mutant (7).

Along with the C signal and its possible relationship to the various dehydrogenases (see above), the E signal may represent another example of the use by the myxobacteria of a highly conserved metabolic reaction or pathway for the purpose of cell-cell communication.

Summary of Signaling

The A signal seems to be the earliest of the five signals that is called into play. It is produced in response to a nutritional shiftdown and then functions as a diffusible monitor of cell density. If cell density is high enough, the next series of developmental events is permitted; if not, low-level growth continues until a cell density sufficient for development is achieved. In a sense, the C signal also functions to monitor cell density but in a different way. As a cell-bound signal, its exchange seems to depend on cell-cell contact and thus to control positioning of the densely packed cells as they aggregate and form fruiting bodies. The fact that E signal exchange also requires cell-cell contact suggests that, like the C signal, it is cell bound. This further suggests that it may play a role in the positioning maneuvers that are a prerequisite for aggregation and fruiting body formation. It is becoming clear, based on the work of Rosenbluh and Rosenberg (201), Mueller and Dworkin (163), and Toal et al. (249), that fatty acids play an important role in developmental signaling.

Table 1 lists the five signaling groups, the nature of the signals, and the nature of the gene products of the genes in which mutations define the signaling groups.

At present, the function of only the A signal in the overall process of aggregation and fruiting body development seems clear.

The principal experimental strategy for studying these five signaling groups has been to clone and sequence the mutated genes and then to deduce the function of the gene products based on sequence homologies. Only in the case of the C signal and possibly the E signal has this approach led to a useful clue as to the nature of the signal itself. In the case of the A, B, and D signals, the deduced gene products bear only an indirect relationship to the putative signal itself. In the case of the A signal, expression of a series of A-dependent Tn*5*-*lac* reporter genes was used as an assay in a successful and conventional biochemical search for the signal. In a similar fashion, it is clear that for the B, D, and possibly the E signals, the molecular search will have to go hand in hand with a biochemical one before the signals themselves are explicitly defined.

For a more detailed discussion of Asg and Csg, see the reviews by Kim and Kaiser (112) and by Kaiser and Kroos (99).

OTHER EXTRACELLULAR DEVELOPMENTAL FACTORS

Glucosamine, Phospholipase, Glycerol, and Autocides

Janssen and Dworkin (94) showed that developmental autolysis, sporulation, and fruiting body formation by one of the original group C mutants described by Hagen et al. (72) could be rescued by a polysaccharide-containing factor isolated from wild-type cells. The factor could be efficiently replaced by 2 to 3 mM mannosamine or 20 mM glucosamine. The possible involvement of glucosamine as a developmental signal re-emerged with the finding by Mueller and Dworkin (163) that glucosamine induces massive lysis of the population when

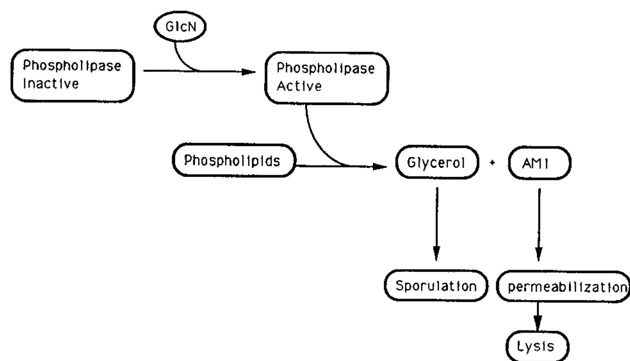

FIG. 5. Proposed model for glucosamine regulation of developmental autolysis and sporulation. Reprinted from reference 163 with permission of the publisher.

added to growing cells. Most surprisingly, 60 to 70% of the surviving cells convert to bona fide myxospores. Thus, the effect of exogenous glucosamine seems to mimic the normal results of development, i.e., lysis and sporulation. The induced developmental autolysis is associated with an increase in the levels of phospholipase and of the autocide AMI. The subsequent myxosporulation of the survivors is associated with a transient increase in the level of intracellular glycerol, itself an inducer of myxosporulation (49). Recently, Frasch and Dworkin (62) have shown that normal development of fruiting bodies and myxospores is associated with a transient increase in the intracellular concentration of glycerol. Thus, the model that has emerged proposes that glucosamine, or a similar amino sugar, is part of a developmental signal that results in elevated levels of a phospholipase. This results in the hydrolysis of phosphatidylethanolamine, the major phospholipid of the outer membrane of *M. xanthus* (174), with the consequent release of AMI and glycerol. The AMI is then responsible for the developmental autolysis, and the glycerol is responsible for myxosporulation. The model is illustrated in Fig. 5.

INITIATION SIGNAL

While earlier work had indicated that limiting growth by witholding a number of essential or unessential amino acids is sufficient to induce development (44, 77, 147), pyrimidine deprivation has essentially no effect (113). Development under conditions of amino acid deprivation is correlated with an increase in the intracellular levels of guanosine penta- and tetraphosphate ([p]ppGpp) (146). Singer and Kaiser (230) have recently shown that if the levels of [p]ppGpp are elevated by the expression in *M. xanthus* of a cloned *relA* gene from *E. coli*, using a light-inducible promoter, developmental gene expression and the early stages of development are induced. This provided additional evidence that these highly phosphorylated nucleotides may play a role in the early stages of transduction of the extracellular nutritional signals.

EXTRACELLULAR APPENDAGES

Two appendages that are attached to the outer surface of *M. xanthus* are pili and fibrils. Interest in these organelles has been generated by the fact that both are involved in contact-mediated cell-cell interactions (223).

Pili

Pili were first demonstrated in myxobacteria by MacRae and McCurdy (144). The pili of *M. xanthus* are polarly located, less than 10 nm thick, and up to 10 μm long (97). The fact that pili are required for social motility has been convincingly demonstrated in Kaiser's laboratory (see the Motility section). Nevertheless, the precise role that pili play remains unclear. A number of laboratories have been unsuccessful in their attempts to physically isolate and characterize the pili of *M. xanthus*. However, Wu and Kaiser (263) have recently cloned and sequenced three genes from *M. xanthus* which encode amino acid sequences with substantial similarity to genes of the type IV pilus biosynthetic pathway in *Pseudomonas aeruginosa*. These genes are contained within the *sglB* locus of *M. xanthus* and, when mutated, result in the loss of S motility (82). The sequence similarities suggest that the three genes code for PilA, a putative pilin precursor, and PilS and PilR, members of the two-component regulatory system of the NtrB/C family in *P. aeruginosa* (79), and prove that pilus assembly and social motility are not merely correlated but are part of a cause-and-effect relationship.

These are the first of the S (social) motility genes that have been characterized; none of the A (adventurous) motility genes have yet been characterized (see the Motility section).

Fibrils

Cells of *M. xanthus* possess extracellular organelles called fibrils. These are peritrichous filaments that may be as long as 50 μm (Fig. 6). The diameters of the fibrils fall into two approximately equal size distributions, 15 and 30 nm (8). The fibrils are composed of approximately equal amounts of protein and carbohydrate (9) and have been shown to play a role in cell-cell interactions (47, 223).

Fibrils on *Myxococcus fulvus* were originally described by Fluegel as myxonemata, using India ink deposition and simple microscopy (57). Arnold and Shimkets (4) were the first to demonstrate that the fibrils play a role in cell-cell cohesion and social behavior in *M. xanthus*. They showed that treatment of the cells with the diazo dye Congo red prevents the appearance of fibrils on the cells, and cells thus treated lose the ability to cohere, show social motility, or form fruiting bodies. They also showed that a class of mutants termed *dsp* (for dispersed growing) lack fibrils and are likewise unable to show cohesion, social motility, and fruiting body formation. Arnold and Shimkets concluded that the fibrils supply the cohesive force that allows the cells to manifest these social and developmental behaviors (5).

Behmlander and Dworkin (9) showed that the fibrils are present, as such, on living cells and are not fixation or dehydration artifacts. They are produced when the cells are grown at a high cell density and are polysaccharide structures with associated proteins.

A set of monoclonal antibodies directed against the cell surface antigens of *M. xanthus* (63) were used to characterize the protein composition of the fibrils. One of these monoclonal antibodies (mab2105) reacts specifically with the protein component of the fibrils (10). This protein, designated IFP-1 (integral fibril protein), comprises the major protein of the fibrils and consists of five multimers with a common epitope. The possibility that the fibrils play a role in development is supported by the finding that the ratio of the multimers changes dramatically during development (10). Chang and Dworkin added support to the idea that the fibrils indeed play an integral role in the social behavior of *M. xanthus* by demonstrating that isolated, purified fibrils, when added to a developmentally

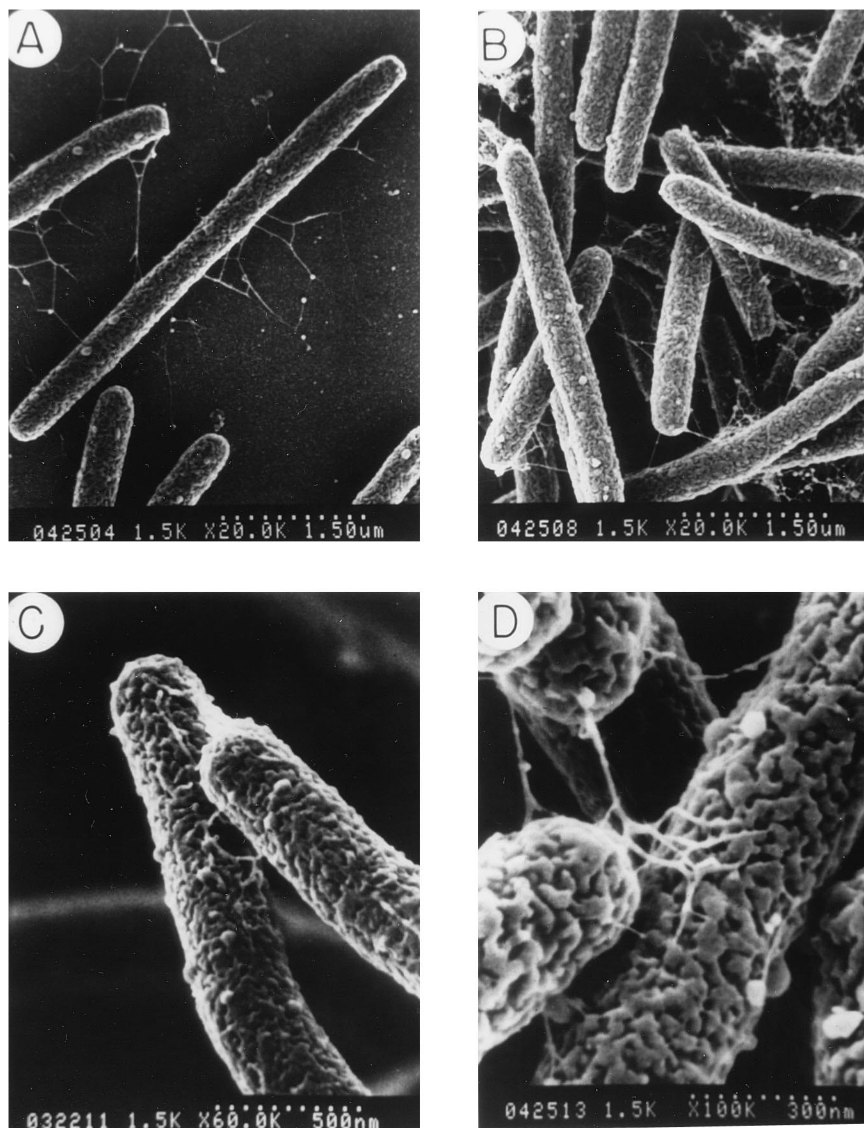

FIG. 6. Low-voltage scanning electron micrographs of vegetative cells of *M. xanthus* grown on a solid surface in submerged culture. (A) Individual cells and fibrils. (B) Cluster of cells with extensive fibrillar connections. (C) Cells from submerged culture anchored to the substratum at the ends not shown. (D) High magnification of the cell surface and fibril anchoring point. The corrugated surface probably indicates shrinkage of the cells during fixation and critical-point drying. Reprinted from reference 8 with permission of the publisher.

and socially defective *dsp* mutant, fully restore development and cohesion (25).

It has been conceptually simple to imagine (in the absence of any evidence) that the role of the fibrils in social behavior is to bind cells physically to each other. However, recent evidence suggests that this may not be the case. As part of an attempt to study a putative fibril receptor, Chang and Dworkin (26) isolated a series of secondary *dsp* mutants whose inability to cohere could no longer be rescued by added isolated fibrils. The notion was that these (*fbd*) mutants lack a putative fibril receptor. When the *fbd* mutants were examined, they turned out to have some unexpected properties. Despite the fact that, like the parent *dsp* mutant, they still lack fibrils, they had nevertheless regained group motility and partial development, i.e., they were able to undergo developmental aggregation and partial myxosporulation but unable to complete fruiting body formation (26). Thus, the mutants present an apparent contradiction: i.e., the loss of fibrils due to the *dsp* mutation is clearly correlated with the loss of cohesion, group motility, and development, yet the *fbd* mutant, which lacks fibrils, regains group motility and the ability to aggregate. Chang and Dworkin (26) have proposed the following model to reconcile these apparently contradictory data. The evidence accumulated by Kaiser's lab has indicated quite clearly that pili are required for social motility (263). Rosenbluh and Eisenbach took this one step further (200) and suggested that both pili and fibrils are required for social behavior. Chang and Dworkin have proposed that the *fbd* mutant (which forms pili but not fibrils) contains a mutated fibril receptor which is constitutively in the "occupied" configuration. This both prevents binding of fibrils (thus blocking rescue of cohesion by the fibrils) and falsely signals the cell that the fibril receptor is occupied. Thus, in effect, both the fibril and the pilus functions for social behavior are satisfied in the *fbd* mutant (26).

Guespin-Michel and Monnier (69) have raised the interesting possibility that the extracellular matrix and its associated fibrils may play a role in the binding of extracellular proteins. One may imagine that those proteins are either extracellular hydrolytic enzymes used by the cell to generate low-molecular-weight substrates or extracellular signals transmitted from cell to cell.

While it seems clear that the extracellular appendages of *M. xanthus* play important roles in the social behavior of the organism, the mechanism of how this is accomplished remains to be established.

FRUITING BODY FORMATION

The myxobacterial fruiting body is the developmental culmination of a series of events set in motion by the changing nutritional and physical environment. In order for a population of essentially single cells to accomplish this morphogenetic event, the cells must perceive the environmental change and then transform that perception into a series of events involving aggregation, construction of the multicellular fruiting body, and cellular conversion of the rod-shaped vegetative cells to the round, resistant, metabolically quiescent myxospores. During these processes, the cells monitor their cell density, control the timing of a series of developmental events, engage in tactic behaviors, undergo developmental autolysis, and carry out a three-dimensional spatial orientation.

Cell Density

The first evidence that cell density played a role in the developmental behavior of *M. xanthus* was the finding that germination of glycerol-induced myxospores is density dependent. The requirement for high cell density could be replaced by P_i, which is excreted by the cells and serves as a quorum sensor (185). However, in view of the artificial nature of glycerol myxospores (see the Glycerol Spores section), the significance of this finding was not obvious. Later, Wireman and Dworkin (260) reported that aggregation is cell density dependent. Shimkets and Dworkin (222) confirmed these findings and showed that the developmental requirement for a high cell density could be bypassed in the presence of 50 mM adenine or adenosine. Furthermore, during development, the cells excreted substantial amounts of a variety of adenine-containing compounds. These results recalled an earlier finding by Campos and Zusman (24), who showed that a number of adenine-containing compounds induce cells to go through partial development under conditions in which the cells normally grow vegetatively. Shimkets and Dworkin (222) thus concluded that adenosine plays a role as a cell density sensor. This view was challenged by Manoil and Kaiser (148), who isolated a mutant of *M. xanthus* that is resistant to the toxic adenine analog 2,6-diaminopurine. Low-density fruiting in this mutant is not rescued by adenosine despite the fact that the mutant is otherwise developmentally competent. Furthermore, adenosine is not able to rescue development in an *asg* mutant or to induce expression of an *asg*-dependent Tn*5-lacZ* reporter gene (125). Kaiser and Kroos (99) have summarized their assessment of the situation by concluding that if adenosine is indeed a density-sensing factor, it is not obligatory. However, its role in relation to the A signal, starvation, and cell density sensing merits further study.

Kim and Kaiser (111) did a series of experiments that show that the C factor acts as a developmental timer that responds to cell density. They examined the effect of added C factor on aggregation, sporulation, and the expression of a *csgA-lacZ* transcriptional fusion and showed that there are two distinct stages in the role of the C factor. In the first, the relatively low cell density at the preaggregation stage of development results in the generation of low levels of the signal, which are then sufficient to trigger aggregation. This results in a substantial increase in cell density, which apparently amplifies the transcription of the *csgA* gene. The combination of the higher cell density and an increased rate of *csgA* transcription results in substantially higher levels of C factor, which then signal the later stages of development, including sporulation. Li et al. (135), using a different strategy, confirmed and extended these conclusions. They generated a series of successively larger deletions of a large regulatory area upstream of *csgA*, which control *csgA* transcription. Inhibition of *csgA* expression is proportional to the extent of the deletion. They measured three parameters of development—rippling, aggregation, and sporulation. Each is reduced to a different extent by the nested deletions: rippling requires ~20% of maximum *csgA* expression, aggregation requires ~30% expression, and sporulation requires ~82% expression, reflecting the temporal sequence of the three developmental events.

Thus, both the A and the C signals monitor cell density. The scenario that seems to emerge is as follows. Both signals play a role in the early stages of low cell density before aggregation. If the cell density is too low, the concentration of A signal is correspondingly low and the cells continue to grow. At a sufficiently high cell density, enough A signal will have accumulated, and the early aggregation events are set in motion. The nature of the separate roles of the two signals and their possible interactions at this stage are unclear. When aggregation is completed, the cell density is then sufficiently high for full expression of the C signal, allowing the subsequent stages of sporulation and fruiting body formation.

Eastman and Dworkin have proposed that ADP-ribosylation also plays a role in the complex process of cell density sensing (52). They have shown that ADP-ribosylation occurs in *M. xanthus* and that the pattern of ribosylation changes during development. They found that nicotinamide, in a manner similar to that previously demonstrated for adenosine (222), stimulates cells at low density to develop, which correlates with the in vivo effects of nicotinamide on ADP-ribosylation. Thus, they propose that ADP-ribosylation, along with C and A signaling, is part of the network of regulatory events that monitor cell density (52).

Role of Motility

Kroos et al. (120) found that neither *mgl* mutants, which have a single mutation in a key motility gene, nor nonmotile double mutants containing mutations in both the *A* (for adventurous) and *S* (for social) motility genes are able to develop or to express those reporter genes normally expressed late in development. This pattern of defects in development and gene expression is similar but not identical to that manifested by the set of *csg* mutants, and they thus suggested that motility per se was required for the Csg-mediated cell-cell interaction. This observation was presaged by Hodgkin and Kaiser (82), who found that 18 of 39 unselected but different S^- motility mutants fail to undergo development, and confirmed by Kroos et al. (121), who found that 3 of 8 developmentally defective mutants are S motility defective. (Subsequently, it has been shown that one of those three putative S mutants is actually an *frzF* mutant [103].) Thus, the general understanding has emerged that there is a strong relationship between motility and fruiting body development. Part of that relationship has been clarified by Kim and Kaiser (107, 108), who proposed that

cell motility allows the cells to be juxtaposed in a cell-cell alignment that is necessary for the presentation of the C signal from one cell to the other. However, the specific roles of social and adventurous motility have yet to be worked out.

Aggregation

Fruiting body and myxospore formation is invariably preceded by the movement of swarms of cells into centers referred to as aggregates. Those movements were dramatically illustrated by a number of Reichenbach's time-lapse motion picture films that showed streams of myxobacteria moving into aggregation centers (e.g., reference 192). While the stimuli that actually induce that aggregation or the clues that guide it are unknown, a series of early experiments suggested that some sort of diffusible factor(s) played a role in inducing aggregate formation. In 1954, Lev reported that fruiting bodies of *Chondromyces exiguus* and *M. xanthus* produce substances that stimulate fruiting body formation (134). Jennings (95) showed that a concentrated aqueous extract from fruiting bodies of *Myxococcus virescens* induces cells to form fruiting bodies under nutritional conditions that do not otherwise permit development. McVittie and Zahler (162) showed that cells of *M. xanthus* layered on a sheet of cellophane that was placed over fruiting bodies of *M. xanthus* form fruiting bodies that are juxtaposed over the underlying fruiting bodies. They excluded the possibility of elasticotaxis (235) by substituting glass beads for the fruiting bodies and showing that these are ineffective. Finally, Fluegel (58) also used a cellophane membrane to obtain the same result with *Myxococcus fulvus.*

What emerged from these old experiments is that some sort of chemical substance is involved in triggering aggregation and thence fruiting body formation. Nevertheless, there is still no experimental evidence that developmental aggregation per se involves a chemotactic response or the perception of any kind of chemical gradient. However, most recently, Stevens (240) has constructed a theoretical model for aggregation of myxobacteria that depends on the following of slime trails plus the production of a chemotactic substance whose diffusion is limited by the production of high concentrations of extracellular slime.

In the past, it has been tempting to view the process in the myxobacteria as somehow analogous with the cyclic AMP (cAMP)-induced chemotactic movement of the myxamoebae of *Dictyostelium discoideum* into pseudoplasmodial aggregates, but such comparisons must be treated with caution. In the case of *D. discoideum*, during the swarming growth of the vegetative myxamoebae, the cells are separated from each other, and a relatively long-range signal is needed to draw them together into an aggregate. In contrast, in the case of the myxobacteria, aggregation is not only preceded by but absolutely dependent on a condition of extremely high cell density. In fact, the vegetative cells are piled in organized layers atop one another. It thus seems likely that cell-cell communication that is mediated by contact also plays some sort of role in signaling the time and the place of aggregate formation.

An alternative way of thinking about the problem was suggested by the work of Vasquez et al. (254). On the basis of a series of beautiful scanning electron micrographs of aggregating *Stigmatella aurantiaca*, they proposed that aggregate formation is preceded by the circular movement of swarms of cells, producing concentric or nearly concentric rings of cells (Fig. 7 and 8). A similar observation was subsequently made by O'Connor and Zusman (170), who also noted that spiral patterns of swarm movement precede aggregate formation in *M. xanthus*. White has proposed (256) that at some point in time and space, the random turning of the swarms becomes converted into a coralling mode that traps the cells in an aggregate. Shi and Zusman (214) have shown that the A (adventurous) and the S (social) motility behaviors of *M. xanthus* respond differently to different physical properties of an agar surface. They found that single cells manifesting A motility show optimal gliding on a firm, dry surface (1.5% agar) and are essentially unable to move on a wet, soft surface (0.3% agar). On the other hand, cells undergoing S motility show the opposite behavior. They suggested that this differentiation may function in a fashion analogous to the swimming/swarming differentiation of *Vibrio parahaemolyticus*, by which the cells respond to changes in substratum viscosity by alternating between two different flagellar states (228). It is feasible that the gradual accumulation of polysaccharide slime during preaggregative swarming may at some point be perceived by the swarms and may trigger a change in the motility pattern that results in coralling and aggregation.

However, the most exciting insights into the possible mechanism of developmental aggregation emerged during the 1995 Annual Meeting on the Biology of the Myxobacteria. Emphasis shifted from chemotaxis as a strategy for moving cells into an aggregate to one based on cell-cell contact, signal perception, inhibition of reversal frequency, and trapping. Thus, Søgaard-Andersen et al. (233) have proposed that head-to-tail contact between cells of *M. xanthus* results in generation of the C signal, which in turn induces methylation of the FrzCD protein and a resultant reduction in the reversal frequency of gliding. The result is the formation of trails of cells moving into aggregation centers reminiscent of those demonstrated by the time-lapse motion picture films of myxobacterial aggregation (192).

Shi et al. (217) also showed that cell-cell contact affects the rate of reversal of gliding and that this reduced reversal frequency is correlated with FrzCD methylation. They have proposed that the reduced reversal frequency together with slime trail following results in aggregate formation. The model presented by Shi et al. rejects the notion of a head-to-tail orientation of the cells and attributes the reduction in reversal frequency simply to the cells accumulating in groups, but they do not explain how this initially comes about, nor does the Søgaard-Andersen et al. model explain what directs the streams of cells to converge at a common center.

Nevertheless, in a general sense, what has emerged is reminiscent of the early, original discovery by Englemann (56) of the phototactic behavior of *Bakterium photometricum*. He illuminated a microscope slide culture with a spot of light and observed that when the cells move from the light into the dark, they rapidly reverse their direction (Schreckbewegung). Thus, they are eventually trapped in the light spot. In other words, the regulation of reversal frequency in response to a signal is the operational parameter that results in cell accumulation.

Peripheral Cells

As part of their attempts to understand the events that occur during fruiting body formation, O'Connor and Zusman (171–173) have described a portion of the population of developing cells of *M. xanthus* that they refer to as peripheral cells. These are those rod-shaped cells representing 1 or 2% of the population that have been left out of the developing aggregate and undergo neither autolysis nor sporulation. O'Connor and Zusman have shown that these cells express many but not all of the developmentally regulated genes. They have proposed that these cells are a specifically differentiated segment of the developing population and that they play some undefined role in myxobacterial development. Alternatively, their data are con-

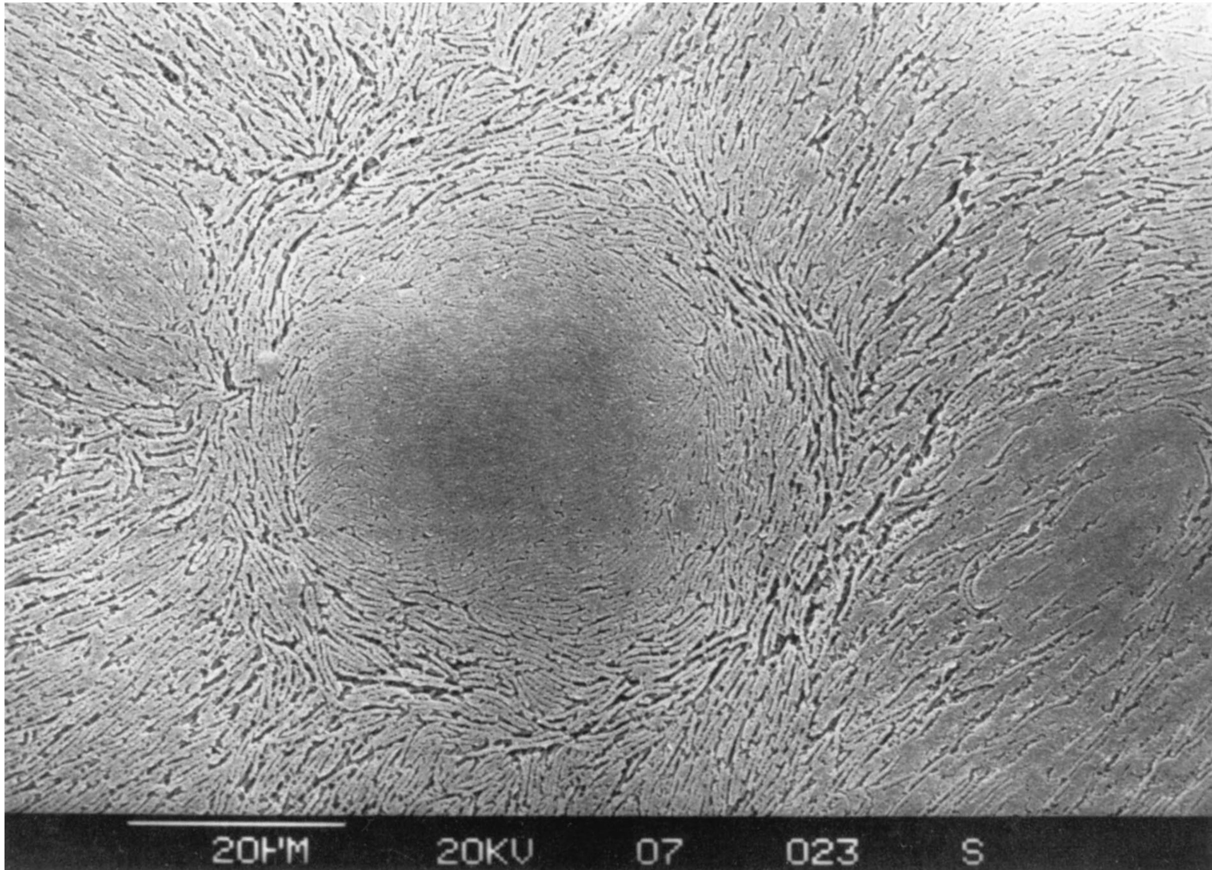

FIG. 7. Very young aggregate of *S. aurantiaca* on filter paper. Reprinted from reference 254 with permission of the publisher.

sistent with the simpler explanation that these are the small fraction of vegetative cells that have not been drawn into development but rather have entered stationary-phase physiology. It has recently become clear that the transition from the growth to the stationary phase involves a specific, programmed shift to a physiological state more adapted to starvation than to growth (229). One might view this, in the case of *M. xanthus*, as a back-up response to starvation that is an alternative to the developmental response.

Fruiting Body Morphogenesis

Morphogenesis, in its literal sense, is the aspect of development that has most eluded the efforts of developmental biologists. Understanding of the one-dimensional nature of the genomic program has not led to an understanding of three-dimensional macroscopic structure. This is ironic in the sense that it is the three-dimensional structure of the myxobacterial fruiting body (Fig. 1) that initially captured the interest of most myxobacteriologists.

The most exciting approach has come from the three-dimensional analysis of the fruiting body of *M. xanthus* by confocal fluorescence microscopy and Tn*5*-*lac* reporter genes (208, 209). Sager and Kaiser labeled cells of *M. xanthus* with a lipophilic fluorescent probe and used the confocal microscope to make a series of nondestructive optical sections through the fruiting body. Their results reveal that the fruiting body is divided into two concentric, hemispherical domains (Fig. 9). The outer domain is densely packed with rod-shaped cells that move in concentric clockwise and counterclockwise streams. These cells reverse their paths less frequently than do growing cells or cells outside the fruiting body. The inner domain contains the less densely packed, nonmotile myxospores (208). They then showed that these domains are differentiated functionally as well as morphologically (209). A collection of mutant cultures containing transcriptional *lacZ* fusions to 80 developmentally regulated genes were induced to form fruiting bodies, and the temporal and spatial distribution of gene expression was determined. Eight of the fusions show expression in the inner domain of the fruiting body (Fig. 10A), while one fusion shows expression initially in the outer domain (Fig. 10B). The location and timing of that expression coincide with the appearance of patches of immature myxospores. As the fruiting body matures, both the patches of myxospores and the pattern of gene expression extend into the inner domain until the fruiting body is uniformly filled with myxospores expressing the *lacZ* fusion gene. An ingenious series of reconstruction experiments support the authors' contention that the movement of the myxospore precursor cells to the interior of the fruiting body is a result of their passive transport by those motile rod-shaped cells that have not undergone morphogenesis. They further suggest that the initiation of myxospore morphogenesis in the outer domain is initiated by the C signal.

This work, for the first time, assigns a specific function for motility in fruiting body morphogenesis. In addition, it connects specific gene expressions, and eventually the corresponding gene products, to spatial and temporal segments of fruiting body morphogenesis.

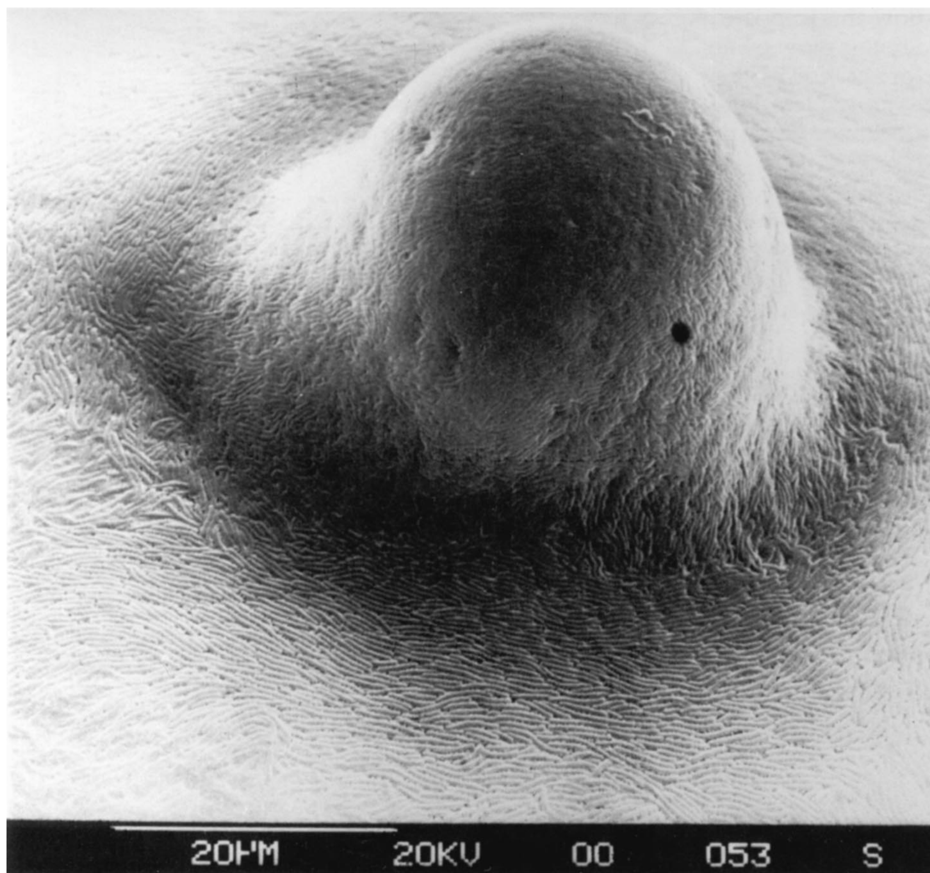

FIG. 8. More advanced aggregate of *S. aurantiaca* on filter paper than that shown in Fig. 7. Reprinted from reference 254 with permission of the publisher.

DEVELOPMENTAL AUTOLYSIS

Wireman and Dworkin described the massive autolysis that occurred during fruiting body formation in *M. xanthus* (260, 261). During the process of aggregation and early mound formation, 65 to 90% of the cells lyse, depending on the particular conditions of development. The surviving cells complete fruiting body formation and eventually convert to myxospores. It is not clear whether the death of a substantial fraction of the population during development is programmed and thus analogous to mammalian apoptosis or is a stochastic phenomenon, with cell fate subject to spatial and temporal variables. Qualls (184) has observed a similar behavior in developing cells of *S. aurantiaca*.

Shimkets (218) has proposed a number of possibilities that could explain the function of developmental autolysis. The products of the lysed cells may be a source of nutrients to allow the further development of the survivors. This would be consistent with the common ability of the myxobacteria to lyse other cells and to grow on the scavenged products (190). Wireman (259) has in fact shown that sporulation of *M. xanthus* can be induced only in the presence of high concentrations of autolysis products or of exogenous nutrients. Another approach has attempted to rationalize the fact that protein S, a protein that appears on the surface of myxospores, does not contain a hydrolyzed leader peptide sequence. Thus, it is difficult to see how it is transported out of the cell. Accordingly, Teintze et al. (244) have proposed that developmental autolysis of the vegetative cells results in the release of protein S, which is then free to self-assemble on the myxospore surface of the nonlysed cells.

O'Connor and Zusman (169) have suggested that developmental autolysis is an artifact and a result of the manipulation of the cells during development. However, a series of experiments by Rosenbluh and Rosenberg (201) and by Rosenbluh et al. (203) confirmed the relationship between autolysis and development under conditions that subjected the cells to essentially no experimental manipulation. Rosenbluh and Rosenberg showed that if cells are placed in liquid culture under conditions of starvation analogous to those that induced development on a solid surface, there is a 90% decrease in the optical density of the culture, with the surviving cells, representing 10 to 15% of the initial population, converting to bona fide myxospores. Mueller and Dworkin (163) subsequently showed that the decrease in optical density of the inducing culture is due to actual lysis of cells. In a definitive experiment that essentially eliminated any putative artifacts due to experimental manipulation of the so-called fragile, prespore cells, Rosenbluh et al. (203) encased vegetative cells in agarose microbeads (approximately 25 μm in diameter) and then placed them under conditions of starvation in liquid shake cultures. When the cells encased in the beads attain a sufficiently high cell density, approximately 90% of the cells undergo autolysis, and approximately 50% of the surviving cells sporulate. Finally, Laue and Gill (129) showed that the tan and yellow cells that are the result of phase variation seem to represent a differentiated population; the tan cells, which are in the mi-

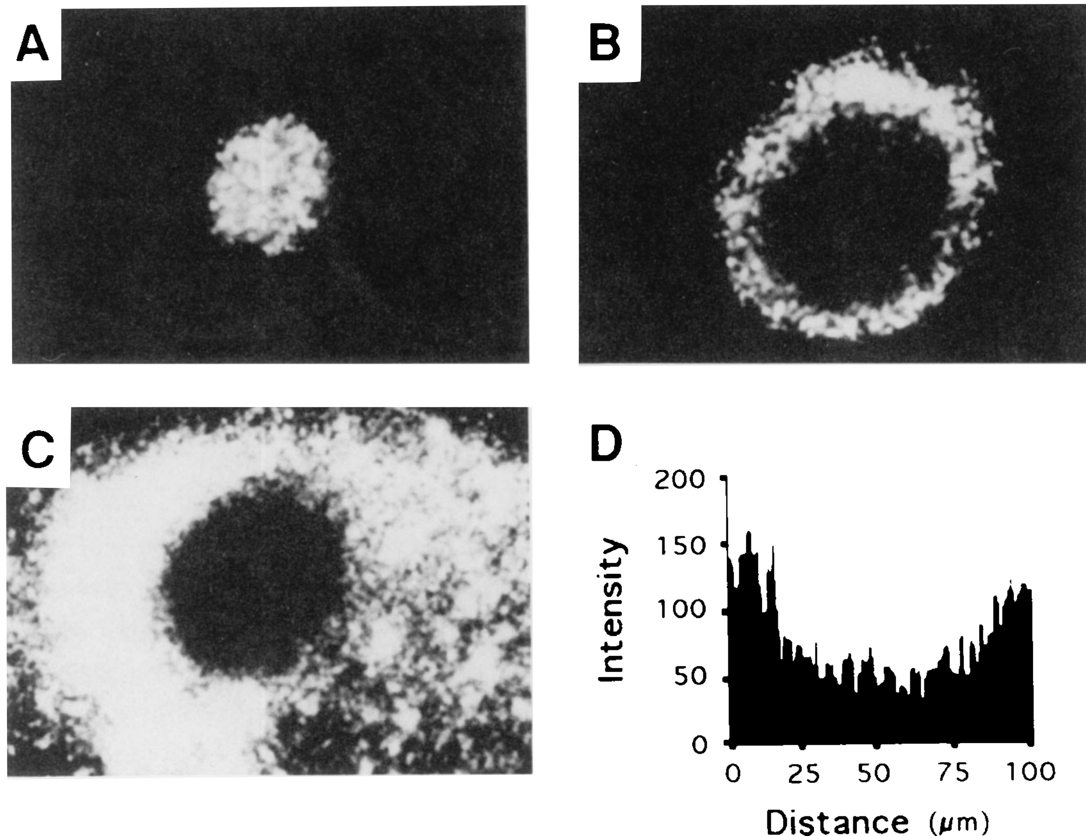

FIG. 9. (A to C) Fluorescence confocal microscopy of optical sections of a fruiting body of *M. xanthus* at 24 h of development. Sections were taken at 20 μm above the base (A), at 6 μm above the base (B), and at the base (C). (D) Quantitative gray scale intensity scan along a diameter of the confocal section shown in panel B. Reprinted from reference 208 with permission of the publisher.

nority in the population, are essentially resistant to autolysis and probably represent the precursors of the myxospores. The yellow cells, on the other hand, undergo massive autolysis. The different roles of these two cell types in development are consistent with the notion that the autolysis that occurs during development plays a functional role in the developmental process (see the Phase Variation section below).

The accumulated evidence supports the original contention that autolysis and sporulation are closely related, and it is now generally accepted that autolysis plays a normal, albeit unexplained, role in the development of *M. xanthus*. It should be pointed out, however, that in the absence of mutants selected explicitly on the basis of their inability to undergo autolysis, there is as yet no compelling evidence for an obligatory, causal relationship between autolysis and development.

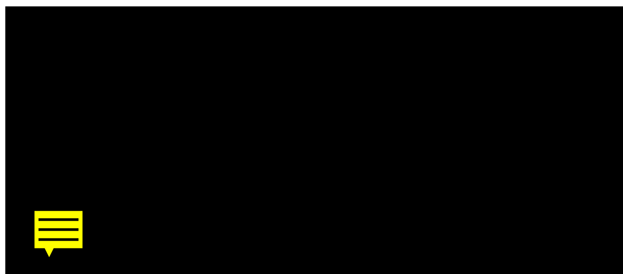

FIG. 10. (A) Spatial differentiation of β-galactosidase expression in a 46-h developing fruiting body of *M. xanthus* examined by bright-field microscopy (×25). The expression of a developmentally regulated gene (*4459*) is restricted to the inner domain of the fruiting body. (B) Spatial expression of a developmentally regulated *lacZ* fusion (*7621*) in a fruiting body of *M. xanthus*. Examined with bright-field optics at 48 h of development. Expression is limited to the inner part of the outer domain of the fruiting body. Reprinted from reference 209 with permission of the publisher.

TACTIC BEHAVIOR

There are at least two aspects of the tactic behavior of *M. xanthus*. One relates to the ability of the cells to perceive and respond to chemical gradients. There have been conflicting reports about the chemotactic ability of *M. xanthus* (48, 216). The second is the ability of the cells to perceive the presence of physical objects (45).

Chemotaxis

From a behavioral point of view, because the cells glide on a solid surface rather than swim in liquid, it has been difficult to demonstrate chemotactic behavior in a fashion strictly analogous to that in *E. coli*. In fact, Dworkin and Eide (48) showed that *M. xanthus* does not respond chemotactically to moderate gradients of a wide variety of defined chemicals as well as of complex mixtures. However, Shi et al. (216) devised a compartmentalized petri dish that generated extremely steep gradients. In addition, they used cultural conditions that substantially increase the rate of gliding. Under these conditions, they were able to show that a colony of cells expands asymmetri-

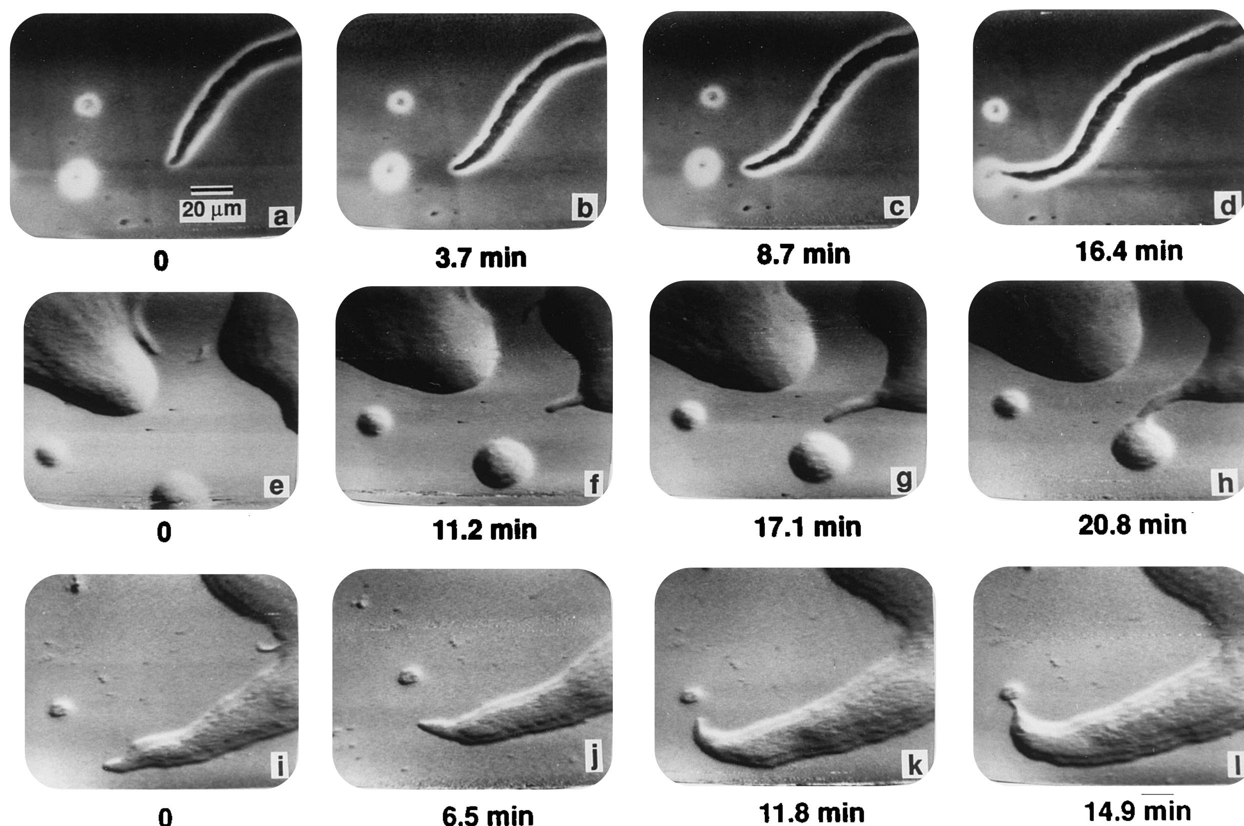

FIG. 11. Movement of flares of *M. xanthus* toward clumps of *Micrococcus luteus*. (a to d) Phase-contrast microscopy; (e to l) Nomarski interference microscopy. The numbers beneath the photographs represent the time elapsed after the first (time zero) photograph. Reprinted from reference 45 with permission of the publisher.

cally, with a bias in the direction of increasing concentrations of complex mixtures of nutrients such as Casitone and yeast extract; conversely, the colony expands away from a variety of repellents such as isoamyl alcohol. They interpreted this as a chemotactic response.

While they were able to demonstrate that the cells show an adaptation response to specific repellents (215), no evidence was presented for an adaptation response to the putative attractants. In addition, the response to attractants is puzzling. First, no defined chemical was found to serve as an attractant; only complex growth media (e.g., Casitone and yeast extract) are effective. In addition, there is an up to 10-h lag in the presence of a gradient of complex attractants before the cells manifest any motility.

In a preliminary report, not yet subjected to peer analysis, Tieman et al. (248) examined the behavior of individual cells of *M. xanthus* in a slide culture system that generated a stable linear gradient of Casitone. A statistical analysis of the cells' motility revealed no evidence of chemotactic behavior. This may represent conflicting results, or it may reflect the differing tactic behaviors of swarms as opposed to individual cells.

From a theoretical point of view, there are some obstacles to formulating a mechanism of chemotaxis in *M. xanthus*. The relatively slow rate of movement of the cells in comparison to the rate of diffusion of molecules makes it difficult to construct a memory-based temporal sensing model (48), and the small size of the cells makes a spatial sensing model equally unlikely, as was pointed out for *E. coli* by Macnab and Koshland (142). It seems obvious that a model for the chemotactic behavior of *M. xanthus* that is simply analogous to that of *E. coli* is inadequate. Furthermore, if bona fide chemotactic behavior does indeed exist in *M. xanthus*, there is no evidence that it plays a role in developmental movement (see the Aggregation section above).

There is, however, convincing molecular and biochemical evidence that, with regard to the signal transduction pathway, there is a great deal of similarity between *M. xanthus* and *E. coli*. The story began to unfold with the discovery of an interesting and important group of mutants designated frizzy. The frizzy mutants are not only aberrant in their colonial morphology but are also unable to coordinate their activities sufficiently to construct normal fruiting bodies (267). An analysis of the motility of wild-type cells (strain DZF1) revealed that the cells reverse their direction of gliding approximately once every 6 to 8 min. In the case of the frizzy mutants, however, most of the mutants reverse their direction only once every 2 h, while one group changed direction every 1 or 2 min (12). A molecular analysis of the locus revealed that it comprises seven genes, *frzA*, *B*, *CD*, *E*, *G*, *F*, and *Z* (13, 215). The *frz* genes have now been cloned and sequenced and their gene products have been partly characterized, and it is clear that collectively they show a striking similarity to the signal transducing system for chemotaxis in *E. coli* (215). The functions of the *frz* gene products are as follows. FrzA is homologous to CheW, which is part of the MCP-CheA-CheW complex in *E. coli*; FrzCD is homologous to Tar, the aspartate receptor and one of the MCPs of *E. coli*. McCleary et al. (158) showed that *S*-adenosylmethionine methylated a glutamate residue of FrzCD. FrzE is especially interesting as it contains sequences homologous to both CheA and CheY (156) and acts as both a kinase and a phosphatase.

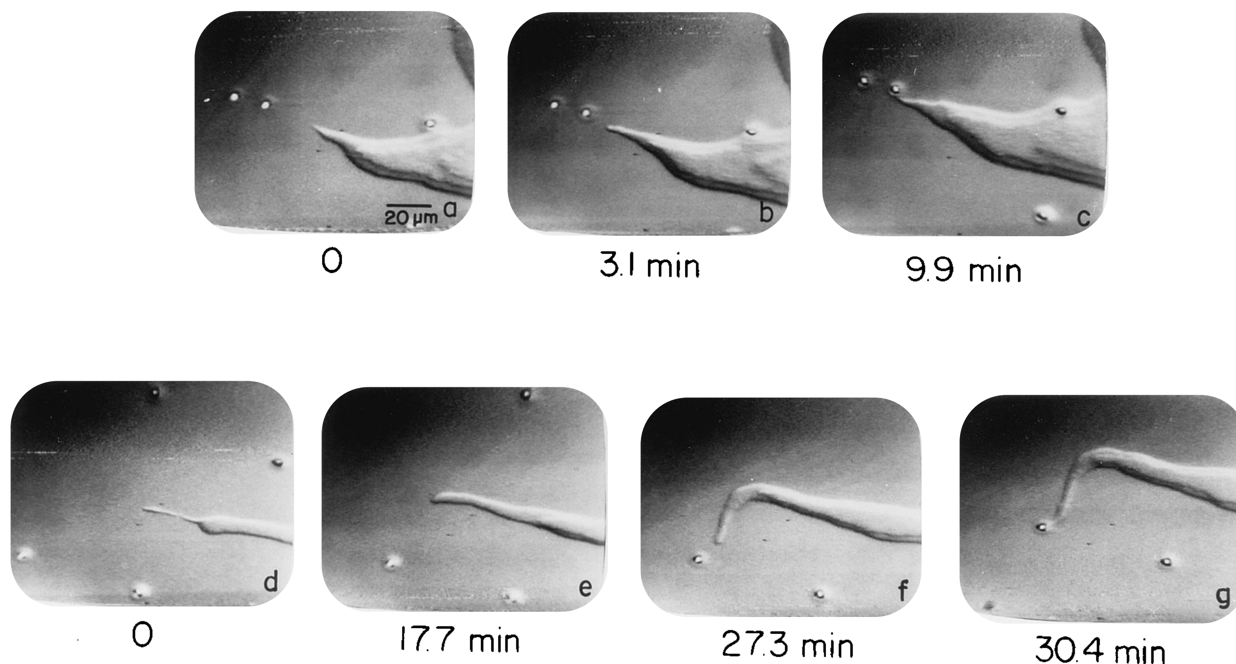

FIG. 12. Movement of flares of *M. xanthus* toward 5-µm-diameter polystyrene latex beads. Nomarski interference microscopy. The numbers beneath the photographs represent the time elapsed after the first (time zero) photograph. Reprinted from reference 45 with permission of the publisher.

It was suggested that it functions as a sensor as well as a response regulator (157). *frzF* is homologous to *cheR* and, in an analogous fashion, its gene product catalyzes the methylation of FrzCD (158). *frzG* is homologous to *cheB*, which codes for a methyl esterase involved in adaptation and whose activity is regulated by phosphorylation and dephosphorylation. FrzZ contains two domains homologous to CheY, which in *E. coli* directly induces clockwise flagellar rotation and thus tumbling of the cells. Finally, *frzB* has no equivalent in the enteric complex, and conversely, *M. xanthus* contains no homolog to CheZ, a phosphatase that acts on CheY-P and controls tumbling. The notion that seems to be emerging is that the Frz complex constitutes a signal transduction system that regulates the rate of reversal of single cells in response to environmental clues (216). Thus, while the details and perhaps even the strategy of tactic behavior in *M. xanthus* may be substantially different from those of the enteric bacteria, it is quite clear that the pathways for connecting the signals to the behavior contain strikingly similar components.

Tactic Perception of Physical Objects

Roger Stanier (235) first described the tendency of myxobacteria to orient their direction of motility to lines of stress in an agar substratum. He showed that if cells of *Chondrococcus exiguus* (now *Corallococcus exiguus*) are placed randomly on a thin sheet of agar that was subjected to lines of stress caused by compression and expansion, the cells align their motility along the stress lines and subsequently aggregate and form fruiting bodies along the stress lines. He called the phenomenon elasticotaxis after the original term elasticotropism, which was used by Jacobsen to describe the directed growth of *Kurthia zopfi* through stretched gelatin gels (93). Subsequently, Dworkin reported that swarms of *M. xanthus* are able to perceive the presence of objects at a distance and to orient their movement toward these objects (45). The study began as an attempt to determine, by the use of time-lapse photomicroscopy, whether swarms of *M. xanthus* were chemotactic toward prey bacteria. When it appeared that indeed they are (Fig. 11), as a control, the clumps of cells of *Micrococcus luteus* to which the swarms were attracted were replaced by 5-mm-diameter polystyrene latex beads (Fig. 12) or Zorbex glass beads (Fig. 13). Surprisingly, the swarms of *M. xanthus* are attracted to the beads as effectively as to the prey bacteria, indicating that the cells are sensing something other than a concentration gradient of a chemoattractant. A statistical analysis confirmed that the encounters between the swarms and the beads are not random but are indeed directed, over distances as great as 50 µm. The mechanism of this long-range perception of objects has not been elucidated and remains a mystery.

MOLECULAR BIOLOGY

The genome sizes of two genera of myxobacteria have been determined and have been found to be the largest known among the prokaryotes. Chen et al. (28) determined the genome size of *M. xanthus* by pulsed-field gel electrophoresis and showed it to be 9,454 kbp. Neumann et al. (166) used the same approach to show that the genome sizes of a number of *S. aurantiaca* isolates varied from 9,200 to 9,870 kbp and that that of the *Stigmatella erecta* genome is 9,710 to 10,010 kbp. Given that the genome size of other bacteria varies from 585 kbp for *Mycoplasma genitalium* (241) to 8,700 kbp for *Bradyrhizobium japonicum* (122), the function of the additional DNA is an obvious question. The large genome size is not a function of that subgroup of the δ proteobacteria that are phylogenetically related to the myxobacteria, as the genome size of the genus *Bdellovibrio* varies from 1,950 to 2,550 kbp (212, 252) and that of the sulfate-reducing bacterial genus *Desulfovibrio* varies from 1,630 to 1,720 kbp (183). Furthermore, there are at least two arguments against the notion that the additional information is necessary for storing the developmental information. First, other developing prokaryotes such as *Bacillus cereus* have a genome size of only 5,700 kbp (115). Second, Kroos et al.

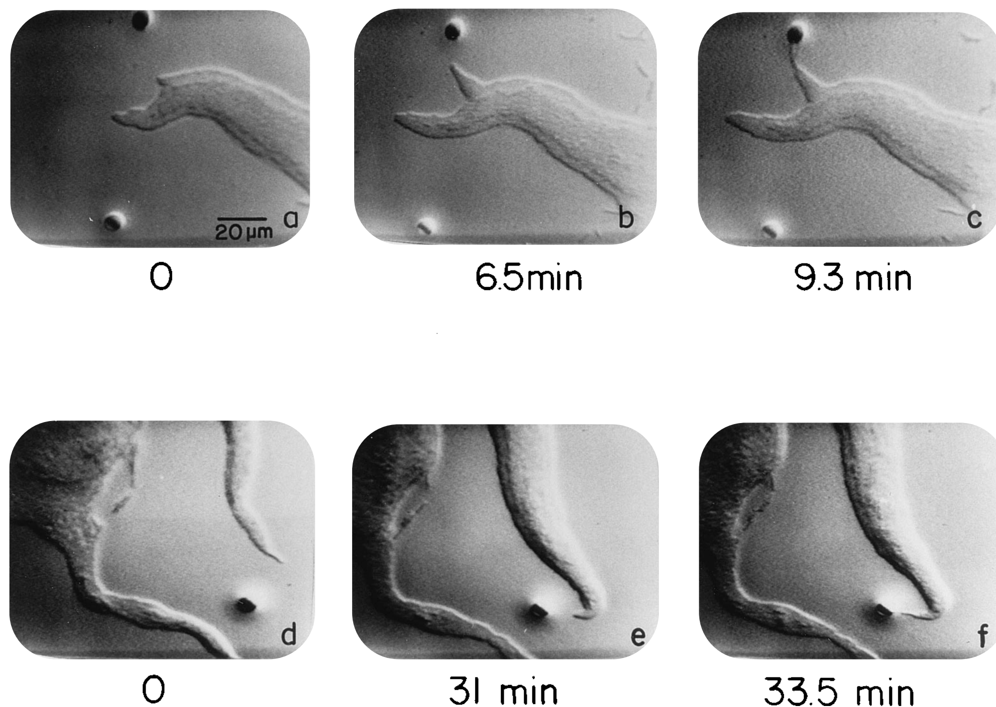

FIG. 13. Movement of flares of *M. xanthus* toward 8-µm-diameter glass beads. Nomarski interference microscopy. The numbers beneath the photographs represent the time elapsed after the first (time zero) photograph. Reprinted from reference 45 with permission of the publisher.

(119), using Tn*5*-*lac* transposons as reporters of developmental genes, showed that only 8% of the genome of *M. xanthus* increased its transcriptional activity during development. Either the large genome is coding for a set of functions that have not yet been demonstrated, or it has conferred on the myxobacteria a kind of genetic plasticity that facilitated the evolution of a complex social and developmental phenotype.

Chen et al. (29) have formulated a physical map of the *M. xanthus* genome by hybridization of *Ase*I restriction fragments with a genomic library prepared in yeast artificial chromosomes and *Spe*I-digested genomic DNA. The results indicate that the *M. xanthus* genome is organized in a single, circular chromosome, as shown in Fig. 14.

RETRONS

In 1984, Tom Yee, a graduate student in Masayori Inouye's laboratory, described a unique nucleic acid fragment present in *M. xanthus* in impressively large amounts (500 to 700 copies per cell genome). It was referred to as multicopy single-stranded DNA (msDNA) (264). msDNA was subsequently shown to be a 162-base single strand of DNA joined to a 77-base single-stranded piece of RNA by an unusual 2′–5′ phosphodiester bond (35) (Fig. 15). Later work (92) revealed that the information for msDNA is contained in a single locus containing the genes *msr*, which codes for the RNA strand; *msd*, which codes for the DNA strand; and a large open reading frame coding for reverse transcriptase (RT) (126). Collectively, the locus is referred to as a retron (245).

This was the first demonstration of RT in a prokaryotic cell, but subsequently, RT and msDNA have been found in a number of other bacteria (127, 194).

msDNA and RT have been studied in some detail in *E. coli*. Their distribution among the enteric bacteria is sporadic and heterogeneous. Hertzer et al. (78) have suggested that, based on codon usage indices, the RT genes involved in msDNA synthesis in *E. coli* are foreign to *E. coli* and probably arose via a relatively recent horizontal transfer. In contrast, in the myxobacteria, retron elements have been shown to be essentially ubiquitous, with homogeneous clusters among each of the three major phylogenetic groups of the myxobacteria (195). Furthermore, codon usage analysis is consistent with the notion that, unlike the scenario among the other prokaryotes, retrons were an evolutionarily early and vertical acquisition by the myxobacteria (195).

Retron deletions in myxobacteria have no effect either on growth or on any measurable parameter of development (91). In a like fashion, the absence of retrons in *E. coli* is not correlated with any deficiency in the growth or behavior of the organism (78). Thus, the function of retrons remains a mystery (but see Temin [245] for a speculative discussion of their possible functions).

Lampson (126) has suggested that contemporary myxobacterial msDNA probably evolved from a common ancestor of the *Myxococcus* phylogenetic subgroup, and Temin (245) has proposed that myxobacterial retrons are the evolutionary precursor of all viral and bacterial retroelements. (See the review by Lampson [126] for a more detailed discussion of retron elements.)

PHASE VARIATION

Burchard and Dworkin (17) originally noted that cultures of *M. xanthus* routinely dissociate into two colony types, designated yellow and tan. The physiological significance of this phase variation was noted by Sudo and Dworkin (242), who showed that UV light irradiation of *M. xanthus* results in a surviving population that is enriched in yellow cells. Burchard et al. (20) confirmed that effect and characterized the phase variation more completely. They showed that tan cells convert

FIG. 14. Physical and genetic map of the chromosome of *M. xanthus* DK1622. Reprinted from reference 219 with permission of the publisher.

to yellow with a rather high frequency, while the pigment phenotype of yellow cells is quite stable. They also showed that the yellow pigment has an absorption maximum at 379 nm. While numerous unsuccessful attempts have been made to characterize the pigment, it is clear that it is not a carotenoid (unpublished observations).

Since then, it has become abundantly clear that yellow and tan cells are different physiologically and developmentally. Wireman and Dworkin (260) showed that phase variants had dramatically different abilities to form fruiting bodies and myxospores. At that time, however, they attributed these differences not to the yellow-tan variation but rather to an associated swarming-mucoid colony variation.

During their examination of density-dependent feeding, Rosenberg et al. (198) found that yellow cells grew more rapidly than tan cells but only at low nutrient concentrations.

The first explicit indication that yellow and tan cells played different roles in development came as a result of the finding by Janssen and Dworkin (94) that a small number of tan cells had to be added to a *csgA sglA* mutant in order for complementing mutants, glucosamine, or an unidentified factor to rescue its development (see the Other Extracellular Developmental Factors section). The same section describes the follow-up to that work, which shows that glucosamine, when added to vegetatively growing cells, induces massive lysis and converts the surviving cells to myxospores (163). However, the effects of glucosamine on lysis and myxospore formation are completely dependent on the ratio of yellow to tan cells in the population. The tan cells are substantially more resistant to the glucosamine-induced lysis than are the yellow cells; in addition, glucosamine-induced myxospore formation in populations that are either 100% tan or 100% yellow is essentially nil. At a ratio of 9:1 yellow to tan, myxospore induction is maximal.

Downard et al. (42) have found that one of the pleiotropic properties of the *esg* mutant (see the E Signal section above) is its conversion to the stable tan phase.

A clue as to one possible physiological basis for the different behaviors of tan and yellow cells was provided by the work of Guespin-Michel et al. (70), who showed that tan variants secrete less protein than their yellow counterparts. All of the $Exc^{\pm}$ mutants (defective in protein excretion) are tan, while all Exc^{++} mutants (hypersecretors) are yellow (70).

It is clear that the problem of phase variation in *M. xanthus* has two aspects. The first is the question of the mechanism of the variation, and the second is the matter of the biological role of the yellow-tan phase variation.

In a molecular genetic tour de force, Laue and Gill (128, 129) have addressed both of these questions. First, they identified and cloned a phase-regulated promoter. Second, they used that cloned promoter to construct a mutant locked in the tan phase. The mutation *var-683* was shown to lie in a locus that controls the expression of a *trans*-acting transcriptional regulator of the yellow-phase-specific promoter (128). Subsequently, they used the phase-locked tan mutant to show that the tan and yellow variants play different roles in both growth and development (129).

FIG. 15. Primary and proposed secondary structure for Mx162 msDNA of *M. xanthus*. Mx162 msDNA is composed of a 77-base single-stranded RNA joined to the 5′ end of a 162-base single-stranded DNA. Although other species of msDNA differ considerably in primary sequence, all msDNAs share certain secondary structural features. These secondary structures are as follows: 1, stable stem-loops in both the DNA and RNA strands as a result of complementary base pairing; 2, an rG that forms the 2′–5′ phosphodiester linkage to the 5′ end of the DNA strand; and 3, a short region of base pairing between the 3′ ends of the RNA and DNA chains. The arrow indicates a late processing event that removes part of the 5′ end of the RNA strand to yield a smaller, mature form of Mx162 msDNA. Reprinted from reference 126 with permission of the publisher.

Tan and yellow cells differ substantially in their properties during vegetative growth. Tan cells grow faster in complex media and reach higher cell densities. Upon arriving at stationary phase, tan cells, in contrast to yellow cells, are essentially resistant to autolysis. This is consistent with the observations of Mueller and Dworkin (163) on the relative resistance of the tan cells to glucosamine-induced autolysis, which results in an enrichment of the tan population among old cultures.

Many of the laboratories that have worked with *M. xanthus* have noticed not only that the ratios of tan and yellow cells vary under different circumstances, but that the outcomes of developmental experiments also seem to vary as a function of the yellow-tan ratios (94, 260, 261). It has been a common observation that after development, the population becomes substantially enriched in tan cells. It has also not been clear whether this is a reflection of the conversion of yellow to tan cells by development or reflects a biased recruiting of tan cells as myxospores. Laue and Gill (129) addressed this question as follows. They mixed populations of the phase-locked tan cells with yellow cells, each of which was labeled with a different selectable marker. The mixed population was induced to develop, and the tan members of the resultant myxospore population were found to have been enriched sevenfold over their input ratio. Thus, it seems that myxospore formation is biased toward the recruitment of the tan cells. Next, Laue and Gill showed that the phase-locked tan mutant is severely defective in its developmental abilities. It is unable to cohere or to form aggregates or fruiting bodies. The cells form what seem to be abortive myxospores—round, unrefractile cells unable either to complete development or to revert to vegetative rods. However, when the phase-locked tan mutant is mixed with yellow cells, development proceeds normally, and the tan cells are effectively converted to myxospores. The optimal ratio of yellow to tan cells is approximately 9:1, the same as the optimal ratio for glucosamine-induced myxospore development (163). This is also consistent with previous observations that the extent of developmental autolysis during normal development is approximately 90%, with the surviving 10% (presumably tan cells) converted to myxospores (260, 261).

The idea that the tan cells are myxospore precursors, their inability to form complete myxospores without the assistance of the yellow cells, and the predilection of the yellow cells to undergo autolysis are consistent with the suggestion of Teintze et al. (243) that the function of developmental autolysis (presumably by the yellow cells) is to provide a source of protein S, the myxospore cell surface protein that does not contain a signal peptide sequence. Accordingly, it would be interesting to see if the incomplete myxospores formed by the phase-locked tan cells lack protein S.

The work of Laue and Gill (128, 129) has begun to clarify the annoying puzzle of yellow-tan phase variation by more precisely defining the roles of the two cell types. They have, in fact, made the important suggestion (129) that the phase variation into yellow and tan cells represents a true differentiation of the population into two functionally distinct cell types.

REGULATION AND SIGNAL TRANSDUCTION

Transcriptional Regulation

Expression of *lacZ* arising from transposition of Tn*5-lac*, which generates random transcriptional fusions, has been used to produce an overall picture of the extent of the developmental regulatory network. Of 2,374 mutants thus generated, 36 show increased β-galactosidase expression during development. Extrapolation to the full genome suggests that there are probably a total of several hundred such developmental genes (118, 119). The timing of expression of the developmental genes was determined, as was the dependence of their expres-

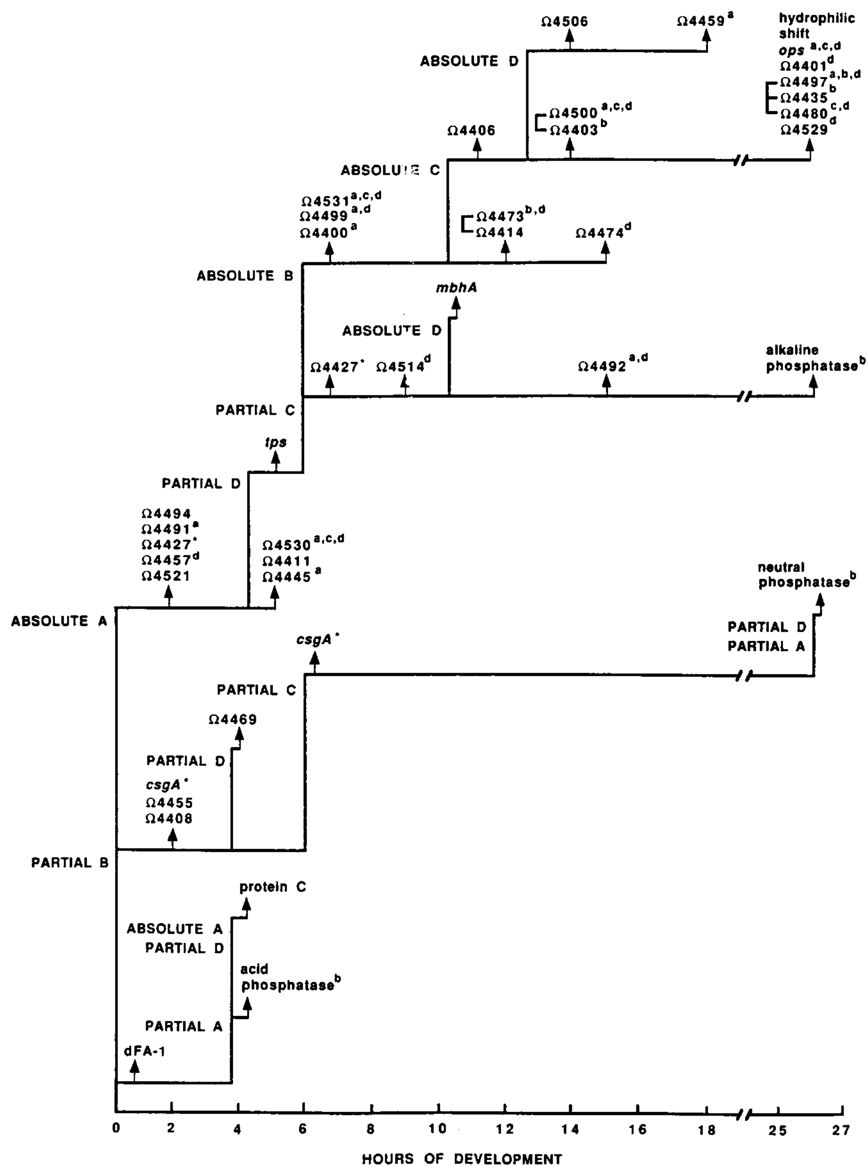

FIG. 16. Dependence of developmental markers on A, B, C, and D factors. Each marker is placed above an arrow positioned along the *x* axis according to the time at which its expression normally begins during development. The position along the *y* axis indicates the marker's dependence on cell-cell interactions, as determined by measuring its expression in *asg*, *bsg*, *csg*, and *dsg* mutants. For example, a marker whose expression is reduced in an *asg* mutant exhibits partial dependence on A factor (abbreviated partial A). Similarly, a marker whose expression is abolished in a *bsg* mutant exhibits absolute dependence on B factor (abbreviated absolute B). Dependences are cumulative, so a higher position along the *y* axis indicates greater dependence on cell-cell interactions. Superscripts a, b, c, and d mean that the marker has not yet been tested in *asg*, *bsg*, *csg*, or *dsg* mutant cells, respectively. Tn*5-lac* insertions are indicated by Ω followed by a four-digit number, and brackets connect insertions that probably lie in the same insertion unit. Insertion 4427 and *csgA* are starred and appear twice in the diagram because they exhibit no dependence on C factor before 6 h of development but a partial dependence on C factor after 6 h. Reprinted from reference 99 with permission of the publisher.

sion on their ability to produce the A, B, C, or D signals. This allowed Kroos and Kaiser (118) to construct a temporal dependence map for the expression of these developmental genes. A recently modified version of this pathway is illustrated in Fig. 16. This diagram illustrates the dependence of developmental gene expression on cell-cell interactions and emphasizes the close regulatory coupling between these two aspects of myxobacterial development.

Regulatory Sites

The regulation of two developmental operons, the *tps* and *ops* complex, which codes for the myxospore surface protein S (37, 41, 106), and the *csgA* gene, which codes for the C signal (135), has been examined.

The *tps* and *ops* genes code for two closely related proteins, S and S1, respectively. Protein S is the major protein and is a component of the outer surface of the myxospore. Protein S1 accumulates in the cytoplasm and represents about 1/10 of the amount of protein S. The genes are highly similar, adjacent to each other in tandem on the chromosome, and separated by a short spacer sequence, and are expressed at different times during development. It is possible that the genes function as backups for each other, but the real reason for their dual presence is not known. The most recent review on protein S was by Teintze et al. (243).

It has been shown that there are two types of regulatory regions for both the *tps* and *ops* genes. These are promoters for the binding of RNA polymerase adjacent to the transcriptional start sites and upstream activation sites (UAS) where transcriptional activators are bound and presumably initiate transcription. Both the *tps* and the *ops* genes have been shown to contain multiple UAS regions, and interestingly, a 180-bp segment of the *ops* UAS has been shown to activate transcription of the *tps* gene (106). Thus, a single transcriptional activation may participate in the regulation of both the *tps* and *ops* genes.

The *csgA* gene, which encodes the C signal, is also regulated by a series of upstream segments of the gene (135). The existence of a number of upstream attachment sites in the context of a large regulatory region may be a particular feature of developmental genes. In the case of the *csgA* gene, it seems especially appropriate, in view of the fact that this gene is not only expressed at different times during development but also must respond to nutrient levels, the B signal, and murein components as well as to its own gene product (135).

Sigma Factors and Protein Kinases

As part of the attempt to understand the nature of the regulation of developmental gene expression, the Inouye laboratory has taken two approaches. One has been to search for analogs in *M. xanthus* of the serine/threonine kinases that play essential roles in eukaryotic development. The other has been to seek developmental σ factors that could play a role similar to that which has been well characterized for sporulation in *B. subtilis* (138). Both of these approaches have been successful: a large family of serine/threonine kinases has been described (164), and a number of sigma factors, including two that seem to be developmentally regulated, have been isolated (2, 3, 85).

The first clue that the differential activity of multiple sigma factors might be involved in the development of *M. xanthus* came with the then puzzling finding that MX-1 bacteriophage infection of *M. xanthus* is interrupted during induction of glycerol spores and the incomplete phage particles become trapped within the spore. Subsequent germination results in release of the lytic phage (18). A similar phenomenon in phage-infected *B. subtilis* (265) was subsequently shown to be a result of a change in the template specificity of RNA polymerase during sporulation (139) and, more specifically, a result of the differential effects during development of a cascade of sigma factors (138).

The first direct experimental evidence for the existence of sigma proteins in *M. xanthus* was obtained by Rudd and Zusman (204), who showed that two closely related proteins, whose molecular weights are similar to that of σ^{70} of *E. coli*, copurify with vegetative RNA polymerase. Inouye (85) then used a probe from the *rpoD* gene of *E. coli* to identify a homologous portion of the *M. xanthus* chromosome and showed that the gene that she subsequently cloned and sequenced from that segment is highly similar to the *E. coli* gene for σ^{70} as well as the gene for *B. subtilis* σ^{43}. This gene is now referred to as *sigA* (2). Since then, the genes for two additional sigma factors have been demonstrated. Using a highly conserved region of the *sigA* gene as a probe, Apelian and Inouye (2, 3) were able to identify, clone, and sequence two additional sigma factor genes, *sigB* and *sigC*. Experiments with *lacZ* fusion reporter genes showed that *sigB* is expressed from the onset of myxosporulation to late development; SigC, on the other hand, appeared at a very early stage of development. A *sigB* deletion mutant undergoes normal fruiting body formation but produces spores lacking protein S1, the gene product of *ops*. The spores thus formed, unlike the parent culture, gradually lose their viability as they age (2). *sigC* deletion mutants develop more or less normally (the fruiting bodies are somewhat distorted). However, unlike the parent strain, the cells initiate development in the presence of high levels of nutrient. Apelian and Inouye (3) thus suggested that *sigC* functions to prevent development in the absence of an appropriate nutritional downshift.

In an experimental approach similar to that used to demonstrate the *M. xanthus* sigma factors, Inouye's laboratory has shown that a family of eukaryotic-like serine/threonine kinases exist in *M. xanthus* (86). They used probes derived from the highly conserved consensus sequences of subdomains VI and VIII of eukaryotic serine/threonine kinases as PCR primers. A number of the PCR products from the *M. xanthus* chromosome were likely candidates, and these were used for subsequent cloning and sequencing. Munoz-Dorado et al. (164) characterized one such gene, *pkn-1*, and showed that it indeed has substantial similarity to the catalytic domains of the eukaryotic kinases. When *pkn-1* was placed in *E. coli*, the *pkn-1* product was autophosphorylated at its serine and threonine residues. A *pkn-1* deletion mutant was found to be defective in its ability to form myxospores. A subdomain sequence of *pkn-1* was used as a further probe, which revealed an additional 23 bands on a Southern blot of an *Xho*I digest of *M. xanthus* chromosomal DNA (266). There are now a total of 26 putative kinase genes in *M. xanthus*, most of which have been cloned and 8 of which have been partially or completely sequenced (164). The details of this family of kinases and their interactions with a possible sigma cascade represent an exciting area of study in the regulation of *M. xanthus* development.

In view of the numerous parallels between eukaryotic and myxobacterial physiology, there have been occasional informal speculations that G-protein-mediated signal transduction pathways might exist in myxobacteria. After the demonstration by Caillon et al. that *S. aurantiaca* contains inositol phospholipids (23), Benaïssa et al. showed that the synthesis and degradation of these inositol phospholipids in *S. aurantiaca* are stimulated during Ca^{2+}-induced cohesion (11). They also showed that a phospholipase C activity increases during this process and is stimulated by the presence of GTPγS, an inhibitor of G protein cycling (66). This, of course, raises the possibility that a G-protein-like pathway may exist in *S. aurantiaca*.

Eastman and Dworkin (52) have shown that endogenous ADP-ribosylation occurs in *M. xanthus* and that the pattern of proteins that are thus modified changes during development. They have thus suggested that ADP-ribosylation is yet another covalent modification that may play a role in developmental regulation of *M. xanthus*.

For a more detailed description of those regulatory genes that have been characterized, see Downard and Kroos (38). For a complete description of the kinase cascades and sigma factors in *M. xanthus*, see Inouye and Inouye (86).

RIPPLING

It is the complex social behavior of the myxobacteria that originally caught the attention of most of us who work with them. None of these behaviors is more intriguing or puzzling than the process called rippling. This phenomenon was discovered by Hans Reichenbach, during his time-lapse photomicroscopic studies of *Corallococcus* and *Myxococcus* spp. (192), and was called rhythmische Vorgänge (rhythmic oscillations) (187). Prior to aggregation, the cells move in a series of equally spaced travelling ridges to form a highly organized, periodic pattern (Fig. 17). Neither the function of rippling nor its mechanism is understood. The first detailed study was by Shimkets

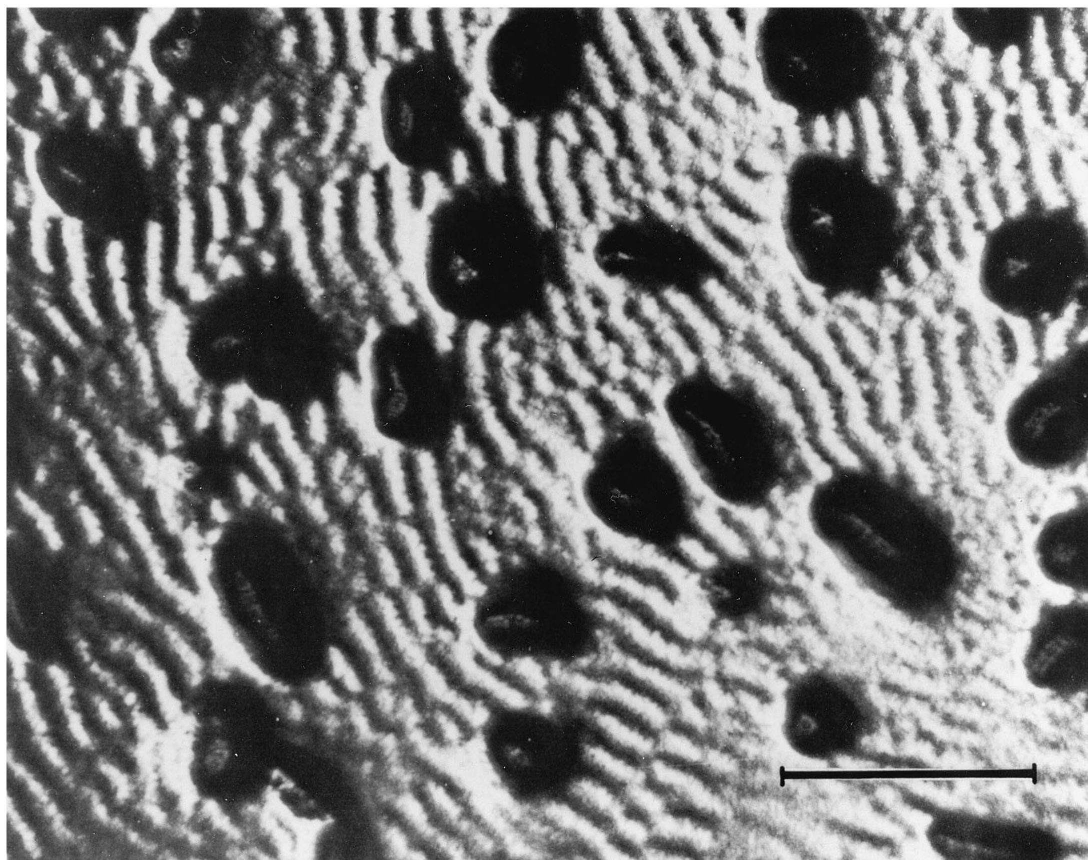

FIG. 17. Ripples generated during development on clone fruiting agar. The dark masses are fruiting bodies. Bar, 400 μm. Reprinted from reference 224 with permission of the publisher.

and Kaiser (224, 225), who showed that rippling could be induced by fragments of murein and that there is a close correlation between rippling and development. In addition, rippling requires the activity of both the A and the S motility systems (224). Since then, Sager and Kaiser have described the phenomenon more clearly and explicitly (210), and their observations have provided some important clues as to the mechanism of rippling. The ripples often appear as circular waves that spread outward in domains. When one wave encounters a wave from another domain moving in the opposite direction, the two waves appear to interpenetrate one another and to pass through each other unchanged, without any interference. This puzzling behavior was explained when it was shown that the opposing waves of stacked, moving cells do not actually interpenetrate and pass one another. Rather, when cells in countermigrating ripples meet each other in a head-to-head collision, the cells reverse their direction of movement. So, in effect, the cells in the opposing ripples, upon encountering each other, exchange directions and reflect off each other, and the waves appear to continue their travel unchanged.

Mutants defective in A or B signaling are able to ripple, but *csg* mutants are not (226). It had been previously shown that the C signal, which is a small protein associated with the cell surface, is required for rippling (135, 224). Sager and Kaiser (210) also showed that the head-to-head collisions between individual cells travelling in waves initiate C signaling and that the C signaling causes the cells to reverse the direction of their gliding. Thus, their model relates cell collisions, initiation of C signaling, and increases in the rate of reversal of cells travelling in ripples. Recall that Søgaard-Andersen et al. (233) proposed that head-to-tail collisions between cells initiate C signaling and subsequent aggregation (see the Aggregation section). It is feasible that *M. xanthus*, like *E. coli* (145) and *Caulobacter crescentus* (1), does have a head and a tail but that these are reversible, depending on the direction of movement, and that head-head and head-tail contacts play different roles in rippling and aggregation.

Many questions remain. What induces rippling? What is the role of murein fragments? How are the cells initially organized into the ripples? What are the forces that prevent the ripples from dispersing? Perhaps most puzzling, what is the function of rippling? Rippling is usually associated with fruiting body formation but is not required for it, i.e., Rhie and Shimkets (193) have shown that while mutants carrying suppressors of the *csgA* mutation have regained the ability to form fruiting bodies, they are still unable to ripple.

MOTILITY

The myxobacteria move by gliding on a solid surface and share this property with at least 6 of the 10 phylogenetic groups of the bacteria (234). The mechanism of gliding motility is unknown, and the reader is directed to Burchard (16) for a review of the literature on the subject. The only recent work on the mechanism of gliding motility is by Lünsdorf and Reichenbach (141), who recently claimed to have demonstrated a motility organelle in *M. fulvus*. However, their evidence is inferential and not at all compelling.

Cell motility plays an important role in development and morphogenesis in eukaryotic systems. It is clear that it is also indispensable for development and social behavior in the myxobacteria; nonmotile mutants are unable to form fruiting bodies (155), and of course, they cannot manifest the social behaviors such as swarming and rippling that are characteristic of wild-type myxobacteria.

The discovery of adventurous (A) and social (S) motility by Hodgkin and Kaiser (82) focused attention on the regulatory and social aspects of myxobacterial motility. This work demonstrated that there were two separate genetic systems that controlled motility. We now know that there are at least five classes of mutations that affect gliding motility (76). These are the *A* and *S* genes, *mgl* (mutual gliding), *frz* (frizzy), and *dsp* (dispersed).

Mutations in any of the genes controlling the S (social) system result in cells that are able to move as individuals but are reduced in their ability to glide as groups. Similarly, mutations in one of the *A* (adventurous) genes result in cells able to move only as groups (81). Mutations in *mgl* result in the complete loss of motility. The *mgl* locus was cloned (236) and sequenced (238) and shown to comprise two genes, *mglA* and *mglB*. The essential role of the *mgl* locus for gliding motility led to the hope that its gene products were either components of the gliding machinery itself or part of a global regulator of gliding. However, immunoelectron microscopy and immunoassay of subcellular fragments showed that MglA was located in the cytoplasm (74) and thus unlikely to function as a component of the gliding motor itself. In order to determine if MglA acted as a global regulator of A and S motility, MacNeil et al. (143) inserted Tn*5-lac* transposons in the *A* and *S* genes, generating transcriptional reporters of these genes. When these insertions were introduced into mgl^+ and *mgl* mutant strains, there was no difference in the levels of β-galactosidase produced, indicating that *mglA* did not regulate the transcription of those *A* and *S* genes.

The predicted amino acid sequence of MglA indicates a similarity with the family of GTP-binding proteins, and specifically with the consensus site for GTP binding and hydrolysis (75). Thus, even though it seems not to be involved in regulating the transcription of the *A* and *S* motility genes, its similarity to the family of G proteins suggests a possible role in signal transduction.

Mutations in the *mglB* gene disrupt motility and result in substantially reduced levels of the MglA protein (75). While segments of the predicted amino acid sequence of MglB show some similarity to one of the calcium-binding sites of yeast calmodulin (75), the function of MglB is still unclear.

For a discussion of the genetics of gliding motility in *M. xanthus*, the reader is directed to the recent review by Hartzell and Youderian (76).

Since it is necessary for cells to move into aggregation centers as a prelude to fruiting body formation, since the exchange of C factor requires that the cells move into close juxtaposition to each other (109, 110), and since morphogenesis of the fruiting body itself requires cell movement within the fruiting body (208, 209), the relationship between motility and development is indisputable (see the Role of Motility section). In addition, motility is obviously necessary for rippling (see the Rippling section).

What is the nature of the social interactions among cells during social motility? The first clue came as a result of the important observation that some S^- (*tgl*) mutants can temporarily regain S motility if they are placed within one cell length of a tgl^+ cell (82). The second clue came with the observations that the *tgl* mutant cells are invariably lacking polar pili and

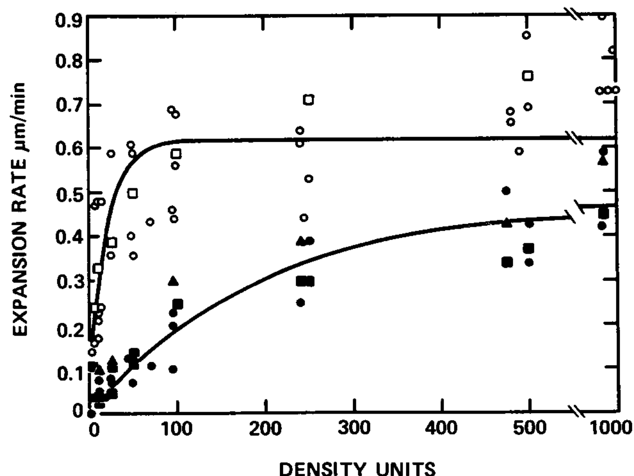

FIG. 18. Rate of swarm expansion versus cell density for three A^- S^+ strains (solid symbols) and two A^+ S^- strains (open symbols) of *M. xanthus* at 33°C. Reprinted from reference 98 by permission of the publisher.

that after the nongenetic complementation by tgl^+ cells, the temporary reappearance of social motility is accompanied by the equally transient ability to synthesize pili (97). Rosenbluh and Eisenbach (200) showed that the mechanical removal of pili from *M. xanthus* mimics the effect of a mutation in the S system but has no effect on A motility. And Wu and Kaiser (263) have recently shown that a mutation in the gene for pilus synthesis causes a loss of S motility. It thus became clear that polar pili are associated in some nontrivial way with social motility. However, the possibility that social motility is simply A motility with the cells held together by their pili could be excluded by the motility of A^-S^+ mutants. (See the Pili section for a further discussion of the possible role of pili.)

Given the pervasive social quality of myxobacterial behavior, S motility intuitively seems to make sense. However, what is one to make of A motility, i.e., the so-called ability of cells to move as single individuals? The following evidence suggests that adventurous motility may not be as independent of cell-cell interactions as first believed. It has been a common observation that while A^+ cells can move as individuals, their normal behavior is to remain in close proximity to the main swarm. Cells that do leave the swarm as individuals soon reverse their direction (without turning) and reenter the swarm (255). The first nonmotile mutants whose motility could be temporarily restored by the proximity of a wild-type cell were the *cgl* subgroup of the *A* mutants (80). And Kaiser and Crosby (98) showed that the rate of A motility (using swarm expansion as a parameter), like S motility, is dependent on cell density, i.e., on the proximity of other cells (Fig. 18); note, however, that the shapes of the two dependency curves are different. Thus, the expansion rate as a function of cell density for the A^+S^- cells reaches a maximum at a relatively low cell density (100 Klett units, or about 2×10^8 cells per ml). At this cell density, the cells are about 20 µm apart, far outside the range where cell-cell or cell-pilus interactions are likely. This is in contrast to the situation with the A^-S^+ cells, for which the threshold seems to be reached at 1,000 Klett units, or about 2×10^9 cells per ml. At this cell density, the cells are about 2 µm apart. These calculations are consistent with the notion that for S motility, the cells must be within pilus range of each other; for A motility, however, stimulation of the rate of motility can take place over distances as great as 25 µm. Kaiser and Crosby (98)

have suggested that A motility may require a motility-enhancing factor that does not depend on pili. One possibility is that the main swarm may release such a diffusible factor, which has a range of about 20 μm. Alternatively, it may be that the cells must periodically return to the swarm in order to replenish a rapidly turning over supply of the motility-enhancing factor. In either case, dependence on such a factor would guarantee that individual cells would never be separated from the main swarm by too great a distance.

CAROTENOIDS AND LIGHT

In 1966, Burchard and Dworkin (17) showed that blue light has two distinct effects on *M. xanthus*. If the cells are in the exponential phase of growth, light results in the induction of carotenoid synthesis which accelerates as the cells reach stationary phase. On the other hand, cells in stationary phase that had been grown in the dark (and were thus devoid of their photoprotective carotenoids) undergo massive photolysis when exposed to blue light. The photosensitizing pigment was shown to be protoporphyrin IX, which in stationary-phase cells is present at 16 times the concentration found in exponentially growing cells (17). The results of action spectra for photolysis (21) and for carotenogenesis (19) strengthened the conclusion that protoporphyrin IX is the photosensitizing molecule and that both photolysis and carotenogenesis are mediated by the same photosensitizer. A mutant constitutive for carotenoid production was used to provide evidence that the carotenoids in *M. xanthus* act as photoprotective pigments, as has been demonstrated in numerous other systems (22).

Since then, a series of elegant experiments have capitalized on the power of molecular biology and molecular genetics and have begun to uncover an elaborate scheme for the regulation of carotenogenesis and to illuminate the process whereby light energy is transduced into a regulatory signal (see the recent review by Hodgson and Murillo [84]). Carotenogenesis in *M. xanthus* is controlled by the *car* regulon. The genes comprising the regulon are listed in Table 2 and are distributed among at least three unlinked chromosomal regions. The current model for the regulon, as suggested by Hodgson and Murillo (84), is illustrated in Fig. 19 and is as follows. Blue light interacts with the photosensitizing pigment, protoporphyrin IX, to generate an excited triplet state, with the concomitant release of singlet oxygen ($^1O_2$). CarQ and CarR exist in a complex embedded in the cytoplasmic membrane. The excited oxygen causes the dissociation of the complex by an unknown mechanism, resulting in the functional release of CarQ. Lonetto et al. (137) have shown that the *carQ* gene is strongly homologous with a family of (thus far) eight σ factor genes, present in a group of phylogenetically diverse bacteria, that regulate extracytoplasmic functions and respond to extracellular signals. Thus, CarQ is presumably acting as a σ factor and CarR as an anti-σ factor (160). CarQ then causes the increased expression of the *carQRS* and the *carC* promoters. *carC* codes for phytoene dehydrogenase, the enzyme responsible for the sequential dehydrogenations that result eventually in the fully unsaturated carotenoid pigments (61, 152). The relationship between the expression of the *carC* gene cluster and the growth and nutrition of the organism has been examined by Fontes et al. (61). They have shown that the light activation of the *carC* locus occurs only when the cells reach the stationary phase of growth or are starved of a carbon source. This provides an explanation of the earlier observation that *M. xanthus* accumulates carotenoids mainly in the stationary phase of growth (17). The *carS* gene product along with the *carA* gene allows the expression of the genetically unlinked *carB* cluster, which bears a homologous relationship to the phytoene synthetases of other bacteria. This results in the eventual synthesis of the esterified carotenoid myxobacton, which quenches the signal that inactivates the anti-σ factor CarR (83). Ruiz-Vazquez et al. (205) have shown that there is a cluster of genes between the *carA* and *carB* genes that code for the synthesis of one and possibly two enzymes involved in the terminal steps of carotenoid synthesis. The authors suggest that this cluster of genes is transcribed from a single, light-activated promoter under the control of the *carA*, *carQ*, and *carR* genes.

The last gene in the model is *carD*, which has turned out to be a most interesting element in the regulon. *carD* mutants are blocked in their ability to express the regulatory *carQRS* operon as well as the structural *carC* cluster. They are also blocked in development and in the ability to express A factor- and C factor-dependent reporter genes. Furthermore, the *carD* mutant can be phenotypically rescued by being mixed with wild-type cells, indicating that the transfer of a paracrine signal is involved in the process. The genetic data are consistent with the idea that CarD is required to activate the photoinducible promoters of the *carQRS* operon and the *carC* genes (168). These results reflect an interesting and puzzling relationship between carotenoid synthesis and development. The *carD* gene has been sequenced, and it is homologous to a eukaryotic transcriptional activator (164a).

The work on the *car* regulon illustrates the immense ability of the molecular approach to dissect and clarify an extremely complex regulatory network.

PROTEIN SECRETION

The myxobacteria secrete a greater diversity of proteins and to a far greater extent than is characteristic of other gram-negative bacteria that have been examined (70). This is undoubtedly a reflection of the fact that they depend on the secretion of a variety of hydrolytic enzymes in order to feed on high-molecular-weight macromolecules (197); in addition, their social behavior depends on their ability to exchange at least one high-molecular-weight extracellular signal (99).

It is interesting that although about 20,000 Tn*5* insertion mutants have been screened in Guespin-Michel's laboratory, no mutants of *M. xanthus* that are completely blocked in their ability to secrete proteins have been isolated (167). This suggests, as one explanation, that such a mutation would be lethal for a myxobacterium. In a sense, that is not surprising, as growth, social behavior, and development all depend on the export of proteins as either degradative enzymes (198), extracellular signals (99), or structural components of myxospores (68, 159, 165). Alternatively, multiple secretion systems may exist. In all of the secretion mutants that have been isolated, there is a partial and general reduction ($Exc^{\pm}$) or an increase (Exc^{++}) in all secreted proteins, but no single protein is differentially affected (70).

There is a complex and as yet unresolved relationship between protein secretion and development. Two $Exc^{\pm}$ mutants whose mutations fall into two loci, *excA* and *excB* (which are also tan variants), are unable to form fruiting bodies and are reduced in their ability to form myxospores. In addition, signaling mutants (*asgA*, *B*, and *C*) and one *bsg* and one *dsg* mutant behave somewhat like the $Exc^{\pm}$ mutant with regard to protein secretion (177).

There is some indirect evidence that signal peptides may be involved in protein export by *M. xanthus*. The sequence of the gene coding for protein U (68) bears a slight resemblance to the signal sequence of enteric bacteria, and *M. xanthus* is able to express the genes and export the products of genes coding

TABLE 2. *M. xanthus car* genes[a]

Type of gene and name	Phenotype	Carotenoid content	Description
Regulatory			
carQ	Car$^-$	None in light or dark	First gene of light-inducible *carQRS* operon. CarQ is required for activation of p^{carQRS} and p^{carC}.
carR	Car^c	Wild type in light and dark	Second gene of light-inducible *carQRS* operon; translationally coupled to *carQ*. CarR is membrane bound and inactivates p^{carQRS} in dark. Light inactivates CarR. Mutations in *carQ* are epistatic to *carR* mutations.
carS	Car$^-$ (Δ*carS*)	ND[b]	Third gene of light-inducible *carQRS* operon; translationally coupled to *carR*; required for light activation of *carB*.
	Car$^+$*carB*^{c} [*carS*(Um)]	ND	*carS* nonsense mutation (umber) causes constitutive expression of *carB* in light and dark but no Car(Con) phenotype.
carD	Car$^-$ Agg$^-$ Fru$^-$	ND	Distinct genetic locus. CarD is required for expression of *carQRS* operon and hence all structural *car* genes. CarD is also required for expression of several developmentally regulated genes.
carA	Car^c	Dark, phytoene; light, wild type plus excess phytoene	Linked to *carB* cluster of structural genes. Mutation *carA1* causes constitutive expression of *carB* in dark and light while reducing light activation of *carC*. *carA1* effect on *carB* is epistatic over effects of *carQ* or *carD* mutations.
Structural			
carB	Car$^-$	ND	ORF[c] with homology to phytoene synthetase genes of other bacteria; grouped with other structural genes in single, light-inducible operon linked to *carA*. Mutations at *carQ*, *carS* (deletion), or *carD* block expression of operon. Mutations at *carR*, *carS*(Um), and *carA* cause light-independent expression.
carC	Car$^-$	Dark, no carotenoids; light, all-*trans* phytoene	Distinct genetic locus. ORF has homology to phytoene dehydrogenase genes. Activated by light only after cells enter stationary phase. Mutations in *carQ* or *carD* block light activation. Mutations in *carR* cause light-independent expression. Mutations in *carS* have no effect on *carC*. Mutation *carA1* reduces stimulatory effect of light on *carC* expression.

[a] Reprinted from reference 84 with permission of the publisher.
[b] ND, not determined.
[c] ORF, open reading frame.

for foreign proteins that contain signal peptides (14, 15). On the other hand, at least two excreted proteins, protein S (165, 244) and the myxobacterial hemagglutinin (196), lack signal peptide precursors.

The failure to obtain any mutants that are completely Sec$^-$ has been a serious impediment to fully characterizing the process of protein export and secretion. As an alternative strategy, workers in Guespin-Michel's laboratory have studied the secretion of a variety of foreign proteins by *M. xanthus*. Thus, the secretion of the periplasmic acid phosphatase of *E. coli*, encoded by the *appA* gene (133), and five pectate lyases from *Erwinia chrysanthemi* (15) has been studied in *M. xanthus*.

Letouvet-Pawlak et al. (133) placed the gene for the *E. coli* periplasmic pH 2.5 acid phosphatase, which is fully secreted in its native host, into *M. xanthus*. Secretion of the acid phosphatase thus expressed in *M. xanthus* is limited by its diffusion through the periplasmic space and thence through the outer cell membrane. When the acid phosphatase gene was placed in the Exc$^\pm$ mutant, secretion was substantially delayed; conversely, in the Exc$^+$ mutant, secretion was accelerated. Thus, they have proposed that the limiting (and perhaps controlling) steps in the secretion of some proteins by *M. xanthus* may be their diffusion through the periplasm and the outer membrane. On the other hand, the pectate lyases are readily secreted by the *M. xanthus* recombinants (15), leading Guespin-Michel and Monnier (69) to conclude that protein secretion by *M. xanthus* lacks the specificity characteristic of most other secretory systems.

The composition of the outer membrane of *M. xanthus* has been shown not only to differ from that of other gram-negative bacteria (174) but also to change substantially during development (175). This is consistent with the notion that the diffusion properties of the outer surface of *M. xanthus* differ from that of other gram-negative bacteria, change during development, and may play a role in the regulation of protein secretion. In this context, it is interesting to recall the proposal of Rosenbluh

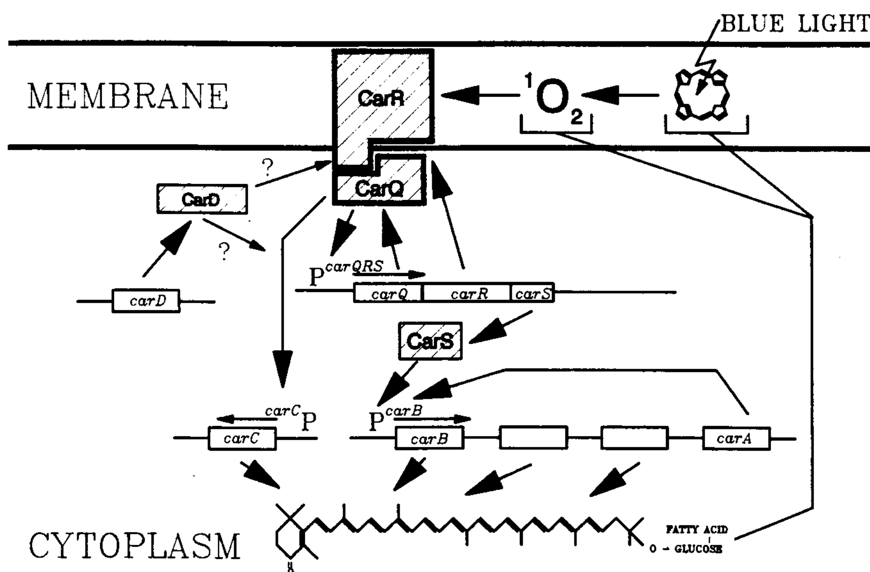

FIG. 19. *car* regulon of *M. xanthus*. Reprinted from reference 84 with permission of the publisher.

and Rosenberg (202) that the autocide AMI, produced during development, functions to increase the permeability of the cells, allowing the release of cell-signaling molecules.

In any case, it is clear that, as with a number of other aspects of the physiology of *M. xanthus*, new and unusual physiological mechanisms may be at work.

ANTIBIOTICS

The myxobacteria have been shown to be a rich source of new antibiotics. Among the thousands of strains that have been screened, 55% of the bacteriolytic myxobacteria and 95% of the cellulolytic myxobacteria have yielded bioactive compounds. From these, over the past 15 years, Hans Reichenbach's group has isolated and identified nearly 300 antibiotics, comprising about 50 basic structural groups. Most of these have turned out to be new antibiotics with occasionally unique modes of action (191). In addition, Rosenberg's laboratory has been studying the regulation of the biosynthesis of antibiotic TA, produced by *M. xanthus* (251, 253). Antibiotic TA is a broad-spectrum antibiotic with the unusual property of sticking tenaciously to surfaces, either of substrata or of cells (199). This has made it an obvious candidate for clinical applications requiring a high, persistent local concentration of antimicrobial activity (149, 150). Antibiotic TA is probably one of the many variants of myxovirescin, a class of antibiotics produced by many strains of *M. xanthus* and *M. virescens* (191).

Reichenbach and Höfle (191) have pointed out a number of general principles that apply to antibiotic production by the myxobacteria.

(i) Unlike other groups of bacteria, e.g., the genera *Bacillus* and *Pseudomonas*, which in general produce only one chemical class of antibiotics, the myxobacteria produce aromatics, heterocyclics, alkaloids, quinoids, peptides, isoprenoids, macrocyclics, and polyenes. In addition, a new class of boron-containing macrodiolides, the tartrolons, has recently been described (211). In this sense, the myxobacteria resemble the actinomycetes.

(ii) The overwhelming majority of myxobacterial antibiotics are new chemical compounds; only 3 of the 50 basic structures that have been characterized had been found in other organisms.

(iii) A single strain of myxobacteria may produce many variants of a basic compound. Thus, one strain of *M. fulvus* produces 35 different myxothiozols, one strain of *M. virescens* produces 20 variants of myxovirescin, and one strain of *Sorangium cellulosum* produces 50 variants of soraphen.

(iv) The ability to produce a particular antibiotic is characteristic of a particular strain and not of a species or of a genus. Since there are only about 40 species of myxobacteria but an unlimited number of strains, this is an important consideration. However, production of the same compound may cross species, genus, and family lines.

(v) A single strain may produce a number of different classes of antibiotics.

As is the case for other groups of antibiotic-producing organisms, the function of antibiotic production by the myxobacteria is unknown. They share with *Bacillus*, the actinomycetes, and the fungi the fact that they go through a developmental cycle. However, the fact that nonproducing *Bacillus* (104) and *Streptomyces* (27) mutants can still undergo apparently normal development suggests that that connection is no more than a correlation.

It is possible that, in view of the fact that the bacteriolytic myxobacteria are predators of and feed on other bacteria, antibiotics are part of the mechanism by which they kill their prey. However, this does not explain the prevalence of antibiotic production among the cellulolytic myxobacteria.

Alternatively, a common rationalization for antibiotic production is that the antibiotics play a role in intermicrobial competition. Apart from the fact that there is no convincing experimental evidence for this, it is not obvious that the levels of antibiotic achieved under natural conditions are sufficient to act as an effective control.

It is interesting that the myxobacteria, like the streptomycetes, have an exceptionally large genome, 9,454 to 10,010 kbp (28, 166), which allows them the luxury to code for the biosynthesis of a large number of complex secondary metabolites.

For a more detailed description of myxobacterial antibiotics, the reader is referred to the recent review by Reichenbach and Höfle (191).

MYXOSPORE MORPHOGENESIS

During myxospore morphogenesis, vegetative rods are converted to round, encapsulated, optically refractile, metabolically quiescent, resistant resting cells. There is essentially no information available on the physiological or metabolic changes that occur during this process, and correspondingly little information about the regulatory or molecular processes that would be expected to accompany these changes is available. Originally, it was hoped that these processes could be studied during glycerol induction of myxospores (see Glycerol Spores section), which was an experimentally more convenient system than was available for fruiting body myxospores. Indeed, a fair amount of information was obtained about murein changes, intermediary metabolism, spore coat synthesis, and macromolecular changes (see reference 257 for a review of this information). With the realization that there were important differences between glycerol-induced and fruiting body myxospores (268), emphasis shifted to the examination of the surface proteins of fruiting body myxospores. Among these, the most intensely studied has been protein S, a 19-kDa major protein component of the thick spore coat that surrounds the fruiting body myxospore (see the review chapters by Downard and Kroos [38] and by Dworkin [47]). Protein S has two high-affinity Ca^{2+}-binding domains and shares sequences both with calmodulin (88) and with bovine crystalline lens protein (262). Protein S and the closely related protein S1 are coded for by the *tps* and the *ops* genes, respectively. These highly similar genes are adjacent to each other and are separated by a short spacer sequence (89, 90). Protein S is synthesized early in development and appears eventually on the surface of the myxospore (88); protein S1, on the other hand, is synthesized late in development and accumulates inside the spores rather than on their surface (40, 244). Mutations that block the formation of protein S delay development but eventually have no effect on the formation of fruiting bodies or myxospores (116). Thus, the actual function of protein S remains unknown.

Protein U, another spore coat protein, is synthesized during the late stage of development (87) and is secreted and assembled on the surface of the myxospore. It differs from protein S in that it is secreted as a pro-protein U with a signal peptide (68). Thus, there are at least two pathways of excretion of spore coat proteins; why this should be so is not obvious.

A third major component of the myxospore coat, protein C, has recently been described (159). Its molecular mass is 30 kDa, it is synthesized earlier than protein S, and, like protein S, it appears as two closely related species.

McBride and Ensign (153) made the interesting observation that vegetative cells of *M. xanthus* contain trehalose, a common storage carbohydrate in microorganisms that has been shown to play a role in heat and desiccation resistance (33). McBride and Zusman (154) subsequently showed that the level of trehalose increases considerably in glycerol-induced and fruiting body myxospores. The trehalose is rapidly metabolized upon myxospore germination and thus may be serving one or both of two functions—as a stress protectant in the spores or as an energy source during germination.

GLYCEROL SPORES

In 1964, Dworkin and Gibson described a technique for inducing the rapid and synchronous conversion of vegetative rods of *M. xanthus* to myxospores (49). The addition of relatively high concentrations of glycerol or any one of a number of other related compounds (206, 207) induced vegetative cells to convert to round, optically refractile, resistant cells in about 120 min. These cells were able to germinate, and thus this provided a convenient experimental technique for beginning the study of myxobacterial morphogenesis. As indicated in the preceding section (Myxospore Morphogenesis), the discovery that the myxospore coat protein S was absent from glycerol-induced myxospores led to a more careful comparison of glycerol spores and fruiting body spores and to a more cautious use of glycerol spores in studying developmental events.

A number of developmental genes have been tested to determine if they are expressed during glycerol-induced myxosporulation. Of the two similar genes, *tps* and *ops*, that code for the myxospore surface proteins S and S1, respectively, only the later-expressed, upstream gene, *ops*, is expressed during glycerol induction (40), and only 1 of 13 Tn*5-lac* developmental insertions was expressed during glycerol induction (117). Apelian and Inouye (3) have shown that the *sigC* gene, which appears to regulate the developmental response to a nutritional downshift, is expressed in glycerol-induced spores. It is thus clear that glycerol-induced myxospores lack some important structural features of mature fruiting body myxospores and that the process of glycerol induction bypasses much of the normal developmental program. Nevertheless, glycerol induction of myxosporulation is valuable for determining whether the inability of a developmental mutant to form myxospores reflects a regulatory lesion or is a result of a fundamental inability to undergo myxospore morphogenesis. In addition, both the morphogenetic and the regulatory changes that occur should be instructive in terms of understanding the ability of a cell to change from an actively metabolizing, growing cell to a resistant resting cell.

TERRITORIALITY

It has been a common experience of those who have sought to isolate myxobacteria from the fruiting bodies that appear on cultivated rabbit dung or pieces of bark that the fruiting bodies invariably appear as clusters of a single type and that these clusters are always well separated from those of other species or genera. Given the social quality of myxobacterial behavior, two obvious questions arise. First, will swarms of two different myxobacteria that encounter each other merge and form a chimeric fruiting body? I.e., can the myxobacteria distinguish self from nonself? The second question concerns the mechanism that prevents the establishment of clusters containing mixtures of different fruiting bodies; i.e., what are the mechanisms of myxobacterial territoriality?

Walter Fluegel was the first to systematically address the question of fruiting body distribution. He showed that fruiting bodies of *M. fulvus* induced to form under conditions of submerged culture (59) are evenly distributed. He suggested that there must therefore be a spacing mechanism (60).

Smith and Dworkin have recently examined the questions of self/nonself recognition and the mechanism of the establishment of territoriality (232). They have shown that even the closely related species *M. xanthus* and *M. fulvus* will not form mixed fruiting bodies. Furthermore, they have shown that each of these two species produces an extracellular factor(s) that inhibits the growth of the other. The properties of these factors are consistent with the possibility that they are bacteriocins. Smith and Dworkin (232) have suggested that this may be an example of an ecological function for bacteriocins. In a similar fashion, swarming *Proteus* strains often fail to merge with each other and form lines of demarcation referred to as the Dienes phenomenon (36). Similar to the situation with the establishment of myxobacterial territoriality, the Dienes phenomenon has been shown to be due to the elaboration of protocines,

bacteriocins produced by the *Proteus* swarms (213). In both of these groups of social bacteria, the mechanism of self/nonself recognition may be largely based on the success of their bacteriocins and may determine which of two closely related and presumably competing species will occupy a particular territory.

MATHEMATICAL MODELING

There are two groups attempting to generate insights about myxobacterial aggregation and social movement by means of mathematical modeling. Pfistner (178) has used the parameters of gliding velocity and turning frequency to construct a mathematical model that describes the one-dimensional behavior of swarms or "streets" of cells of *M. xanthus*. Stevens (239) has been able to construct a cellular automaton model that generates rudimentary aggregates based on only the production of large quantities of slime trails. In order for the model to generate true aggregates, it was necessary to add a diffusing chemoattractant to the model (240). However, recent experiments (see Aggregation section above) suggest that a diffusing chemoattractant is not involved in developmental aggregation.

MYXOSPORE GERMINATION

It is interesting that the process of spore germination never seems to attract the kind of detailed attention that is usually lavished on spore formation. This has historically been the case with bacterial endospores and is proving to be the case as well with myxospores. Germination of *M. xanthus* myxospores was first described by Thaxter (247). A series of morphological (51) and physiological studies were done on the germination of glycerol-induced myxospores (96, 185, 186), but this approach was abandoned when it became clear that there were important physiological differences between glycerol-induced and fruiting body myxospores (100). Since then, there have been two publications on the germination of fruiting body myxospores. The first of these reported the kinetics of morphological changes and loss of resistance and the effects of calcium and amino acids as inducers of germination (54); the second reported the isolation of a set of seven germination-defective mutants and the cloning of one of the mutated genes (55).

STIGMATELLA AURANTIACA

Most of the work on the developmental and social biology of the myxobacteria has been done with *M. xanthus*. Nevertheless, the fruiting body of *S. aurantiaca* is a substantially more complex structure. In addition to an essentially acellular stalk, the cell mass is further differentiated into a cluster of sporangioles, each of which contains an undetermined number of resistant resting cells (Fig. 1B). It was the challenge of trying to understand the morphogenesis of this beautiful and complex structure that originally led David White to carry out most of the pioneering work on the physiology and microbiology of *S. aurantiaca*. In a like fashion, Schairer's laboratory is currently working out methods for genetic and molecular analyses of *S. aurantiaca.*

In addition to his electron microscopic description of aggregation and fruiting body formation (see the Aggregation section), White's laboratory made two other important contributions concerning the role of visible light and of the *S. aurantiaca* pheromone. They showed that *S. aurantiaca*, unlike *M. xanthus*, requires visible light for optimal fruiting body formation when the cell densities are below a threshold (258). The light apparently increased the sensitivity of the cells to a pheromone that is produced during aggregation (237). The pheromone is a volatile, branched alkane derivative with a molecular weight of about 200, containing only C, H, and O (210a). While it appears to differ structurally from the increasing family of quorum-sensing autoinducers (43), it seems to fulfill a similar function of cell density monitoring.

As a first step toward establishing a genetic analysis for *S. aurantiaca*, Glomp et al. used membrane mating between *E. coli* and *S. aurantiaca* to generate a series of Tn*5* transposon mutants. The transposon vehicle was either an RP4 plasmid or a small multicopy plasmid (67). More than 600 mutants have thus been obtained, some of which are blocked at different stages of development (182). It has also been possible to generate a series of Tn*5-lacZ* transconjugants as promoter probes (182) and thereby to detect the expression of a number of developmentally regulated genes. As part of the attempt to begin to understand the regulatory mechanisms in *S. aurantiaca*, Skladny et al. (231) used an antibody directed against the σ^{80} protein of *M. xanthus* to detect a similar polypeptide in vegetative cells of *S. aurantiaca*. The gene coding for the *S. aurantiaca* SigA has been cloned, sequenced, and expressed in *E. coli*, and the gene product has been characterized.

Neumann et al. (166) constructed a physical map of the *S. aurantiaca* chromosome by pulsed-field gel electrophoresis and confirmed that the size of the genome of *S. aurantiaca* is indeed 9.35 Mbp. It is likely that studies on *S. aurantiaca* will eventually provide the opportunity to examine the molecular basis of the formation of complex multicellular structures.

EUKARYOTE-LIKE PROPERTIES OF MYXOBACTERIA

Computer-assisted homology searches over the years have uncovered a considerable number of bacterial genes that bear substantial similarity to their eukaryotic counterparts. The myxobacteria, however, despite the fact that they are no more closely related phylogenetically to the Eukarya than to the Bacteria (140), show an especially high number of similarities with the Eukarya. Munoz-Dorado et al. (164) showed the presence of serine-threonine kinases in *M. xanthus*. These kinases had previously been thought to be characteristic solely of the Eukarya (53). Protein S (referred to in the section on Myxospore Morphogenesis) shares some properties with bovine brain calmodulin; two of its internal domains contain a sequence of nine amino acids that is similar to the Ca^{2+}-binding sequence of calmodulin (89). There is an even more striking similarity between protein S and the β and γ crystallins of the vertebrate eye lens, and it has been suggested that these crystallins may have evolved from myxobacterial proteins (262). Baker (6) has shown a statistically significant sequence similarity between C factor and human 17β-hydroxysteroid dehydrogenase and has suggested that they evolved from a common ancestor. In fact, Kohl et al. (114) have demonstrated the presence of steroids in *Nannocystis exedens*, which is one of the phylogenetically distinct lines in the myxobacteria (140, 227). Inositol phosphates have been shown to play a role as second messages in signal transduction processes among the Eukarya (66). Benaïssa et al. (11) have demonstrated a phosphatidylinositol cycle in *S. aurantiaca* and have shown a GTPγS dependency of a developmental phospholipase C, suggesting that a G protein may play a role in the process. Finally, the startling demonstration of the presence of RT in *M. xanthus* (91, 92) and in myxobacteria in general (194) has left little doubt that the myxobacteria have played some kind of role in the evolution of eukaryotic multicellularity.

EPILOGUE

The past 20 years or so have seen an impressive and gratifying accumulation of fundamental information about the myxobacteria. Much of the basic biology of at least one member of this group of bacteria, which exemplify social behavior and developmental complexity, has been defined. We are beginning to understand the signaling processes during development, many of the underlying regulatory processes are yielding to the exciting advances in genetics and molecular biology, and the technology for probing the organism from its surface to its genome is well in hand. The challenge that faces us is now to begin to understand the more complex multicellular behavioral and developmental phenomena that originally intrigued many of us who work on the myxobacteria.

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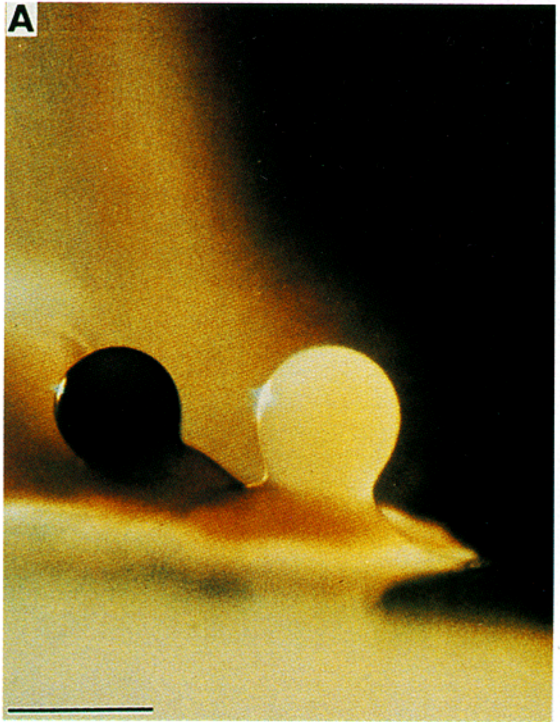
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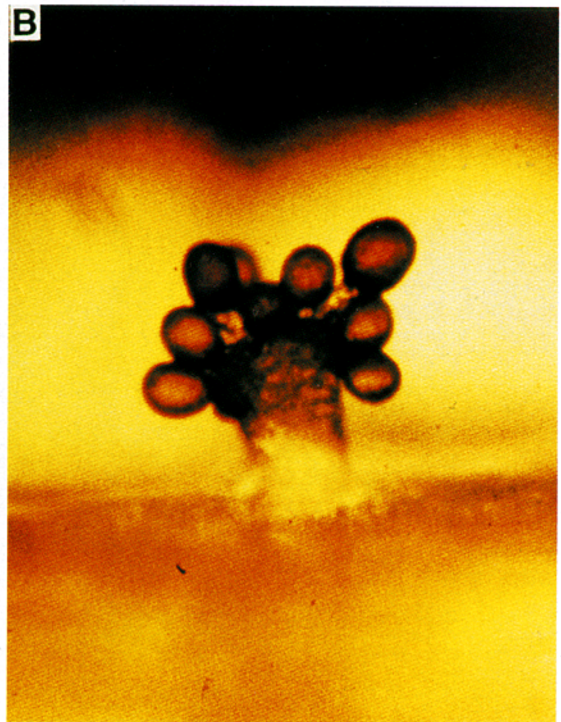
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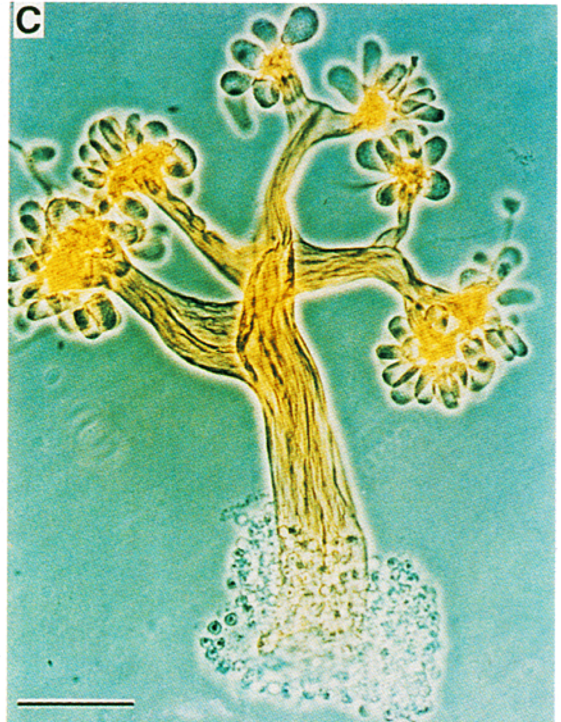
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