For one reason or another, the exploration of the universe plays a role for us today somewhat like that played by the exploration of the earth in the time of Columbus. In many nations throughout the world, these explorations awaken the imagination of the public, they gain the sometimes generous support of their governments, and they attract the most strenuous efforts of the men and women who undertake the explorations. One great difference is that, while the exploration of the world set the nations of Europe at each other's throats, the exploration of the universe has tended to bring them together. In my own area of subatomic physics, we have the example of CERN, the greatest international scientific collaboration of history. In the Canary Islands we see the productive cooperation of Denmark, Ireland, Germany, the Netherlands, Spain, Sweden, and the United Kingdom, all working together, as on a happy ship, to find new worlds.

In closing, I want to go back to one more point about cosmology. You may have noticed that, despite all these brave words, I have not explained the origin of the universe. The reason, of course, is that this is a matter about which scientists still have no clear idea. We can trace the history of the present period of expansion back to its first million years, or its first three minutes, or its first ten billionth of a second, but we still do not know if time really began just a little before then, or if so, then what started the clock. It may be that we shall never know, just as we may never learn the ultimate laws of nature. But I wouldn't bet on it.

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Cell Interactions in Myxobacterial Growth and Development

Martin Dworkin and Dale Kaiser

Cell interactions dominate the activity of the nervous, immune, and endocrine systems of animals. Ever since the seminal work of Hans Spemann (*1*), it has become increasingly evident that cells of animals and plants rely on interactions also to guide their proper development in embryos (*2, 3*).

Among the variety of known interactions, four distinct patterns have emerged (*4*):

1) Cells may secrete substances that are freely diffusible. Such substances may signal other cells that are near or far—depending on the anatomy, amounts secreted, and the stability of the secretions.

2) Cells may secrete nondiffusible substances, such as an extracellular matrix, that modify the behavior of adjacent cells.

3) Cells may bind other cells and influence a bound cell by direct contact.

4) Cells may join their cytoplasms by means of localized fusions or pores. Some junctions, thus formed, allow cytoplasmic mixing; others permit only certain substances to pass.

Although many cell interactions are known in animals and plants, it is less widely appreciated that bacteria also benefit from the advantages of cell interactions. One group of bacteria in particular, the myxobacteria, actually live as rudimentary multicellular organisms and engage in various cell interactions; both diffusible substances and cell contact interactions appear to have roles in the myxobacterial life cycle. In this article we delineate the facility with which molecular genetics, biochemistry, and cell biology can be applied to the study of bacteria.

The Myxobacteria

Myxobacteria move, feed, and lie dormant in organized multicellular masses. In fact, the structural complexity of myxobacterial fruiting bodies (Fig. 1) led to their mistaken identification as fungi (*5*), until Thaxter (*6*) recognized their prokaryotic nature. The fruiting bodies of myxobacteria are the products of multicellular development and cellular differentiation. The swarming motility of myxobacteria, their cooperative feeding, and their development of fruiting bodies are based on interactions between cells. The interactions are precise because, for example, the structure of the fruiting

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bodies of each species are sufficiently consistent to serve as taxonomic characters (Fig. 1) (*7*).

Despite the apparent complexity of their feeding, swarming, and fruiting behavior, myxobacterial cells have the structural and chemical simplicity of gram-negative bacteria. They can be grown in large cultures of uniform cell type. Their genome consists of 5700 kilobase pairs of DNA (*8*). The methods of recombinant DNA and transposon genetics have been applied to myxobacteria. As consequences of their simple cellular structure, relatively small genome, and the facility with which genetics can be applied to them, it may be possible to define in molecular terms how these cells interact with each other. Before reviewing what is known of their cell interactions, we briefly summarize their multicellular development and related aspects of their biology. A general review on myxobacteria is now available (*9*).

Myxobacteria live in the soil where they feed on insoluble macromolecules. They degrade protein, microbial cell walls, cellulose, and other polymers with secreted and cell-bound hydrolytic enzymes. The efficiency of feeding on a polymeric substrate, like the protein casein, more than doubles with increasing cell density, and growth occurs only if initiated from a large inoculum (*10*). Thus a dense population of myxobacteria is able to pool the action of digestive enzymes from many individual cells, so that the group feeds like a microbiological "wolf-pack" (*11*). For this reason, apparently, the ability of cells to assemble and to stay together in multicellular masses has been selected in the course of evolution. The close genetic relation of all the families of myxobacteria has been shown by comparison of the sequences of their 16*S* RNA's (*12*). Although other bacteria, like *Cytophaga*, also feed in multicellular groups, only the myxobacteria lie dormant in multicellular groups.

The life cycle of *Myxococcus xanthus*, the most commonly studied myxobacterium, can be divided into two phases (Fig. 2). One phase is vegetative growth. Cells in a rich medium grow exponentially and divide by binary transverse fission. Most of the laboratory strains of *M. xanthus* in common use grow in a dispersed state in liquid medium–enzymatic hydrolyzate of casein, for example—with a generation time of 4 hours and can reach a final population density of 5×10^9 cells per milliliter. They will also grow in a minimal defined medium; *M. xanthus* is unable to synthesize only three amino acids—leucine, isoleucine, and valine. They form colonies from single cells on solid media, and in general are amenable to all the manipulations commonly used for the study of bacteria (*9*).

Summary. During their complex life cycle, myxobacteria manifest a number of cell interactions. These include contact-mediated interactions as well as those mediated by soluble extracellular signals. Some of these interactions are well-defined; in addition, the tools for molecular and genetic analysis of these interactions in *Myxococcus xanthus* are now available.

Vegetative growth ceases and there is a transition to the developmental phase of the life cycle if several conditions prevail. These conditions are: (i) depletion of nutrient (*13*), (ii) presence on a solid surface, and (iii) high cell density (*14*). Then, many thousands of cells move to aggregation centers where they assemble multicellular structures that have species-specific shapes (*7, 15*) (Fig. 1).

After the period of multicellular morphogenesis, there follows a period of cellular morphogenesis. The initially rod-shaped (vegetative) cells differentiate into round, optically refractile (*16*), metabolically quiescent (*17*), and resistant myxospores (*18*). Normally, vegetative rods convert to myxospores as one of the last stages in the development of a fruiting body. It is possible, however, to circumvent formation of the fruiting body and to induce more than 95 percent of the vegetative rods to convert to myxospores in liquid culture by adding 0.5*M* glycerol (*19*) or 0.7*M* dimethyl sulfoxide (*20, 21*), or any of a number of other inducing agents. The spores thus formed lack the outermost coat of fruiting body myxospores, an aggregate of protein S (*21*). However, glycerol spores are heat, radiation, and desiccation resistant, and they contain spore protein U (*21*), suggesting that they are closely related to fruiting body spores.

Before sporulation and the completion of fruiting body development, many cells lyse (*22*). Only the cells that escape lysis can become myxospores. The time of lysis and the number of lysing cells depends on the species and on the nutritional conditions; in *M. xanthus*, for example, 90 percent of the cells lyse just prior to sporulation (*14, 22*). An analogy may be drawn with the formation of endospores by *Bacillus*. In that case, the mother cell donates molecules and as-

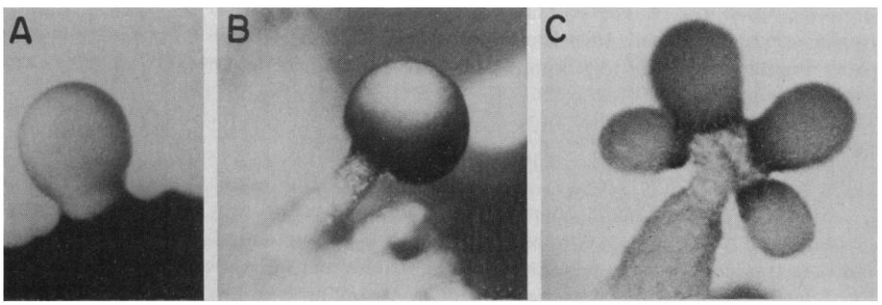

Fig. 1. Fruiting bodies of (A) *Myxococcus fulvus* (*15*), (B) *Myxococcus stipitatus* (*15*) and (C) *Stigmatella aurantiaca* (*79*). The magnifications are, respectively, ×285, ×245 and ×370.

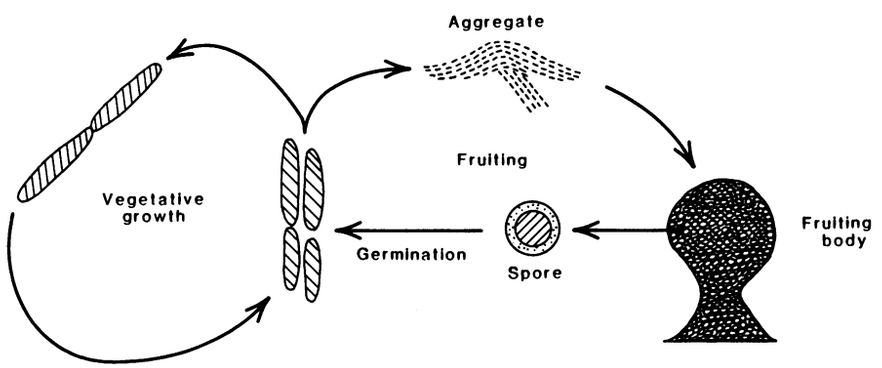

Fig. 2. The life cycle of *Myxococcus*.

Table 1. Genetics of motility.

Movement pattern	Genes		
		No.	Type
A motility: Isolated cells move	System A	23	*cgl, agl, mgl*
S motility: Cells move only when close together	System S	10	*tgl, sgl, mgl*

semblies of molecules to its developing forespore. Eventually, the mother cell lyses, releasing a mature spore. In myxosporulation, by contrast, an entire cell differentiates into a spore; instead of part of the cell lysing while the remaining portion sporulates, part of the population lyses and the survivors sporulate. The analogy suggests that products of lysis may contribute to the differentiation of the sporulating cell. In fact, recent experiments indicate that the outermost coat of fruiting body spores assembles from protein S released by cells that lyse (*23*).

Many biochemical changes occur during fruiting body development. Of 30 major soluble proteins of *M. xanthus* that can be resolved in one dimension on a sodium dodecyl sulfate (SDS) polyacrylamide gel, at least one-quarter change their rates of production during development (*24, 25*). New proteins are synthesized; some proteins increase over vegetative levels; the synthesis of others is arrested at specific developmental times. Meanwhile, there appears a new population of messenger RNA (mRNA) molecules with half-lives of 20 to 30 minutes compared to 3 minutes for vegetative cells (*26, 27*). About one-third of all developmental protein synthesis in *M. xanthus* appears to be encoded in such stable RNA molecules.

Two proteins that occur only during development have been studied in detail; both are translated from species of long-lived messengers. Protein S first appears at 3 to 6 hours after the development period has begun and rises to a maximum rate of synthesis at 24 hours (*28*). At its peak, protein S accounts for 15 percent of total protein synthesis. Initially soluble, protein S assembles late in development to form an outer coat of the developing myxospore. Protein S has been purified and crystallized. The crystals diffract x-rays out to 3 angstroms resolution (*29*), thus promising a detailed view of the structure of this protein. The gene for protein S has been cloned and sequenced, and from the DNA sequence a molecular weight of 19,000 has been deduced for the protein (*30–32*). Comparison of the nucleotide sequence of the S gene with the amino-terminal sequence of protein from spores reveals no signal peptide (*28, 31*). This may be connected with release by lysis, although the protein has been found on the surface of rod-shaped cells (*28*). Protein S requires Ca^{2+} to assemble on spores, and the amino acid sequence shows striking homology to calmodulin, particularly in the vicinity of the putative calcium binding site (*31*). The gene for protein S is present in tandem with a second gene that encodes a protein whose amino acid sequence is 88 percent homologous to that of protein S (*30*). In recent experiments, the second protein was found inside spores (*23, 32*).

A second developmental protein, H, is a hemagglutinin whose production rate is at a maximum near the midtime of the fruiting process—the time of tight cell-cell aggregation (*33*). The hemagglutinating activity of H can be inhibited by the glycoprotein fetuin or by a glycopeptide from fetuin. H binds more strongly to developing *M. xanthus* cells than to vegetative cells, and fetuin blocks the binding to vegetative cells but not to developing cells (*34*). Monomers of purified protein H have a molecular weight of 28,000 and agglutinate red cells (*34*). Protein H antigen is found on the surface of cells, specifically at the two ends (*35*). The data are compatible with the idea that H is a multivalent lectin specific for binding sites present on developing cells; however, the actual role of protein H in development is not yet known (*20*).

A mature spore-containing fruiting body, from the biological point of view, is a resting colony (*36*). Its multicellularity ensures that, when food returns and the spores germinate, a new phase of vegetative growth will be initiated by a community of cells able to feed cooperatively. It may be apparent from this brief review that myxobacteria resemble slime molds in many ways, despite their fundamentally different cell structures.

Interactions That Control Motility

Social motility. Myxobacteria are motile; they glide, but do not swim (*15*). Their gliding consists of a smooth motion without rotation in the direction of the cell's long axis, a motion punctuated with occasional stops and reversals of directions (*37*). Their gliding is restricted to surfaces, and the ability to move on surfaces, including the surface of other cells, permits myxobacteria to feed on insoluble particulate material and to build multicellular fruiting bodies. Myxobacteria possess no flagella or other organelles of locomotion that are visible in the electron microscope. At present, there is much interest in the mechanics of gliding (*38–42*) because it is not yet understood in any gliding organism. Although we do not yet understand the mechanism we do know something about how gliding is regulated in *Myxococcus*.

Investigation of more than 100 motility mutants of *M. xanthus* has revealed two distinct components in the control of movement of wild-type cells: one called "A," for adventurous motility, and the other called "S," for social motility (*43–46*). The two components are under the separate genetic control of two multigene systems: system A (23 loci specifically required for the expression of A motility), and system S (more than ten loci required for the expression of S motility). Each component can function without the other to render cells motile. With the sole exception of *mgl* mutants, single mutations affect either A motility or S motility but not both. The genetic control of motility in *M. xanthus* is summarized in Table 1. Wild-type has both sets of genes and exhibits a combination of A and S motility.

Cell interaction appears to be the basis for S motility. This has been investigated in strains that have a mutation in one or more of the genes of system A so that their motility is only type S (*43, 47*). Cells of such S-motile strains are nonmotile when they are well separated from each other, yet they move at wild-type rates when they are touching or are in the immediate vicinity of other cells (*43, 48*); hence the name "social." Myxococcal cells have pili (also called fimbriae) at one cell pole (*49*). Pili are correlated with S motility in three ways: (i) Among the wide variety of motility mutants all wild-type and all S-motile strains have pili. No strain lacking pili exhibits S motility (*44*). (ii) Certain nonpiliated mutants (*tgl* mutants) become temporarily piliated when they are mixed with tgl^+ cells and during this period they also become S motile (*43, 44*). (iii) The critical separation for S-motile cells is around 3.5 μm; when they are more distantly separated, they fail to move; when they are closer, they may move (*48*). *M. xanthus* pili are approximately this length. Thus a need for contact between cells or between

cells and pili may account for S motility.

Motility stimulation. About one-tenth of the nonmotile mutants of *M. xanthus* are conditional mutants of a novel kind; mutant cells become transiently motile after contact with nonmutant cells or with cells of a different mutant type (*45*). This process is called motility stimulation because it is phenotypic and not genetic. A stimulated cell moves transiently—perhaps 100 μm—and no stably motile strains are generated. Stimulation has been observed only when donor and recipient cells were allowed to touch each other. When donor and recipient cells were placed on agar in patches that were close but not touching, there was no stimulation even though the two types were separated by less than 50 μm, which is about eight cell lengths (*45*).

Stimulation is an example of extracellular complementation, a property of cell interactions (discussed below). In motility system A, several types of stimulatable gliding mutants, *cglB*, *cglC*, *cglD*, *cglE*, and *cglF*, and the nonstimulatable *agl* and *mgl* mutants can be distinguished by stimulation tests (*45*). Any mixture of two mutants of different stimulatable types causes movement, but mixtures of two different mutants of the same type remain nonmotile. Either stimulatable or nonstimulatable mutants can be stimulation donors, but only a stimulatable mutant can be a recipient. For example, any $cglB^+$ strain, whether motile or nonmotile by virtue of mutations at other motility loci, can stimulate a $cglB^-$ mutant. In motility system S, *tgl* is the only stimulatable type and any tgl^+ strain can stimulate a tgl^- mutant (*43, 46*).

The specificity of stimulation is revealed by the fact that all mutants of a given stimulation type map to the same genetic locus (*43, 45, 46, 50*). For example, the mutations found in all 14 mutants that were classified by stimulation tests as type *cglB* map in one cluster. All 14 mutations are 90 percent cotransducible with Tn5 insertion Ω1932 and define the *cglB* locus. Similarly all *cglC* mutants map to one locus, all *cglD* mutants to another, all *cglE* to another, and all *tgl* mutants to another. There is only one *cglF* mutant and it maps to a site that is distinct from the other loci. Only stimulatable mutants and no other mutant types have been found at these loci. Stimulation is thus locus-specific, but not allele-specific.

Stimulation has been studied with nonmotile mutants for technical reasons; stimulated motility is transient and it stands out better against a nonmotile background. However, the facts that stimulated motility is quantitatively similar to normal wild-type motility and that wild-type cells can be stimulation donors argue that stimulation may be involved in the swarming of wild-type cells and possibly in the formation of fruiting bodies (*45*).

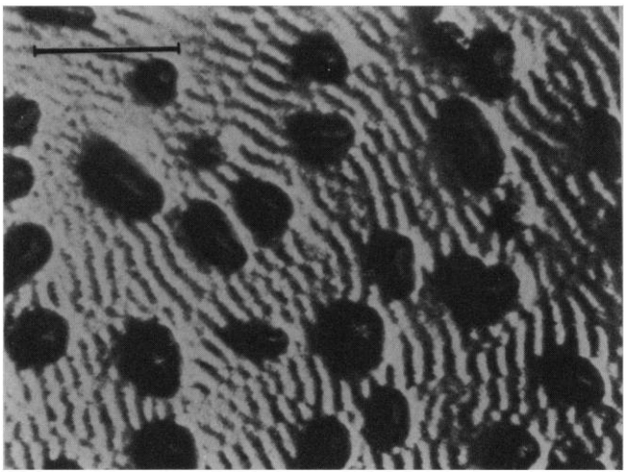

Fig. 3. Scanning electron micrograph showing ripples and fruiting bodies of *Myxococcus xanthus* (*52*). The bar represents 400 μm.

Rippling. Ripples are rhythmic gliding movements that arise in dense fields of cells (Fig. 3). The phenomenon was discovered by Reichenbach (*51*) in time-lapse microphotographic studies of fruiting body development in *Myxococcus* and was later observed by him in *Stigmatella* and in *Archangium* (*15*), suggesting a general occurrence in myxobacteria. Ripples were also found during predatory lysis by *Myxococcus* of *Micrococcus luteus* on water agar (*51*). Ripples of *M. xanthus* have a period of about 15 minutes (which explains why their discovery awaited time-lapse studies) and a wavelength of 50 to 100 μm. Trains of ripples appear to emanate from localized areas in a swarm and to spread outward. Scanning electron microscopy of ripples in *M. xanthus* clearly shows that cells are accumulated in the crests of ripples and are depleted from the intervening troughs (*52*).

The coordination of movement of many cells in ripples implies interactions. Cells within the same crest are very close to each other, perhaps in contact, and they move in unison (*52*). Adjacent crests of cells are about 10 cell lengths apart, and they maintain this distance while they move. This property gives a series of adjacent crests the overall appearance of a train of traveling waves.

Rippling, like the development of morphologically normal fruiting bodies, requires both A and S motility systems. Shimkets and Kaiser (*52*) discovered that rippling could be induced in quiescent cells by an extract of rippling cells, and that peptidoglycan is the ripple-inducing activity in these extracts. First, they observed that the activity is associated with the cell envelope fraction of disrupted cells. Second, peptidoglycan purified from *M. luteus* or *Corynebacterium poinsettiae* will induce rippling, but peptidoglycan from *Methanobacterium* spp, which is structurally different (*53*), will not. Third, a mixture of four components of *M. xanthus* peptidoglycan, *N*-acetylglucosamine, *N*-acetylmuramic acid, diaminopimelic acid, and D-alanine are able to induce ripples (*54*). Related compounds such as L-alanine and D-glutamic acid are inactive. Induction by peptidoglycan or its components explains why *M. xanthus* forms ripples during fruiting body development since many cells lyse during development (*22*) and release their murein. Also, the predatory lysis of *M. luteus* would release its peptidoglycan, thus accounting for rippling under those conditions. *M. xanthus* produces three murein hydrolases: lysozyme, amidase, and peptidase (*55*), which would be expected to cleave the released peptidoglycan into fragments.

Extracellular Complementation

If there are multicellular interactions in a population, it should be possible to obtain mutants deficient in the generation, transmission, or receipt of cell-to-cell signals. Furthermore, any mutant whose development is blocked by its inability to generate or transmit such a signal should be rescued when either the signal itself or the cells providing the signal are made available to the mutant. Multicellular interactions can, in fact, be explored by a complementation test with mixtures of whole cells. The test, called "extracellular complementation" to distinguish it from the more widely used genotypic complementation, can: (i)

identify mutants defective in a cell interaction, particularly mutants that are defective in an interaction "donor" function, (ii) group such mutants according to the step in which they are defective, and (iii) provide a bioassay for molecules that may be lacking in a mutant. Developmental geneticists have described extracellularly complementable cell mutants as "nonautonomous" or "reparable."

Stimulation of motility described above is one example of extracellular complementation in *M. xanthus*. Another involves a set of developmental mutants that behave as if they are unable to generate a cell-to-cell signal (*56*). These mutants exhibit normal vegetative growth, but when they are placed under conditions that would induce the wild-type to form fruiting bodies, they fail to develop normally and do not sporulate. They are able to sporulate, however, if they are mixed with wild-type cells or with appropriate other mutants. Pairwise testing of 57 mutants divided them into four (extracellular) complementation groups such that sporulation occurred upon challenging by starvation a mixture of two mutants belonging to different groups but not a mixture of two mutants belonging to the same group.

Extracellular complementation of these mutants is not the result of genetic exchange between cells in the mixtures because spores formed by the fruiting of a mixture of cells retained the genotypes of the original cells used (*56*). Furthermore, the fact that wild-type cells could complement the mutants indicates that signal generation is a property of the normal cells and presumably occurs normally during development. The mutants are not auxotrophs; they grew on a minimal medium like the wild type (*56, 57*). Examination of the developmental phenotype of mutants representing the four groups (called *spoA*, *B*, *C*, and *D*) showed that members of each group are distinguished by the stage in the fruiting process beyond which they fail to develop. These properties of the four groups of developmental mutants suggest that they are defective in the production or release of extracellular factors required in myxobacterial development. Each group of mutants retains the ability to develop normally, if provided with the necessary factor or factors.

Ordinarily, myxosporulation takes place within a maturing fruiting body. However, as described above, the early multicellular steps can be by-passed and myxosporulation can be induced directly by addition of glycerol or other substances to a growing culture of vegetative cells (*19*). Although glycerol-induced spores are not identical to fruiting body spores, their basic similarities suggest that each cell contains a genetic program for changes in cell shape and for the acquisition of resistance properties characteristic of spores. This unicellular program would normally be activated at the appropriate time late in the development of fruiting bodies by earlier events. According to this view, glycerol would somehow activate the unicellular program directly, bypassing the normal requirements for starvation, high cell density, and a solid surface. These three conditions would thus appear to be required for the multicellular, cooperative part of development, and possibly for modifying the structure of the spores that are formed, by addition of protein S for example.

Extracellularly complementable mutants of the *spoA*, *B*, *C*, and *D* type fail to sporulate under conditions that normally induce multicellular development, but all can be induced to sporulate by glycerol (*56, 57*). Retention of glycerol inducibility implies that these mutants retain their program for cell shape change and acquisition of resistance properties. It also implies that the failure of these mutants to sporulate under starvation conditions that induce the wild-type to form fruiting bodies is the consequence of earlier defects in a developmental pathway that depends on cell interactions.

SpoC

The *spoC* class of extracellularly complementable mutants has been studied in most detail. Three original *spoC* mutants, though isolated independently, have the same phenotype. When starved under conditions that would induce wild-type to form fruiting bodies, *spoC* mutants synthesize developmental proteins S and H (*57*), but they fail to ripple (*52*), to lyse (*54, 58*), or to sporulate (*52, 57*). The similarity in developmental phenotype of the members of this group of mutants suggested they had related genetic defects and progress in analyzing their molecular genetics was facilitated by the introduction of transposon Tn5.

A *spoC* mutation, like a *spoA*, *B*, or *D*, blocks the development of spore-filled fruiting bodies, but leaves the growth of cells and colonies unchanged. Properties such as these are difficult to embody in a scheme for positive genetic selection favoring the mutation. This general limitation on the handling of mutations that alter multicellular properties can be overcome in general by linking to the mutation being examined a genetic marker that is subject to selection in single vegetative cells. In bacteria, transposable drug resistance elements, like Tn5, are particularly useful for this purpose. Tn5 is a 5.7-kilobase segment of DNA originally isolated from *Escherichia coli* (*59*). Like other bacterial transposons, Tn5 inserts itself into a DNA molecule, becoming an integral part of the recipient chromosome. Tn5 includes a gene encoding the enzyme kanamycin-neomycin phosphotransferase; consequently cells that carry Tn5 phosphorylate kanamycin and become resistant to it.

Coliphage P1 has a broad host range (*60*) that includes *Myxococcus* (*61*). When *M. xanthus* is infected with a P1 that carries Tn5, the transposon can move to the *Myxococcus* chromosome, generating stable kanamycin-resistant strains (*62*). From a library of such kanamycin-resistant transductants one can readily isolate Tn5 insertions that are cotransducible by myxophages Mx4 (*63*) or Mx8 (*64*) with any particular mutation (*62*). In the case of *spoC*, Tn5 insertion Ω1519 was found to be linked to *spoC* 731 (*57*). Transductional crosses then showed that three other mutations of group C were also linked to this Tn5 insertion, and to the same degree, although they had arisen independently and are recombinationally distinct. The *spoC* mutants thus define a single genetic locus. Linkage to Tn5 at site Ω1519 permitted transfer of each of the group C mutations into the same wild-type strain so that their developmental phenotypes could be compared in the same genetic background. Linkage to Tn5 at Ω1519 also permitted the *spoC* locus to be cloned in *E. coli*, utilizing the ability of Tn5 to express kanamycin resistance in *E. coli* (*59*). A segment of *Myxococcus* DNA containing both Tn5 at Ω1519 and 15 kb of adjacent sequence from a $spoC^+$ strain was thus cloned (*65*). When this DNA segment was returned to a $spoC^-$ strain of *Myxococcus*, it became $spoC^+$, showing that the locus was on the segment that had been cloned. Partial diploids, containing two copies of the *spoC* locus in tandem duplication, were obtained with Tn5. All the heterozygotes $spoC^+/spoC^-$ had a spo^+ phenotype, consistent with the extracellular complementability of the *spoC* mutants (*65*). All the heterozygotes between different $spoC^-$ alleles had a $spoC^-$ phenotype, and thus all belong to the same gene or unit of transcription (*65*). Recently, Shimkets (*66*) has obtained a 1-kb fragment of *Myxococcus* DNA that rescues all four *spoC* mutants. He has also obtained new *spoC* mutants by Tn5 insertion in the 1-kb region.

SpoC mutants fail to form the traveling cell accumulations known as ripples (*52*). Because *spoC* mutants exhibit normal motility, their failure to ripple may reflect a defect in a long-range coordination of cell movement necessary for rippling. Genetically, the sporulation and rippling defects go together; all of more than 575 *spoC* mutants, transductants, and segregants from $spoC^{-}/spoC^{+}$ tandem duplications either were sporulation deficient and ripple deficient, or were proficient for both.

Moreover, the lysis and sporulation defects of all the single site *spoC* mutants can be rescued by addition of a mixture of the same four components of *M. xanthus* peptidoglycan that induce rippling in wild-type cells: *N*-acetylglucosamine, *N*-acetylmuramic acid, D-alanine, and diaminopimelic acid (*52*). Although addition of these components induces wild type to form ripples as described above, the *spoC* mutants, which do not form ripples spontaneously under conditions of development, still do not form ripples when peptidoglycan components are added. Since *spoC* mutants grow vegetatively on unsupplemented minimal medium at rates similar to the wild type, they are not vegetative auxotrophs for murein components. With peptidoglycan components added the number of spores formed by *spoC* mutants and the extent of their lysis is similar to that of wild type during normal development. The high rescue efficiency and the fact that peptidoglycan is released by developmental lysis (*22*), as well as in the process of wall remodeling during sporulation (*67*), suggest that peptidoglycan or its components may normally serve as developmental regulators or special substrates for development. According to this view, added peptidoglycan components allow *spoC* mutants to reenter the pathway of normal development downstream of the step blocked by the *spoC* mutation.

Development Stimulating Factor

Janssen and Dworkin (*58*) were able to rescue developmental autolysis, sporulation, and fruiting body formation in group C mutants that also carry a mutation in motility system S (for example, *spoC sglA*) with an extract from wild-type, developmentally competent cells. This extract (referred to as development stimulating factor or DSF) has been substantially purified and has been shown to have the properties of a polysaccharide or glycopeptide with a molecular size of 3000 to 7000 daltons. The DSF could also be isolated from the group C mutants, despite the fact that they cannot, of course, complement their own development. This unexpected finding suggested that the DSF in the group C mutant is either obscured by some modification in the cell surface or has been rendered inaccessible by some conformational change in the signal molecule itself. DSF was also absent from vegetatively growing cells unless the cells had been subjected to a nutritional shift-down. It was also shown that neither the C signal nor the B signal seemed to be acting as a trigger but rather were required throughout development; this was consistent with the observation that the DSF seemed to be tightly bound to the cells. Solubilization of DSF required either disruption of the cells by sonic oscillation or freeze-thaw cycles or extraction with 1*M* NaCl. It has also been possible to rescue a number of the *spoC* mutants with mannosamine or, somewhat less effectively, with glucosamine (*58*). This suggests that the portion of DSF that is recognized may be mannosamine or a similar sugar residue.

In contrast, the single-site *spoC* mutant (*54*) was not complemented by DSF or mannosamine and showed delayed complementation with glucosamine (*58*) and conversely, the *spoC sglA* mutants were not rescued by peptidoglycan.

Diffusible Signals in *Myxococcus* and in *Stigmatella*

In order for *M. xanthus* to shift from the growth mode to the developmental mode, one of the requirements that must be satisfied is that the cells must be present at a high density (*68*); at low densities, they will not aggregate even though they are starved and present on a solid surface. *Myxococcus* cells excrete adenosine as the extracellular parameter of cell density (*68*). The evidence for this is that exogenous adenosine will allow cells present at a low density to aggregate and form fruiting bodies; at this density, in the absence of added adenosine, they are unable to develop. Furthermore, the cells, when undergoing normal development, excrete adenosine in amounts approximately equivalent to that necessary to induce low-density development. Finally, inhibitors of adenosine biosynthesis, such as hadacidin, prevent normal development and the inhibition is reversed by adenosine (*68*).

Stigmatella aurantiaca is a myxobacterium that forms an extremely complex fruiting body (Fig. 1). Like *M. xanthus*, fruiting body development in *S. aurantiaca* requires a high cell density, starvation, and a solid surface. Unlike *M. xanthus*, however, visible light will allow low density populations of *S. aurantiaca* to aggregate and form fruiting bodies (*69*). Qualls *et al.* (*70*) discovered that if a high-density population of *S. aurantiaca* was placed adjacent to a low-density population in the dark, the latter was induced to aggregate and form fruiting bodies. This led to the isolation and partial purification of a chloroform-soluble lipid material which, when added to a low-density population of cells, could replace the developmental requirement for visible light and thus allow essentially normal development. This material was called a pheromone (*71*). In other words, both light and the pheromone stimulate aggregation and induce the maturation of aggregates of cells into fruiting bodies. Investigation of the relationship between light and the pheromone indicated that, while light does not increase the synthesis of pheromone, it does seem to increase the responsiveness of the cells to the pheromone (*71*). Stephens and White (*72*) suggest that light stimulates the formation of fruiting bodies because it increases the sensitivity of cells to their endogenously produced pheromone.

Stephens and White have also shown that guanine nucleosides and nucleotides could replace the lipid pheromone. In addition, they found that light sensitized the cells to respond to one-fourth the concentration of guanine compounds normally required for stimulation in the dark (*72*). Whether *S. aurantiaca* excretes guanine compounds in a fashion analogous to the cell density-sparing effect of adenosine on *M. xanthus* discussed above has not been determined (*68*). It is not surprising that, given the central role played by cell density in the growth and developmental behavior of the myxobacteria, a variety of signaling mechanisms for the purpose of monitoring cell density exists in these bacteria.

Application of Molecular Genetics to Cell Interactions

Myxobacteria have certain advantages for experimental studies of the molecular basis of cell interactions. Their genome is small enough [5700 kb in *M. xanthus* and *S. aurantiaca* (*8*)] to envision realistically the identification, by saturation mutagenesis, of all genes required for cell interactions. Similarly, it may be possible to determine how the interactions coordinate the development of fruiting bodies. These possibilities rest

on the availability of the following genetic tools in *M. xanthus*:

1) Three different generalized transducing myxophages are available for transferring genes between strains of *M. xanthus*: *Mx4* (*63*), *Mx8*, and *Mx9* (*64*). Multiple mutant strains can be constructed and mutant loci can be mapped by transduction. Mx8 forms lysogens by chromosomal integration (*73*) offering the possibility of forming specialized transducing phage.

2) Genes can be transduced by coliphage P1 from *E. coli* to *M. xanthus* either by generalized or specialized transduction (*61, 62, 65, 74*).

3) The transposon Tn5, carrying a gene that confers kanamycin resistance, can transpose from bacteriophage P1 to many different sites within the *M. xanthus* genome (*62*). Once inserted, Tn5 remains at the same chromosomal position during growth and subsequent transduction. Tn5 also encodes resistance to streptomycin that can be expressed in *M. xanthus* (*75*).

4) There is a procedure to isolate, from a library of many independent Tn5 insertions, those insertions near any particular gene (*62*). These linked Tn5 elements provide selectable markers for strain construction, localized mutagenesis, and chromosome mapping. Genetic work can be focused on a chromosomal region of particular interest.

5) Tandem duplications of predetermined regions of the *M. xanthus* chromosome can be constructed, and can serve as partial diploids for tests of dominance and genetic complementation (*76*).

6) With the use of kanamycin-resistance of an inserted Tn5 for selection, it is possible for nearby *M. xanthus* genes to be cloned in an *E. coli* plasmid. The plasmid and its insert can be transduced back to *M. xanthus* by bacteriophage P1 (*65, 74*).

7) Tn5 lac, a transposon that fuses lacZ expression to exogenous promoters, has been constructed (*77*). Tn5 lac can transpose in *M. xanthus* where it serves as an easily assayable probe of developmental or vegetative promoter activity.

Conclusions

From the time the myxobacteria began to be closely examined, they manifested a variety of cell interactions involving both diffusible and contact-mediated signaling. In this article we have tried to emphasize that a number of these interactions have now been sufficiently defined so that they represent realistic experimental systems. In addition, the techniques for genetic and molecular analysis of these interactions are now available. A number of other interactions (such as developmental aggregation, fruiting body construction, developmental autolysis, and cell-density perception) only await investigation. Finally, it has recently become possible to generate a set of monoclonal antibodies against cell-surface antigens of developing cells of *M. xanthus* (*78*), thus opening up yet an additional strategy for examining these interactions. Examination of the complex set of cell interactions in a relatively well-defined prokaryote and the availability of systems for genetic analyses should allow indepth analyses of the mechanisms and regulation of cell-interactions that have thus far been elusive in higher systems.

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