Morphogenesis and Developmental Interactions in Myxobacteria

These prokaryotes demonstrate complex cellular interactions unique among bacteria.

John W. Wireman and Martin Dworkin

The myxobacteria are a group of prokaryotes that carry out complex developmental sequences involving intercellular communication. A detailed study of the mechanisms of developmental interactions that occur in the myxobacteria has the potential of providing insight into the nature of signaling and cell interactions in other developmental systems, and may also aid in an understanding of the phylogeny of developmental interactions. For example, a comparison of the myxobacteria and the eukaryotic cellular slime molds provides an excellent opportunity to compare the mechanisms of development by two widely different cell types that carry out similar developmental events.

Some recent attempts to approach problems of malignant growth have focused on changing patterns of cell-cell interactions (*1*). In addition, the role of cell-cell interaction in eukaryotes has been the subject of many studies. For example, such studies have dealt with amoebal interactions in *Dictyostelium* (*2, 3*), contact inhibition in mammalian cells (*4*), cell interactions in the immune response (*5*), and formation of developmental patterns (*6*). We now suggest and provide evidence that the myxobacteria can be used to investigate developmental interactions in a prokaryote.

Myxococcus xanthus, the myxobacterium with which most of the recent work has been done, is a long gram-negative rod, capable of aerobic growth in a dispersed state in either complex or defined media (*7*). The cells divide by binary transverse fission and grow with a generation time of about 200 to 250 minutes in complex medium. There are no peculiar problems associated with their cultivation or cloning.

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Life cycle. The unique and distinctive feature of the myxobacteria is that when cells on the surface of a solid medium are deprived of specific nutrients, they shift from growth to development and begin to migrate, by means of a peculiar gliding motility, into aggregation centers. A number of studies have indicated that this is a chemotactic response (*8*), although the matter has not yet been carefully examined. Having assembled, the cells then construct a macroscopic structure called a fruiting body. In some members of the genus *Myxococcus* the fruiting body is a mound of cells covered by slime, slightly elevated from the surface (Fig. 1). Many other members of the order Myxobacterales construct more elaborately differentiated fruiting bodies with a stalk, and often a complex head, containing the resting cells. The most primitive of these differentiated fruiting bodies is *Myxococcus stipitatus* (Fig. 2). The cells inside the head of the fruiting body have developed into myxospores. The myxospores of the genus *Myxococcus* are optically refractile, resistant resting cells (*9*) which, when placed in a suitable nutritional milieu, will germinate into vegetative rods (*10*). The two myxobacteria that represent the peak of fruiting body complexity are *Chondromyces apiculatus* (Fig. 3) and *Stigmatella aurantiaca* (Fig. 4). In these organisms there is a further differentiation; the resting cells are contained in clusters of cysts arranged around the head of the fruiting body. The head itself is borne on a hardened slime stalk with a few embedded cells (*11*). Germination in these organisms consists of the bursting of the cyst and the release of the vegetative bacteria into the surrounding medium. The general properties of the myxobacteria have been reviewed a number of times (*12–15*) and a diagramatic illustration of the life cycle of the most intensively studied myxobacterium, *Myxococcus xanthus*, is presented in Fig. 5.

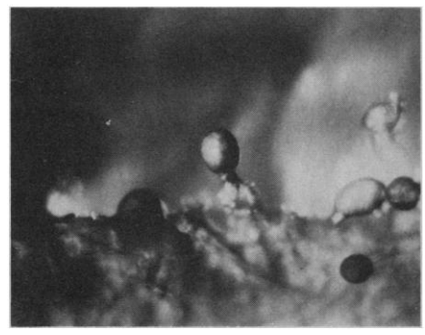
Colonial variation. Burchard and Dworkin (*16*) reported the existence of two colony types when *M. xanthus* FB was plated on nutrient medium which allowed growth and colony formation. These were referred to as "yellow" and "tan," based on the presence in the yellow colony type of a highly polar, acetone-soluble pigment with an absorption maximum of 379 nanometers (*16, 17*). Further examination in our laboratory has revealed that the presence of an additional variable property, that is, colonial morphology (*18*), results in four distinct phenotypes. We refer to these as YS (yellow swarmer), TS (tan swarmer), YNS (yellow nonswarmer) and TNS (tan nonswarmer) (Fig. 6). These four types appear spontaneously, but the conversion from one type to another can be induced or accelerated by treatments such as ultraviolet light irradiation (*9, 17*), mitomycin C, nalidixic acid, and growth at elevated temperatures (*17*). We are currently investigating the properties of the four cell types and the rate of conversion of one cell type to another. This phase variation is of additional interest because strains derived from the various colony types show distinctly different patterns of developmental behavior (see below).

Genetics. Recently several genetic systems for *Myxococcus xanthus* have been described. Zusman's group (*19*) has succeeded in isolating a variety of bacteriophages for *M. xanthus* including at least one with generalized transducing properties. Kaiser and Dworkin have recently reported (*20*) that bacteriophage P1, a generalized transducing phage whose normal host range is restricted to the enteric bacteria, is capable of abortively infecting *M. xanthus* FB. Furthermore, P1CM, a phage carrying the gene for chloramphenicol resistance is able to transfer that resistance to *M. xanthus*. The resistance is highly unstable in the absence of chloramphenicol, thus behaving like plasmid-mediated chloramphenicol resistance in *Escherichia coli* (*21*). Similarly, Parish has been able to transfer a number of drug resistance properties from *E. coli* containing R factors to *M. xanthus* by simply incubating the organisms together (*22*). Whether this involves a conjugal transfer or transformation is not yet clear. In addition, Parish has demonstrated transfer of resistance to rifampicin and 5-fluorouracil between strains of *Myxococcus* (*22*). Thus the means for using intergeneric and intrageneric gene transfer for the study of myxobacterial development may soon be available.

Cellular Morphogenesis

Attempts to determine the biochemical correlates of myxospore development in *M. xanthus* were aided by the discovery that it is possible to short-circuit the normal developmental formation of myxospores and induce their formation directly in liquid culture. This was done by the addition of one of several possible inducers (*23*). Under these conditions, vegetative cells were converted to myxospores rapidly, quantitatively, and relatively synchronously. The compounds that had this effect were those with primary or secondary alcohol groups (such as glycerol, ethylene glycol, butanol, and phenethyl alcohol) (*23*) as well as dimethyl sulfoxide (*24*). The vegetative cells progressively shortened and rounded up until, at about 100 to 120 minutes, the complete vegetative cells were converted to round, optically refractile, resistant resting cells similar to those formed in fruiting bodies. Biochemical changes however, continued to take place for an additional 6 hours at least.

Macromolecular changes. During myxospore induction in *M. xanthus* there are substantial changes in macromolecular synthesis. These changes are directed toward: (i) the structural conversion of the cell, (ii) converting it to a state of metabolic quiescence, and (iii) preparing it for subsequent germination. During myxospore formation DNA synthesis continues until, as has been suggested by Rosenberg *et al.*, existing rounds of replication are completed; no new rounds are initiated (*25*). Net RNA synthesis ceases immediately, although increased turnover takes place (*24*). The data of Okano *et al.* indicate that a small fraction of RNA synthesized during myxospore formation is unique to the myxospore (*26*). In addition, Foster and Parish (*27*) have found differences in the physical properties and in the protein subunit composition of the 30*S* subunit of the myxospore ribosome. Net protein synthesis continues during the morphological conversion; after 2 hours, net protein synthesis ceases but a high rate of protein turnover continues (*28*).



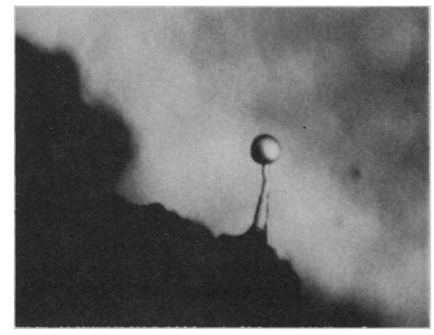
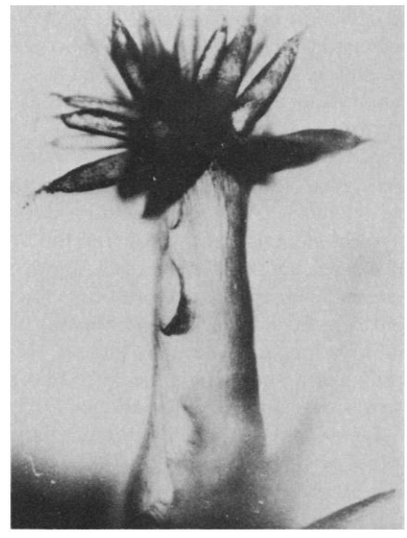


Fig. 1 (left). Fruiting bodies of *Myxococcus fulvus* on filter paper. The fruiting bodies are about 75 to 150 μm in diameter. Fig. 2 (right). Fruiting body of *Myxococcus stipitatus*. The fruiting body is about 200 μm tall (*12*).



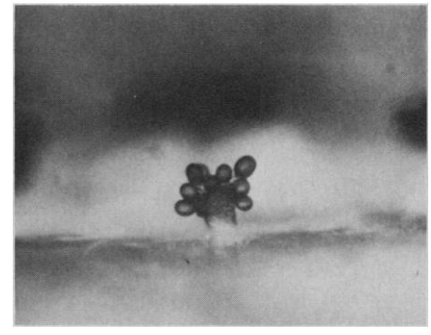


Fig. 3 (left). Fruiting body of *Chondromyces apiculatus*. The fruiting body is about 200 μm tall (*12*). Fig. 4 (above). Fruiting body of *Stigmatella aurantiaca*. The fruiting body is about 150 μm tall.

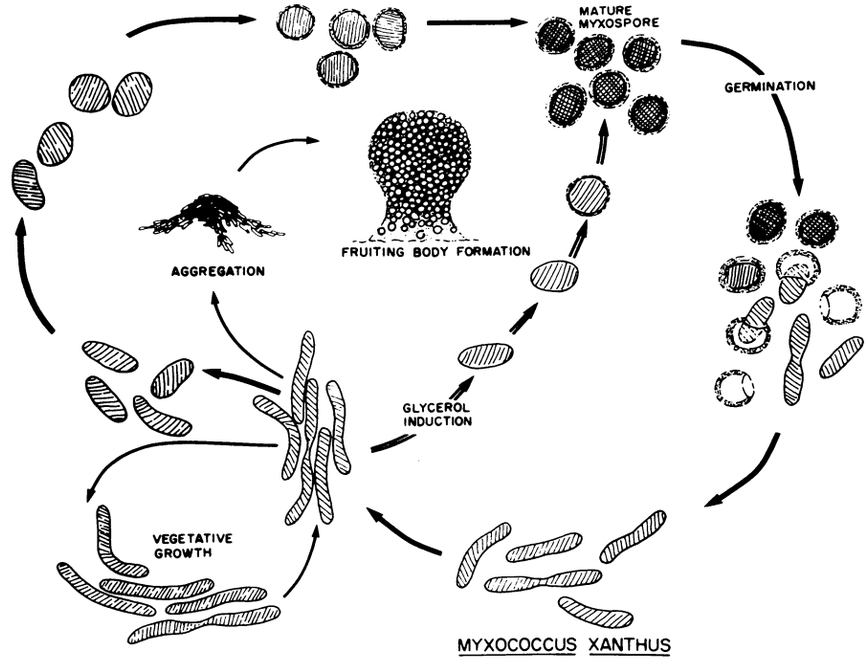


Fig. 5. Schematic life cycle of *Myxococcus xanthus*. The options available to the cell are depicted. Vegetative growth occurs under conditions of nutrient abundance. The cells may be induced to aggregate and form fruiting bodies by nutrient deprivation (inner circle). The outer circle shows some cell stages during the conversion of vegetative rods in early aggregates to mature myxospores in completed fruiting bodies. In addition cells may be induced to convert from vegetative cells to myxospores by the addition of glycerol to cultures growing in liquid media (*23*).

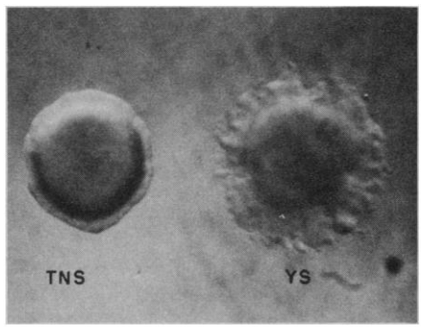


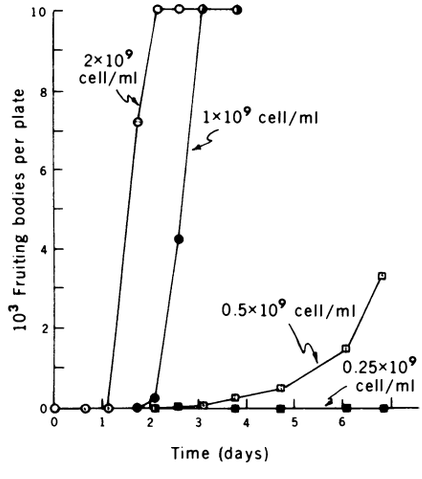
Fig. 6. Representative colonies of *Myxococcus xanthus* on 2 percent Casitone agar (*7*). The colony on the left (TNS) is a 6-day-old tan "nonswarmer" colony and on the right (YS) a yellow "swarmer" colony. The other two colony types, that is, yellow nonswarmer and tan swarmer, are not shown and are similar to the colonies shown here.

Changes in spore coat and cell wall. White *et al.* (*29*) reported that the peptidoglycan of vegetative cells of *M. xanthus* consisted of contiguous or overlapping plates, rather than a continuous bag-shaped macromolecule. This appears not to be the case in the myxospore. In addition, the myxospore peptidoglycan appears to be more highly crossed-linked and to contain covalently bound glucose (*30*). A striking change in the cellular structure during myxospore morphogenesis is the appearance of a spore coat. The coat is largely carbohydrate, and approximately 50 percent of the dry weight of the spore coat is *N*-acetylgalactosamine (*31*).

Enzyme changes. An examination of the activities of crude extracts of vegetative cells and myxospores of *M. xanthus* revealed that both cell types contained glycolytic, gluconeogenic, and Krebs cycle activities. It was of interest that both cell types lacked hexokinase and pyruvic kinase activity (*32*). In addition, vegetative cells and spores contained cyanide-sensitive reduced nicotinamide adenine dinucleotide (NADH)-oxidase, diaphorase, NADH-cytochrome c reductase, and cytochrome oxidase. Examination of the difference spectra of cell extracts revealed the presence in both cell types of flavoprotein and cytochromes b and c (*33*). During glycerol induction of myxospores, a number of enzymatic activities have been shown to change. These are aspartokinase (*34*), isocitratase and malate synthetase (*35*), fructose-1,6-diphosphatase, uridine diphosphate (UDP)-*N*-acetylgalactosamine transferase (*36*), and a broad spectrum phosphatase (*37*). Furthermore, during glycerol induction of myxospores, the rate of oxygen uptake is reduced by about 80 percent, the residual activity being resistant to sodium azide (*38*). At present, it is not possible to state that any of these changes are what Wright has referred to as "critical variables in differentiation" (*39*).

Bacteriophage Mx-1. Mature myxospores of *M. xanthus* are resistant to infection by the virulent DNA phage Mx-1. If vegetative cells are infected during the first 30 to 40 minutes of glycerol induction of myxospores, induction is prevented, and productive infection takes place. If, however, the cells are infected after the first 40 minutes, but before they have acquired optical refractility, a certain proportion of the myxospores finally formed are cryptically infected with the phage and give rise to infective centers upon spore germination (*40*). This phenomenon is similar to one subsequently reported in *Bacillus subtilis* (*41*). In the case of *B. subtilis*, it has been shown that the inability of sporulating cells to complete phage replication is due to an alteration of the sigma factor of RNA polymerase (*42*).

Developmental mutants. It is relatively easy to obtain mutants of *M. xanthus* blocked at some developmental stage (*43*), such as those that do not form myxospores in response to glycerol induction (*13*). Burchard and Parish have recently demonstrated that such mutants are also resistant to induction by ethylene glycol and dimethyl sulfoxide (*44*). When a large number of such mutants was examined for the ability to form fruiting bodies, 109 out of 117 mutants tested were able to do so. Examination of the fruiting bodies formed by the mutants revealed that the cells had converted to myxospores (*44*), suggesting that the mechanism of glycerol induction of myxospores is not identical with that occurring during the normal developmental process. There have been no reports of mutants blocked at intermediate steps of myxospore induction. *Stigmatella aurantiaca* (Fig. 4) forms myxospores which may also be induced by glycerol, as well as by other, chemically unrelated inducers (for example, NaCl, leucine, or dimethyl sulfoxide) (*45, 46*). Reichenbach has isolated a large number of noninducible mutants that he has placed in three groups based on categories of inducers to which the mutants are indifferent (*46*). The properties of myxospores have been reviewed recently (*31, 47*).



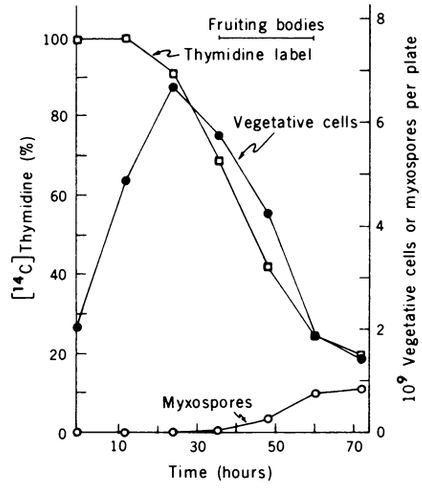


Fig. 7 (left). Effect of initial cell density on the rate of fruiting body formation. Vegetative cultures of wild-type *M. xanthus* FB were washed and resuspended in distilled water at 2×10^9 cell/ml. The cells were diluted as shown and spotted on agar plates (*58*). Each plate contained 17 spots, and the total number of fruiting bodies per plate is plotted as a function of time (*59*). Fig. 8 (right). Changes in cell number and loss of thymidine label during fruiting body formation. A culture was labeled during vegetative growth with [methyl-^{14}C]thymidine (*61*). The washed vegetative cells were resuspended in 2 percent Casitone medium at 3×10^9 cell/ml and spotted on Bacto agar plates (*58*). Each plate contained 30 spots, and duplicate plates were harvested at 12-hour intervals. The total number of vegetative cells and myxospores was counted (*62*), and the remaining acid-precipitable radioactivity was determined (*63*). The bar labeled "fruiting bodies" represents the time interval from the first appearance of fruiting bodies to their maximum development.

Fruiting Body Morphogenesis

Until recently, most of the emphasis on development in myxobacteria has focused on the process of glycerol-induced myxospore formation and germination. Recently, however, a number of laboratories have begun to examine the colonial rather than the cellular morphogenesis. That is, attention has now shifted to the processes of swarming, aggregation, and formation of fruiting bodies. The complexity and organizational precision of these processes make it highly likely that systematic cell

interactions play an integral role in myxobacterial development. Reichenbach (*48*) has used time-lapse photomicroscopy to observe fruiting body formation in various genera of myxobacteria, and observations of these films lead to the inescapable conclusion that the myxobacteria are group oriented during all stages of their life cycle. Swarms of cells attract each other, and an individual cell on the periphery of a swarm of cells remains in close contact with the group; although outward "explorations" do occur, the cell quickly returns to the swarm. During fruiting body construction, the cooperative nature of myxobacterial interactions is even more apparent. There are distinct streams of cells moving into aggregation centers (*48, 49*), and often there are rhythmic oscillations around the aggregation centers (*50*).

These phenomena are strikingly similar to observations that have been made of fruiting body construction by the cellular slime mold, *Dictyostelium discoideum* (*51*). This organism, however, has morphologically distinct developmental stages—such as the aggregation center and the migrating slug—which are not as apparent in myxobacterial development. There are, however, several aspects of fruiting body development in *M. xanthus* that have been identified and that make it possible to construct a preliminary model which may provide some insight and perspective into myxobacterial development.

Initiation of development. The initial stimulus of fruiting body formation is nutrient deprivation (*52*). The myxobacteria feed on decaying organic matter and other bacteria in nature, and presumably there are specific stimuli that result in the cessation of vegetative feeding and growth and the initiation of the developmental sequence. Several studies have shown that deletion of one or more specific required amino acids from a defined agar medium stimulates fruiting body formation (*53, 54*). Furthermore, the end products of one biosynthetic pathway have been implicated in the initiation of fruiting body formation (*34, 55*). The terminal amino acids of the aspartate pathway (*56*) affect aspartokinase activity in vitro and also affect fruiting body formation. Methionine and isoleucine stimulate aspartokinase activity in vitro and inhibit fruiting. Threonine and lysine inhibit aspartokinase activity, but only threonine stimulates fruiting. Campos and Zusman (*57*) observed stimulation of fruiting by the addition of adenine nucleotides, especially adenosine diphosphate (ADP) and cyclic adenosine monophosphate (AMP), to agar containing a low level of nutrients. The most effective stimulus of fruiting, ADP, is also a potent inhibitor of aspartokinase activity in vitro (*55*). Threonine, which alone has the same effect, synergistically enhances fruiting when added with ADP or cyclic AMP. Methionine or isoleucine, which alone inhibit fruiting, abolish the stimulation of fruiting by ADP or cyclic AMP. The stimulation of fruiting by the adenine nucleotides may reflect a developmental control mechanism related to nutrient deprivation, possibly the release of catabolite repression of developmental cistrons.

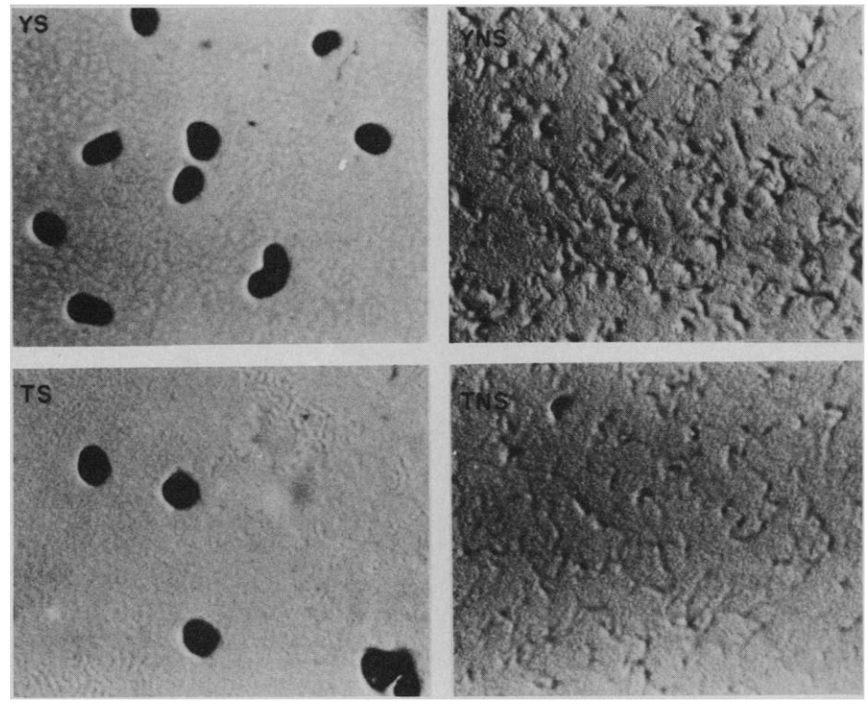


Fig. 9. Development by the four cell types of *Myxococcus xanthus*. Cells were washed and resuspended in distilled water at 1×10^{10} cell/ml (*58*). A 0.2-ml portion was spread on an agar plate and incubated at 32°C for 66 hours. This experiment was the same as that described in Fig. 10. ($\times$ 60)

Many physiological changes are occurring during the shift from vegetative growth to the developmental sequence. A resolution of the questions concerning the regulatory mechanisms that stimulate the shift will require a further clarification of the regulation of amino acid biosynthesis and transport as well as a finer dissection of the overall process of fruiting body formation.

Cell density dependence. Our interest in fruiting body formation in *M. xanthus* has been to define in broad outline some of the developmental events that occur during the process and thus gain some insight into the most appropriate approaches to more detailed studies. Our standard experimental method is to plate washed vegetative cells suspended in distilled water or 2 percent Casitone medium on unsupplemented Bacto agar plates (*58*). Fruiting body formation (*59*) by cells suspended in distilled water shows a marked dependence on the initial density of the cell population (Fig. 7). Cells plated at high cell density construct fruiting bodies rapidly and synchronously with no net vegetative growth (*60*). At lower cell densities, no fruiting bodies are made, although there are sufficient total cells to make about 50 to 100 fruiting bodies per spot, with approximately 2×10^4 to 4×10^4 cells per fruiting body. Early experiments have shown that a diffusible chemical signal is involved in fruiting body formation (*8*); whether there is a requirement for a minimum number of cells to be present to initiate chemical signaling is unknown.

These results, however, are reminiscent of another feature of the developmental process that is dependent on cell density. Glycerol-induced myxospores of *M. xanthus* will germinate in distilled water if they are present at a sufficiently high cell density. The cell density–dependent requirement can be overcome by the addition of orthophosphate to the cell suspension. Furthermore, it has been shown that myxospores suspended in distilled water excrete orthophosphate, and it has been suggested that the concentration of excreted orthophosphate is both a cell density signal and the germination trigger (*10*). Interestingly, the requirement for high cell density for fruiting body formation can be lowered by the addition of orthophosphate to unsupplemented agar medium (*60*).

Cell death. During fruiting body forma-

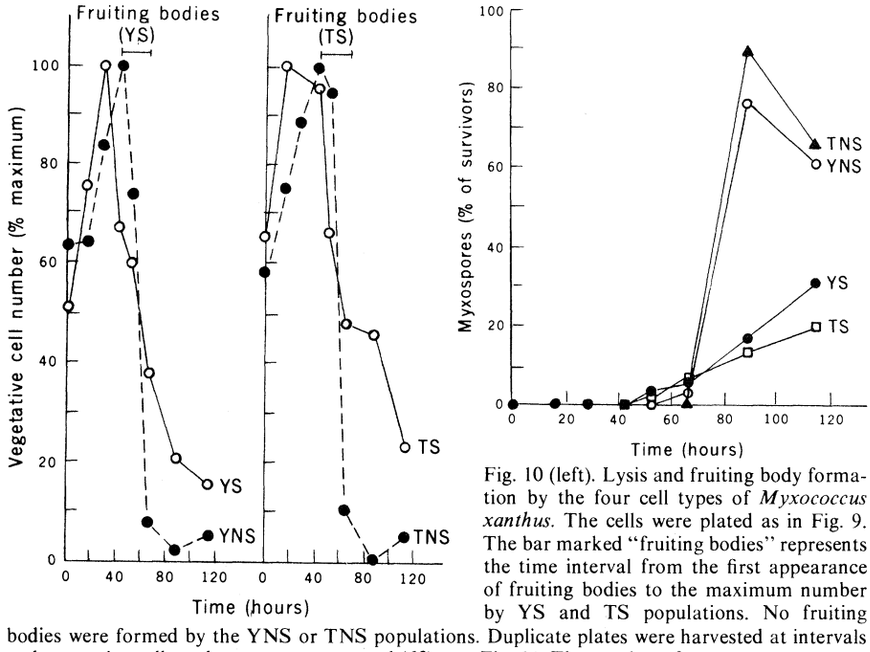


Fig. 10 (left). Lysis and fruiting body formation by the four cell types of *Myxococcus xanthus*. The cells were plated as in Fig. 9. The bar marked "fruiting bodies" represents the time interval from the first appearance of fruiting bodies to the maximum number by YS and TS populations. No fruiting bodies were formed by the YNS or TNS populations. Duplicate plates were harvested at intervals and vegetative cells and myxospores counted (*62*). Fig. 11. The number of myxospores as a percentage of the total surviving cells (that is, the number of vegetative cells plus myxospores) is plotted as a function of time (the same experiment as Fig. 10). The rapid lysis by TNS and YNS populations during myxospore conversion is apparent relative to the gradual lysis during myxospore conversion by TS and YS populations. About the same number of myxospores are formed by all four populations.

tion by wild-type populations of *M. xanthus*, massive cell death occurs (*60*). About 60 to 80 percent of the original vegetative population lyse during fruiting body formation and only about 20 percent survive as myxospores in completed fruiting bodies. We have repeated this observation by several plating methods.

One method of plating, which provides a gradual starvation of cells, is to resuspend washed cells in 2 percent Casitone medium and plate them on unsupplemented Bacto agar. By this method the cells grow for several hours, and gradually the nutrients are depleted by diffusion and growth. If cells are first labeled with radioactive precursors of RNA or DNA during vegetative growth in liquid medium, approximately 80 percent of the label is lost during subsequent fruiting body formation. In the experiment shown in Fig. 8, cells were labeled with [methyl-^{14}C]thymidine (*61*) for several generations during vegetative growth. The plated cells initially were in high nutrient conditions, and by 24 hours maximum growth had been achieved. Approximately 70 percent of the cells present at 24 hours had lysed by 72 hours, and the loss of the labeled DNA followed approximately the loss in cell number (*62, 63*). Whereas the specific mechanism of cell death is unknown, we suggest that regulated cell death has a functional role in the developmental process.

Population differentiation and development. We have also attempted to determine whether there is a developmental role for the four cell types in *M. xanthus* referred to earlier. Colonies of the four types were picked, grown in liquid media, and plated on unsupplemented agar plates. Both the yellow and tan swarmer phenotypes (YS and TS) constructed well-defined fruiting bodies (Fig. 9), whereas the two nonswarmer phenotypes (YNS and TNS) constructed only rudimentary aggregates (Fig. 9). The changes in vegetative cell numbers were followed during the developmental sequence, and a large portion of the nonswarmer populations lysed synchronously and rapidly (Fig. 10), immediately preceding the appearance of mature myxospores. The two swarmer phenotypes also lysed, but the rate of lysis was more gradual, and there was less lysis, as compared with nonswarmer phenotypes. The synchronous lysis of the two nonswarmer phenotypes resulted in almost total loss of vegetative cells within about 15 hours and the appearance of myxospores. Figure 11 shows that the survivors of the nonswarmer populations are predominantly myxospores whereas the survivors of the swarmer populations are still only 30 percent myxospores after 115 hours.

The behavior of mixtures of YS and TNS cells in the wild-type ratio (approximately 1 to 10) is indistinguishable from that of the wild-type population. Fruiting bodies are formed rapidly and completely despite the fact that the majority member of the mixture (TNS) is itself unable to form fruiting bodies. Furthermore, the YS or TS populations are able to construct fruiting bodies and form myxospores within the fruiting body with no apparent requirement for the nonswarmer phenotype.

Germination of myxospores formed in fruiting bodies constructed by the wild-type population (composed of about 10 percent swarmer phenotypes and 90 percent nonswarmer) yielded almost exclusively swarmer phenotypes (90 percent); however, there was no change in the ratio of yellow to tan. That is, YNS and TNS cells massively converted to YS and TS

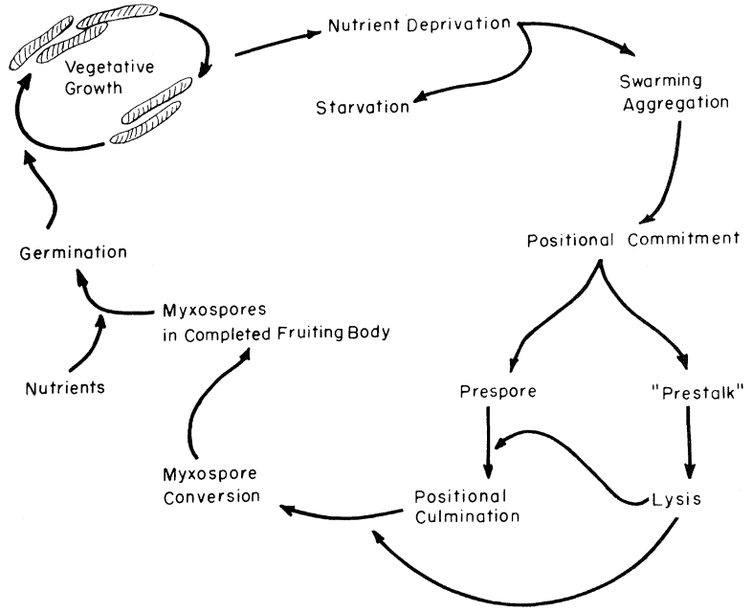


Fig. 12. Diagram of the developmental sequence in myxobacteria. Several of the intermediate steps are hypothetical and are discussed in the text.

cells, respectively, after fruiting body formation and myxospore germination (*64*). However, germination of myxospores formed during development of pure nonswarmer populations of YNS or TNS resulted in exclusively nonswarmer colony types. Conversion of the nonswarmer phenotype to the swarmer required completion of the developmental process and was therefore dependent on the presence of swarmer phenotypes in the original population. It should be emphasized parenthetically that investigations of the physiology or developmental behavior of these organisms must take into account this variability among the cells.

A Preliminary Model

The observations described above represent only the beginning of a dissection of the process of myxobacterial development. It is useful, however, to interpret these observations in a manner that lends some perspective to these complex processes and points to the potential for further studies. Figure 12 represents a schematic hypothesis of several aspects of myxobacterial development.

When vegetative cells are deprived of sufficient nutrients required for further growth, they shift to an alternative mode of behavior. Cells construct fruiting bodies poorly in the absence of exogenous added nutrient, whereas cells provided with limited nutrients or gradually decreasing nutrients are able to complete the developmental sequence rapidly and synchronously. There may be a single nutrient deprivation signal whose regulation is such that threshold levels occur prior to the depletion of the other components required for the completion of development. Alternatively, the nutrient deprivation signal may involve a sensing of time dependent changes in specific nutrients, as has been demonstrated for bacterial chemotaxis (*65*).

We suggest that after nutrient deprivation the concentration of excreted orthophosphate may be a general indicator whereby these organisms determine their own cell density. That is, at sufficiently high cell density or in sufficiently high external concentrations of orthophosphate the aggregation phase of development begins. Nothing is known of the aggregate sensing-responding mechanisms or the chemical signals involved. In *D. discoideum* the aggregation signal is cyclic AMP (*66*). Cyclic AMP or ADP stimulates fruiting by *M. xanthus* (*57*), and the available evidence indicates that these effects may be related to the nutrient deprivation signal rather than being specifically involved as a chemotactic signal for aggregation (*67*).

In addition to diffusible chemical signals, there may be a requirement for other cell-cell interactions involved in swarming and aggregate formation. When we observe the behavior of swarming and aggregating cell populations of myxobacteria, it is difficult to avoid the conclusion that cell interactions play a role in maintaining and guiding the movement of the swarms of cells (*12, 13, 48, 49*). It is characteristic of the myxobacteria that they produce copious amounts of polysaccharide slime, and this occurs during vegetative growth, and also during fruiting body formation. In fact, the thick capsule surrounding myxospores is largely polysaccharide (*31, 47*). It is not unreasonable that cell contact interactions occur either by direct cell contacts or possibly by surface modifications, that is, slime trails.

Aggregation into raised mounds of cells precedes lysis and myxospore conversion. The nature of the stimulus for morphological determination, that is, commitment of a cell to proceed toward ultimate lysis or myxospore conversion, is not understood. It is unlikely, however, that commitment is purely temporal, that is, occurs at some fixed time after nutrient deprivation, since the rate of fruiting body formation may be increased by increasing the initial cell number or by adding orthophosphate to the agar. We would suggest that commitment is likely to be the result of a cell's relative position in the aggregated mound of cells, analogous to the positional determination in the cellular slime molds. In the case of *D. discoideum*, starved amoebas order themselves in the slug as prespore and prestalk cells prior to culmination and fruiting body formation. There has been some dispute concerning the mechanism of determination of prespore and prestalk cells in *D. discoideum*, and it is not yet clear whether, in addition to a reversible positional determination (*3, 68*), there is a sorting out of predetermined cell types (*69*). The prestalk cells eventually lyse and provide the matrix for the stalk. A similar mechanism may be operative in *M. xanthus* development, although not as clearly defined. Stalk formation in other myxobacteria, such as *M. stipitatus*, is much more easily observed than with *M. xanthus*; but no measurement of cell death in other species has been done. Figure 2 illustrates that a large portion of the *M. stipitatus* fruiting body is present as an acellular stalk. In *D. discoideum* about one-third of the cells are prestalk cells (*70*), whereas in *M. xanthus* according to the above hypothesis about 60 to 80 percent of the cells are "prestalk" cells. The lytic products from the "prestalk" cells may provide components for fruiting body construction, that is, slime and stalk, and may also provide lytic products required for myxospore conversion. Regulated senescence and cell death plays a widespread and important role in the development of higher organisms (*71*), and we would suggest that lysis of a portion of the myxobacterial population during the final stages of aggregate formation may be such a regulated aspect of development.

Wild-type populations or pure swarmer cells only form myxospores within the fruiting structure at early times, although at later times individual cells outside of fruiting bodies convert to myxospores. Nonswarmer cells form only small aggregates, and cells within or outside of aggregates convert to myxospores at about the same time. One interpretation of these observations is that the lytic products are primarily sequestered in the slime-covered aggregate, as a result of the position of the lysing cells, and only cells within the enclosed structure are in a sufficiently high concentration to convert to myxospores. The lytic products of nonswarmer cells, however, are not sequestered and are able to diffuse to surrounding cells and effect myxospore conversion, as happens at latter times with the wild-type population. It is possible that swarmer cells produce slime required for fruiting body enclosure and that nonswarmer cells may provide some other necessary function.

The requirement for the presence of swarmer cells for the conversion of the nonswarmer cell type to the swarmer cell type during fruiting body myxospore induction and germination suggests that there may be a direct transfer of chemical information between swarmer and nonswarmer cells, which effects a stable change in phenotype of the nonswarmer. It is not yet possible to define the mechanisms or nature of such interconversion, but we would suggest that the population differentiation in *M. xanthus* has a functional role and may provide an optimum mix of cell types for the most efficient accomplishment of several tasks. An experimental search for both the mechanism of the population transition and their functional roles during vegetative growth and during development will certainly lead to further insights into the nature of communication and cooperation during development.

There are numerous examples of evolutionary adaptations among eukaryotes in which the survival of the individual is subordinate to the survival of the species. The pervasiveness of the interactive behavior in the myxobacteria makes it apparent that there is a considerable adaptive role of the group at the expense of the individual. The

myxobacteria derive nutrients by the action of secreted extracellular enzymes (*72*), and a large group is more effective in optimizing feeding efficiency (*13*). Under normal circumstances the number of myxospores found in the simple fruiting body of *M. xanthus* or the more complex cysts of *S. aurantica* is probably related to a feeding requirement, insuring an optimal swarm size upon germination and the principal function of the colonial aspects of myxobacterial development may be to maintain the potential for a high cell density of the feeding swarm (*13*), that is, a "wolf pack."

Is there, in addition, a requirement for massive cell lysis to insure the satisfactory culmination of *M. xanthus* fruiting bodies? Our observations indicate that there is an obligatory sequence and lysis is a regulated developmental event. The phenomenon of "cooperative" cell death in *M. xanthus* may provide another interesting primitive example of the sacrifice of individual members of the species to insure the survival of the species. We have suggested above that lysis in *M. xanthus* is involved in both fruiting body formation, that is, slime and stalk construction, and myxospore conversion. It would be of some interest to know more about the role of lysis of prestalk cells in the development of the cellular slime molds and whether prestalk cell lysis contributes essential signals for spore formation.

Summary

The myxobacteria represent a unique class of prokaryotes which interact with each other throughout their life cycle. These interactions play a role both in the swarming and feeding process as well as in fruiting body formation. The direct conversion of vegetative cells to myxospores by chemical induction, bypassing the requirement for fruiting body formation, has been a useful system for the study of cellular morphogenesis. The mechanisms of cell-cell interactions during colonial morphogenesis, however, have remained elusive. The myxobacteria, nevertheless, provide an ideal class of organisms for the study of communication and cooperation in a prokaryote. There is a cell density requirement for fruiting body formation in *M. xanthus* during which a large portion of the population lyses. This phenomenon may be analogous to the lysis of stalk cells during fruiting body formation by the eukaryotic cellular slime molds. In addition, there is a reversible population differentiation into four colony types. The two swarmer phenotypes construct fruiting bodies containing myxospores rapidly and the nonswarmer phenotypes are unable to construct fruiting bodies but are able to convert to myxospores. The nature of the cooperative developmental interactions which may occur between these cell types is unknown. Recent advances by several groups make it likely that the developmental interactions in these complex prokaryotes will soon be approachable from a genetic and biochemical basis.

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61. Cells were grown for about five generations to a density of about 6×10^8 cell/ml in 2 percent Casitone medium containing [methyl-^{14}C-]thymidine (0.25 μc/ml). Cells were harvested on ice and resuspended in twice the original volume of 2 percent Casitone medium containing unlabeled thymidine (200 μg/ml) and grown for one generation.
62. Plates were harvested by scraping with a razor blade and rinsing three times with cold distilled water. The number of vegetative cells and myxospores was measured by fixing one portion of the cell suspension (or cell and fruiting body clumps) with 5 percent glutaraldehyde on ice overnight. A 1-ml portion was sonicated for 5 seconds with a Heat Systems-Ultrasonics Sonifier at an output setting of 3. This procedure preserves vegetative cells quantitatively but does not entirely disrupt clumps of myxospores (from fruiting bodies); therefore, vegetative cells were counted in this portion in a Petroff-Hausser chamber. A separate portion of the cell suspension was sonicated for 15 seconds without prior fixing with glutaraldehyde. This procedure completely disrupts myxospore clumps and lyses most of the vegetative cells; therefore, myxospores were counted in this portion.
63. The total radioactivity in the acid-precipitable fraction remaining per plate was determined by precipitation of a portion of the cell suspension with an equal volume of 10 percent trichloroacetic acid (TCA) on ice for 1 hour. The precipitate was removed by centrifugation at 12,000*g* for 10 minutes, and resuspended in 5 ml of cold 5 percent TCA. The precipitate was washed once more with 5 percent TCA and finally washed with 5 ml of 75 percent ethanol. The final precipitate was dried in

air and resuspended in 0.2 ml of distilled water and hydrolyzed overnight at 50°C with 2 ml of NCS tissue solubilizer (Amersham/Searle). To the hydrolyzed sample was added 20 ml of toluene-based counting fluid (PPO-POPOP) and the radioactivity was determined.
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Distributive Computer Networking: Making It Work on a Regional Basis

Effective sharing through a network requires new management and resource distribution techniques.

Ronald W. Cornew and Philip M. Morse

Recent articles in *Science* have dealt with computer networking from a number of perspectives: the national overview for computer and information networks (*1*), the results of free access on an individual campus (*2*), and whether or not an academic computing center should join a network (*3*). In this article we examine the experience of establishing and operating a multisupplier or "distributive" computer network (*4*) and indicate some of the management and resource distribution techniques which the New England Regional Computing Program (NERComP) is finding useful in achieving effective resource sharing on a regional basis. Established in 1971, the NERComP network now serves educational and research computing needs at approximately 40 colleges and universities throughout the six New England states.

The NERComP network is currently unique; it is the only educationally oriented regional network involving multiple supplying institutions which is not contained within a single state or operated by a single quasi-state agency. Its success in achieving a nonsubsidized and completely cost-recovering operation appears particularly relevant to the larger task of establishing such facilities on a national basis, as discussed by Greenberger *et al.* (*1*), Massy (*3*), and others.

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Evolution of NERComP

The New England Regional Computing Program developed from a consortium of New England colleges and universities which, through an IBM grant beginning in 1957, was permitted to use the Model 704 computer located at the Massachusetts Institute of Technology. At that time the MIT computer represented the sole computing resource for these schools. As campus computing proliferated in the early 1960's, many of these institutions began to acquire other sources including, in a growing number of cases, on-campus computers. They continued to make use of the MIT facility, however, because it offered access to a succession of bigger, more capable machines than their own.

By 1967 it was apparent that these colleges and universities, of which more than one-half by then had their own computers, needed a variety of additional services which could not be provided by MIT alone. As they moved toward other suppliers, it became evident that a unified means of accessing the numerous large machines which were then available at academic institutions elsewhere in New England could be very useful. It was this realization that first led to consideration of a network in the region.

In order to prepare its participating institutions for the changes required in accessing a computer by utilizing a network, the consortium, with National Science Foundation assistance, engaged in a number of regional "pump priming" activities in 1968 and 1969. These included a teletype loan program in which a computer terminal and a small supplementary grant were made available to participating schools—typically smaller institutions or those lacking computer resources—to encourage them to try time sharing. The grant was intended to offset most of the costs, including computer charges and long-distance telephone toll charges, for a period of direct connection to one or more of the systems then existing in the region. For some of the medium-sized to larger institutions in New England, the consortium also participated in computer evaluation studies designed to help the institution select equipment that would meet current needs but still be consistent with future network development.

NERComP was incorporated in 1970 as a not-for-profit corporation under the laws of Delaware, which permit formation of a corporation of corporations—a step believed necessary in order to reflect adequately the organizational nature of the consortium. Approximately 40 New England institutions of higher learning participated in the incorporation of the organization. Responsibility to its member institutions was assured through a body of institutional representatives appointed by the presidents of the dues-paying member colleges and universities. These representatives have since met at least once a year. Between general meetings, the business of