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Bacterial Differentiation

The cell cycle of *Caulobacter* is used as a model system for studying the molecular basis of differentiation.

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Living cells, whether they are components of complex tissues and organs or function as unicellular organisms, are not static units but can change morphologically, adapt to environmental conditions by biochemical alteration, or express different functions at different times in the life cycle. A major task of modern biologists is to understand the control of these cell changes. Recent investigations of the genetic control mechanisms which operate in microbial cells indicate that not all the genes in a given cell are translated into protein at the same time (*1*), and that environmental factors can and do influence gene expression (*2*). In a differentiating cell, undergoing a series of biochemical and morphological changes within a regulated time sequence, controls must also exist to ensure the organization and sequential expression of these events during the life cycle of the cell. A persistent notion among developmental biologists has been that organizational control functions are a regular component of the genetic composition of each cell. Important advances in the understanding of cellular differentiation would seem to lie in exploring systems of interacting regulatory genes involved in the programmed expression of well-defined characteristics.

The control mechanisms that are ultimately shown to regulate the process of differentiation in one type of cell may not be applicable to all cells. In both prokaryotes and eukaryotes, however, the observable changes accompanying cell development do reflect variations in the patterns of protein synthesis. It is possible that the complex genetic machinery of the eukaryote demands different or additional controls of protein synthesis (*3*). A full understanding of the regulatory mechanisms of differentiating prokaryotic cells, however, might provide a valid basis for extrapolation to cells of higher organisms. Most importantly, prokaryotic cells that undergo cellular differentiation are accessible to study by the established techniques of bacterial genetics. One such area in which significant contributions to the control of cell regulation have come is sporulation and germination in the genus *Bacillus* (*4*). In these studies it has become apparent that control at the level of selective messenger RNA production (transcription) represents the basic mechanism whereby the cell organizes its development (*5*, *6*). Clues as to the universality of this type of control mechanism may come from the exploration of other readily studied bacterial systems. It is in this context that we have attempted to develop a simple system, using the dimorphic *Caulobacter* bacteria, to study the regulation of cellular differentiation.

Systems for Studying Bacterial Differentiation

Relatively few bacteria carry out a defined morphogenesis, other than cell division, during their normal life cycles. Still fewer bacterial species both differentiate and offer ease of genetic and biochemical manipulation. The criteria that make the use of bacteria reasonable as model systems for cell development include (i) a simple and well-defined differentiation pattern which can be studied in synchronized cell populations, (ii) the ability to grow the cells on defined media, permitting the correlation of biochemical events with morphological development, and (iii) the availability of mutants and a system for the exchange of genetic information. Differentiating bacteria that offer a

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possibility for study include *Bacillus, Streptomyces, Arthrobacter, Myxobacter,* and *Caulobacter.*

The spore-forming *Bacilli* fulfill all of these criteria and have commanded the greatest amount of attention; progress with this system has been extensively reviewed (*4, 5*). These rod-shaped bacteria differentiate by forming heat-resistant and chemically resistent spores which can again become vegetative rods through the process of germination and outgrowth. Sequential changes occurring in a sporulating cell have been carefully defined and include, in addition to morphological alterations (*7*), the appearance of new enzymes (*8*), alterations in electron transport pathways (*9*), and the synthesis of spore-specific substances (*10*). Analysis of mutant organisms has demonstrated that genetic determinants govern these morphological and biochemical changes (*11*). The definition of the sequential expression of specific genes has been greatly facilitated because the outgrowth of cultures of spores to form vegetative cells proceeds synchronously (*5*). During outgrowth, the first gene product is new messenger RNA, followed by the formation of new proteins and enzymes (*5, 12*). This control of sequential expression, which appears to be mediated at the level of transcription (*5*), has been demonstrated to be due to a new type of RNA polymerase which can be isolated from sporulating *Bacillus subtilis.* This polymerase differs from that of the vegetative cells in that the β-subunit of the core enzyme is altered along with an alteration in template specificity (*6*). Thus *Bacilli* offer important guidelines not only to the study of the control of differentiation of bacterial cells, but perhaps of eukaryotes as well.

Other groups of bacteria, such as *Streptomyces, Myxobacter,* and *Arthrobacter,* offer distinct advantages with respect to some of the above-mentioned criteria while they remain deficient in others. Of these groups, the *Streptomyces* can be grown on defined media and are particularly amenable to genetic study. Hopwood and Sermonti have extensively studied the genetics of these bacteria (*13*). The life cycle includes spore germination to form a vegetative cell that develops into a complex mycelium, then septates into aerial hyphae, and then produces single spores that can reinitiate the differentiation cycle. Only recently, however, have there been

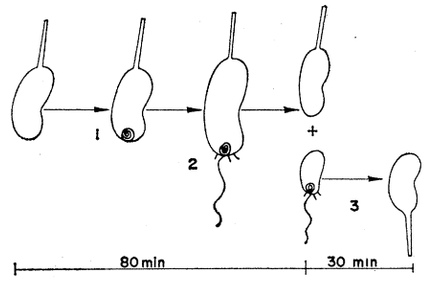

Fig. 1. Representation of the life cycle of *C. crescentus.*

studies on biochemical definition of the differentiation process by examination of mutants blocked in development (*13*). On the other hand, studies with *Myxobacter* have emphasized the isolation of morphological mutants in order to define macromolecular behavior during morphogenesis (*14*). This bacterium can be induced to change from the rod to microcyst form synchronously (*14*); but it cannot be grown on defined media, and a genetic system has not yet been developed. *Arthrobacter* presents an advantage distinct from *Streptomyces* or *Myxobacter* in that its simple differentiation process, which consists of a biochemically and morphologically well-defined sphere-rod transition, is under environmental control (*15*). The main drawback of this system, as in *Myxobacter,* is the absence of a genetic system; consequently no relation between genetic events and phenotypic response has as yet been demonstrated.

The purpose of this article is to demonstrate that *Caulobacter* fulfills the defined criteria and can therefore be used effectively to complement the *Bacillus* and other bacterial systems in providing further insight into control mechanisms operative during prokaryotic differentiation.

Properties of Caulobacter crescentus

Bacteria of the *Caulobacter* genus are aerobic, gram-negative organisms which, like *Pseudomonas,* metabolize hexoses via the Entner-Doudoroff pathway (*16*). A unique feature of *Caulobacter* bacteria is that they carry out obligatory well-defined morphogenic changes during their life cycles (*17*; Fig. 1). The *Caulobacter* are distinguishable morphologically in that they have a stalk at one pole of the cell (*18*). The wall of the stalk is continuous with both the lipopolysaccharide and mucopeptide layer of the cell wall (*19, 20*). Under normal growth conditions a new stalked cell synthesizes an intracytoplasmic structure at the pole opposite the stalk (*16, 19–21*), and this process represents the first step in a cycle of differentiation. It has been suggested that this structure is a membranous organelle that can be distinguished from cell mesosomes by the lack of continuity with the cytoplasmic membrane (*21, 22*). The same type of membranous organelle is found at the stalked pole of the mature cell, as shown in Fig. 2, A and B. We are still unsure of the nature of this organelle, because electron microscopic analysis does not consistently reveal a discrete, membranous structure in the region of the polar organelle (*23*). The second occurrence in the differentiation process is the formation of a single flagellum and several pili also at the pole opposite the existing stalk (Fig. 1) (*19, 20*). Binary fission yields a sessile stalked cell and a motile swarmer cell (Fig. 2C). The daughter swarmer cell must differentiate into a stalked cell before it is capable of cell division. This transition, the third step in the differentiation process, involves the deposition of new cell wall material at the point of attachment of the flagellum to the cell (*20*). The preexisting polar membrane structure now constitutes the interior of the newly forming stalk, where the flagellum can be found as an appendage at the tip of the stalk. Eventually the flagellum falls off and can be recovered in the medium (*24*). Autoradiographic experiments performed by Schmidt and Stanier (*20*) demonstrated that the site of stalk synthesis is restricted to the juncture of stalk and cell. Each step in the differentiation process, therefore, represents the synthesis of a specific structure (polar membrane, flagellum, pili, stalk) at a specific site in the cell, and at a specific time in the cell's life cycle.

The genome of *Caulobacter crescentus* is contained in approximately 3×10^{-8} microgram of DNA per cell (*25*), similar to *Escherichia coli,* a bacterium of approximately the same size. A unique feature of *Caulobacter* DNA is the existence of a satellite band that can be detected only in the stalked cell (Fig. 3). It is not known whether the satellite band is a piece of DNA with a lower guanine plus cytosine (G + C) content or one that has unusual bases. Chemical analysis of total

Table 1. Characteristics of DNA bacteriophage φCbK and its host *Caulobacter crescentus* (*25*). C, cytosine; A, adenine; G, guanine; T, thymine; T_m, melting temperature.

Properties	φCbK	*C. crescentus*
Buoyant density in CsCl	1.53 g/cm³	
Size of head	195 by 64 nm	1 by 2 μm
Size of tail	275 nm	
DNA content	57 percent	3×10^{-8} μg/cell
DNA buoyant density in CsCl	1.721 g/cm³	1.718 g/cm³
DNA $s_{20,w}$	63.5*S*	
DNA T_m*	95°C	94°C
DNA base composition	C = 32.5; A = 17.4 G = 32.7; T = 17.4	C = 33.5; A = 16.5 G = 33.7; T = 16.4

* In saline sodium citrate.

stalked cell DNA labeled with ^{32}P revealed a G + C content of 67 percent, and all of the radioactive material migrated during electrophoresis as either adenine, cytosine, guanine, or thymine mononucleotides (*26*). The presence of the satellite band in stalked cells may therefore represent extra DNA with a low G + C content, which is either physically separated from the bulk of the nuclear DNA or replicated specifically during the stalked phase of the differentiation cycle.

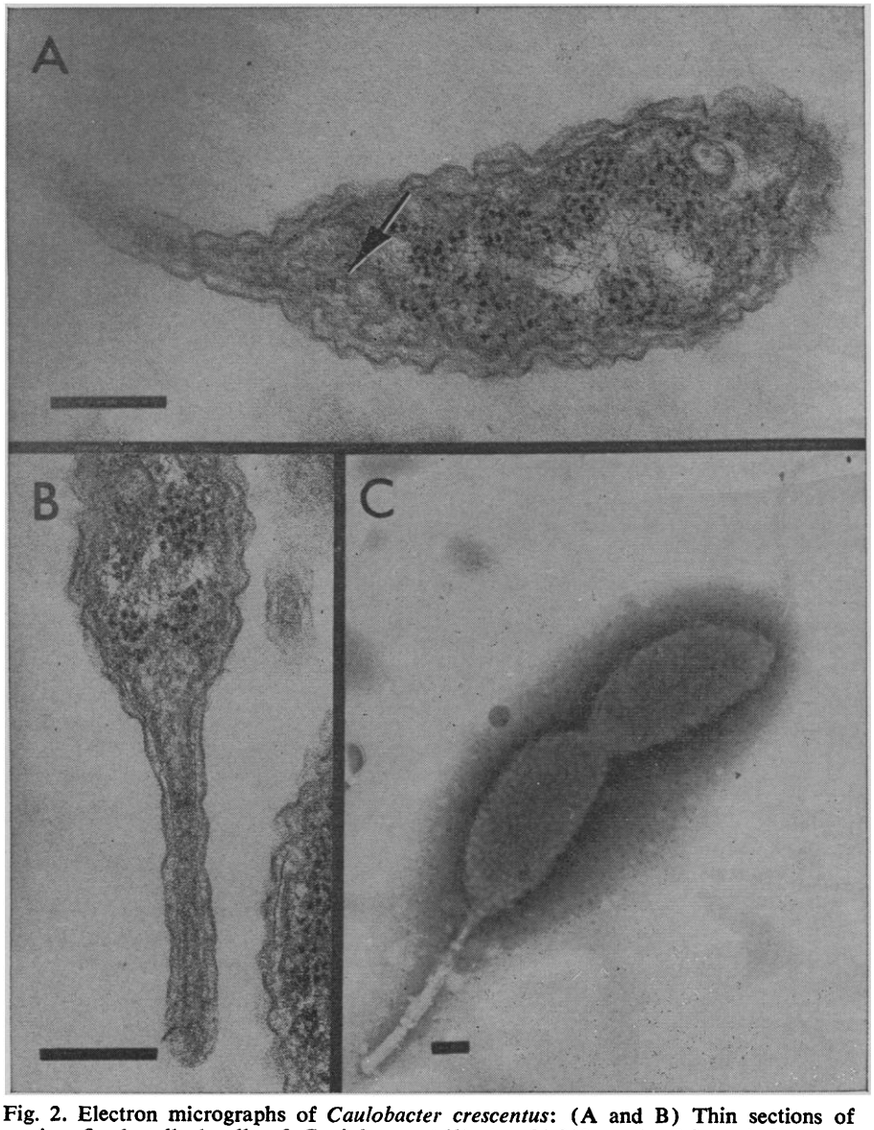

Fig. 2. Electron micrographs of *Caulobacter crescentus*: (A and B) Thin sections of osmium-fixed stalked cells of *Caulobacter* (*41*) (× 81,000; scale, 0.2 μm) [courtesy of Dr. P. G. Model]. A membranous structure (arrow) is evident as the base of the stalk in (A), but not in (B); (C) dividing cell negatively stained with phosphotungstate (× 22,000; scale, 0.2 μm) [courtesy of Dr. P. Atkinson].

Structural changes that occur during the *Caulobacter* life cycle include the synthesis of a new cell wall to form the stalk (*20*) and the temporally controlled appearance of a cell-wall receptor site for a DNA bacteriophage (*27*). In order to correlate such cellular alterations with biochemical events, D. Button, of this laboratory, is studying the chemical composition of the *C. crescentus* cell wall at different stages of the differentiation cycle. In general, the lipopolysaccharide (LPS) component of gram-negative cell walls contains a neutral sugar backbone structure coupled to a structure referred to as lipid A (*28*). The neutral sugar composition of *Caulobacter* LPS was analyzed in populations of swarmer cells and stalked cells and was found to be identical. The most abundant sugar is D-glucose; the remainder of this fraction, as determined by gas-liquid chromatography, includes D-glycero-D-mannoheptose, L-glycero-D-mannoheptose, D-galactose, D-mannose, and an unidentified polyhydroxy compound (*29*). The LPS also contains 2-keto-3-deoxyoctulosonate (KDO), but *o*-phosphorylethanolamine and glucosamine-phosphate, components of LPS in *E. coli* and *Salmonella*, cannot be detected (*29*). Further investigation of the lipid moiety showed that both β-hydroxymyristic acid and lauric acid are absent (*29*). We have concluded from these studies that, although *C. crescentus* is a gram-negative organism, it has no "lipid A" in its water-soluble LPS and that both *Caulobacter* cell forms have the same wall composition.

Caulobacter Viruses

Seven distinct groups of lytic bacteriophages that specifically infect *Caulobacter* have been isolated and classified according to their nucleic acid content, host range, and serology (*30*). In addition, it has been reported that temperate bacteriophages could be induced from several *Caulobacter* strains (*31*). We have analyzed the physical characteristics of both a lytic RNA and DNA bacteriophage, and their relation to their dimorphic host, *C. crescentus*, during various steps in the infection process (*26, 27, 32*).

The RNA bacteriophage ϕCb5 (*32*) is a small polyhedron 23 nanometers in diameter, whose sedimentation coefficient ($s_{20,w}$) is 70*S*, which is composed of a single molecule of single-stranded RNA and a protein coat assembled from two structural proteins. The phage appears to contain the genetic capacity to code for a coat protein subunit, a protein analogous to the R17 maturation protein, and an RNA polymerase not present in uninfected cells (*32*). Bacteriophage ϕCb5 differs from the *E. coli* RNA phages in that it specifically infects *Caulobacter*, contains histidine but lacks methionine in its coat protein, and is extremely salt-sensitive. During the infection process ϕCb5 specifically adsorbs to pili present on the *Caulobacter* swarmer cell (*33*). Susceptibility of the host cell to RNA phage infection during only a limited time in its life cycle poses unique problems in phage propagation, since at any given time in a log-phase culture susceptible swarmer cells compose approximately 15 percent of the population. Difficulties in obtaining liquid lysates of high titer were overcome by allowing small numbers of bacteria to grow for 12 to 16 hours in the presence of 10^{-3} phage per bacterium. Under these growth conditions phage-sensitive swarmer cells are continually being produced by the resistant stalked cell population (*32*).

The DNA bacteriophage ϕCbK was isolated in our laboratory and shown to be among the largest known bacterial viruses (*26*). The phage, composed of protein and DNA, has an elongated polygonal head 195 by 64 nm and a flexible tail 275 nm in length (Fig. 4). The properties of the double-stranded DNA from ϕCbK are similar to those of the host cell with respect to buoyant density, melting point, and G+ C content (Table 1). We analyzed the mode of infection of *C. crescentus* with this bacteriophage and determined that the phage specifically infects the swarmer cell or a predivisional form with swarmer cell characteristics (*27*). Initial evidence that the phage required a specific host property for productive infection came from one-step growth experiments with synchronized populations of swarmer and stalked cells. A longer eclipse period was observed with the stalked cell than with the swarmer cell, suggesting that time was required for the formation of the sensitive cell type (*27*). The hypothesis that adsorption to stalked cells is reversible was confirmed by observations with wild-type cells and two classes of ϕCbK-resistant mutants; the phage is not eclipsed and remains viable (Table 2). Adsorption followed by DNA injection occurs only in the swarmer and predivisional cell forms. It appears, therefore, that the synthesis of a new phage attachment site that permits phage DNA injection illustrates another facet of differentiation in the *Caulobacter* life cycle. This phage receptor site apparently resides in the wall LPS (*29*). Sonically treated extracts of pure populations of swarmer and stalked cells were prepared and extracted with phenol (Table 3; *29*). The LPS was recovered in the aqueous phase, and protein was quantitatively recovered in the phenol phase. Bacteriophage ϕCbK was specifically inactivated by the LPS fraction of the swarmer cell, but not by the same fraction from the stalked cells. Therefore a structural moiety present in the swarmer cell appears to be absent or altered in the stalked cell. Antibodies were prepared against the LPS from wild-type cells and from the bacterial mutant "ϕ resistant I" which fails to adsorb phage (see Table 2). An antigen observed in the wild-type cell was not present in the phage-resistant mutant. The total LPS composition of "ϕ resistant I" was also determined, and it was found to be identical to wild type, with the exception that there was a 20-fold reduction in the amount of D-glucose present in this fraction (*29*).

Table 2. The ability of bacteriophage ϕCbK to absorb, infect, and inject its DNA into swarmer and stalked cells of wild-type and mutant host strains (*27*).

Cell type	Phage adsorption (%)	Phage eclipsed (%)	Phage ^{32}P-DNA recovered in host cell (count/min)
Wild-type swarmer	90	80	558
Wild-type stalked	80	1	19
ϕ-Resistant I swarmer	0	4	96
ϕ-Resistant I stalked	0	3	62
ϕ-Resistant II swarmer	90		64
ϕ-Resistant II stalked	95		69

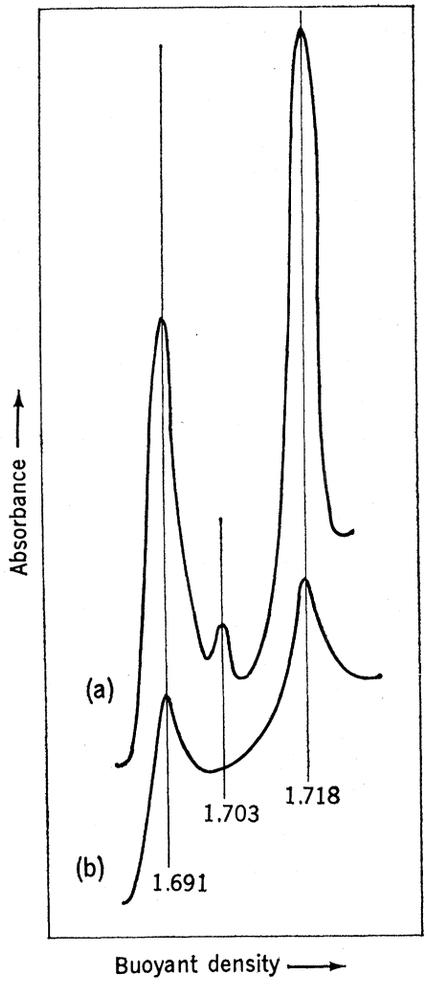

Fig. 3. Microdensitometer tracings of DNA in CsCl after 20 hours of centrifugation at 44,770 rev/min. (A) DNA isolated from stalked *Caulobacter* cells which banded at $\rho = 1.718$ g/cm^3 and $\rho = 1.703$ g/cm^3. (B) DNA isolated from swarmer *Caulobacter* cells which banded at $\rho = 1.718$ g/cm^3. *Clostridium perfringens* DNA ($\rho = 1.691$ g/cm^3) was used as a reference marker [from (*26*)].

Cell Synchrony

The study of an organized sequence of events requires populations of cells doing the same things at the same time. Swarmer and stalked cells can be separated by differential centrifugation (*17*), since the stalked cell offers greater frictional resistance in a centrifugal field than the swarmer cell does. At stationary phase, 95 percent of the cells are stalked. Remaining swarmer cells, as well as cell clusters (rosettes), are removed by low-speed centrifugation. Synchronous growth is

initiated by incubating the stalked cells in relatively dilute cultures at 30°C. We have observed microscopically that by 80 minutes approximately 50 percent of the total cell population consists of motile swarmer cells. Simultaneous measurement of viable count showed that cell division occurs at 80 minutes (Fig. 5A).

Swarmer populations can be obtained when pure stalked cells go through one cell division, and 50 percent of the resulting population exists as swarmer cells. These are centrifuged at low speed, and synchronous growth is initiated by incubation at 30°C in fresh broth. Since we had determined the time required for stalked cells to divide and measured the time required for cell division starting with swarmer cells we deduced the interval required for the transition of swarmer cells to stalked cells (Fig. 5B). Swarmer cell populations, observed by microscopy and viable count measurements, divide 110 minutes after the start of growth. Swarmer cells develop stalks, therefore, in 30 minutes (*34*). Stove and Stanier followed the growth cycle of single clones of *C. crescentus* in microculture and showed that cell transition occurred in 25 to 30 minutes (*17*).

Table 3. Effect of extracts of the host cell wall on infectivity of bacteriophage ϕCbK measured as plaque-forming units (PFU). Bacteriophage ϕCbK was mixed with a known quantity of wall extract and its infectivity was determined. The control PFU was determined by infection of unfractionated cells (*29*).

Source of wall extract	ϕCbK infectivity (PFU)
Control	346
Stalked cells Aqueous phase (10 mg/ml)	320
Stalked cells Phenol phase (10 mg/ml)	368
Swarmer cells Aqueous phase (5.2 mg/ml)	7
Swarmer cells Phenol phase (5.2 mg/ml)	334

We have developed a technique for obtaining relatively pure populations of dividing cells based on the differences in buoyant density of cells in a mixed population. Cells in the log phase of growth in medium containing 0.2 percent glucose are layered on an equal volume of medium supplemented with 20 percent glucose in a simple glass centrifuge tube. Centrifugation for 3 minutes at 37,000*g* suffices to concentrate the dividing cells at the interface between the two glucose solutions. This technique obviates the need for using stationary phase cells in the synchronization process and thus reduces the metabolic fluctuations resulting from adaption of stationary phase cultures.

Most synchronization techniques yield populations of cells growing and dividing with a good deal less than perfect synchrony. The maximum concerted increase in cell number that we have observed is 80 percent (see Fig. 5). This degree of asynchrony, however, still permits us to observe the fundamental changes occurring in the *Caulobacter* life cycle. Synchronized cells, beginning with swarmers, were treated with ^{14}C-labeled amino acids at 10-minute intervals during the 110-minute differentiation cycle, and the newly synthesized proteins were analyzed by electrophoresis in polyacrylamide gels in the presence of sodium dodecyl sulfate (*35*). Thirty minutes into the cycle, at the time when swarmer cells have grown stalks and are no longer motile, a sharp change in the pattern of protein bands became apparent in stained gels and in autoradiograms (*24*). These differences most certainly represent changes in enzyme content and regulatory proteins, as well as in structural proteins. We are now attempting to correlate some of the bands with structural proteins peculiar to the morphogenesis observed in *Caulobacter*. Two of the major bands have been tentatively identified as flagella or pili protein (or both) by comparing gels of either whole swarmer or stalked cells with fractionated swarmer cells and flagella and pili purified from the medium of cultures in the log phase of growth (*24*).

We could have inferred from a gross study of *Caulobacter* morphogenesis that new structural proteins are produced during differentiation, and from a knowledge of molecular genetics we recognize that new enzymes are probably synthesized or activated (or both) for the purpose of building new cell structures. Cataloging the appearance or disappearance of proteins during differentiation permits us to follow cell changes. There still remains the crucial matter of defining the regulation of these changes at the molecular level. The focus of these studies on the defined development of *Caulobacter* is now directed toward an understanding of the specific inducers and regulators of differentiation.

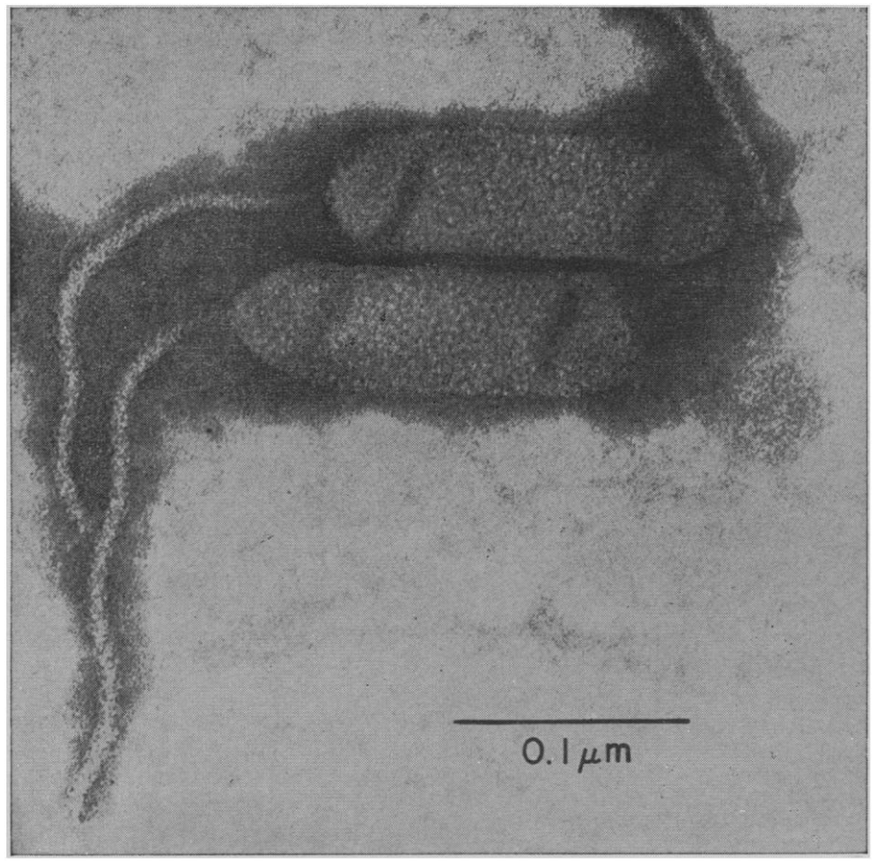

Fig. 4. Electron micrograph of bacteriophage ϕCbK negatively stained with uranyl acetate (× 440,000) [courtesy of Dr. A. K. Kleinschmidt].

Assay for Differentiation in Caulobacter

Study of *Caulobacter* mutants that are blocked at various stages of morphogenesis is a promising approach toward understanding the relation between gene function and the concerted series of events that eventually result in differentiation. In order easily to identify differentiation mutants, we developed a technique with which to monitor the transition between *Caulobacter* cell types in synchronized cultures.

The differentiation assay is based on the fact that RNA and DNA bacteriophage receptor sites are synthesized at specific times in the cell's life cycle. RNA bacteriophage ϕCb5, labeled with radioactive adenine, is retained on Millipore filters only if combined with cells (*36*; Fig. 6A). Since ϕCb5 specifically combines with the pili present on swarmer cells (*33*), the swarmer cell loses the ability to retain labeled phage as it changes into a stalked cell and pili are lost (*34*). The time required for the transition of swarmer to stalked cells was deduced to be 30 minutes (see above). This time span was confirmed by the phage adsorption assay, because the binding of radioactive phage stopped at 30 minutes, along with the loss of cell motility observed microscopically. Thus the RNA phage adsorption assay reflects morphogenic changes occurring in *Caulobacter* and can be used to monitor simply and accurately the transition in synchronized cultures of swarmer to stalked cells.

A complementary phage assay is provided by measuring the appearance of the cell LPS receptor site specific for the injection of phage ϕCbK DNA labeled with [^{3}H]adenine (Fig. 6B). When phage was added to synchronized stalked cells, an increase in the ability to support injection of phage DNA occurred between 50 and 60 minutes. The structural changes occurring in the wall of a stalked cell preparing for cell division can thus be observed and defined by a simple radiochemical assay (*27*).

Caulobacter Mutants

Temperature-sensitive (Ts) mutants are of special value because they permit analysis of genes which control essential cellular functions, such as DNA, RNA, and protein syntheses, cell division, and cell wall synthesis (*37*). The transition of swarmer to stalk cells in *Caulobacter* is obligatory for survival, and therefore the processes of differentiation are essential functions of the cell. Approximately 70 Ts mutants of *Caulobacter crescentus* which are unable to form colonies at 37°C but grow normally at 23°C have been isolated (*38*). These mutants were isolated from cultures treated with the mutagen nitrosoguanidine and, in large part, are of independent origin. The isolation procedure, which excludes a selective step, was based on the method described by Hartwell for obtaining Ts mutants of yeast (*39*). The *Caulobacter* mutants vary in their ability to survive after exposure to the restrictive temperature, since they were never exposed to 37°C during the isolation steps. Mutants which are defective in DNA synthesis, RNA synthesis, and cell division, have been identified. These mutants can be used to study the relation between macromolecular synthesis and the expression of the differentiated state in synchronously growing cultures. Of primary interest, however, are mutants defective in the various aspects of the differentiation process. A mutant that we refer to as TsM continues to increase in mass after a shift to 37°C, but the viable count remains constant for 4 hours after the shift. During this period the cells, which can be observed by phase contrast microscopy, grow erratically, rarely divide, and form long, branched cells. DNA, RNA, and protein syntheses continue at near normal rates. In order to determine whether these cells are expressing functions connected with differentiation at 37°C, we measured their

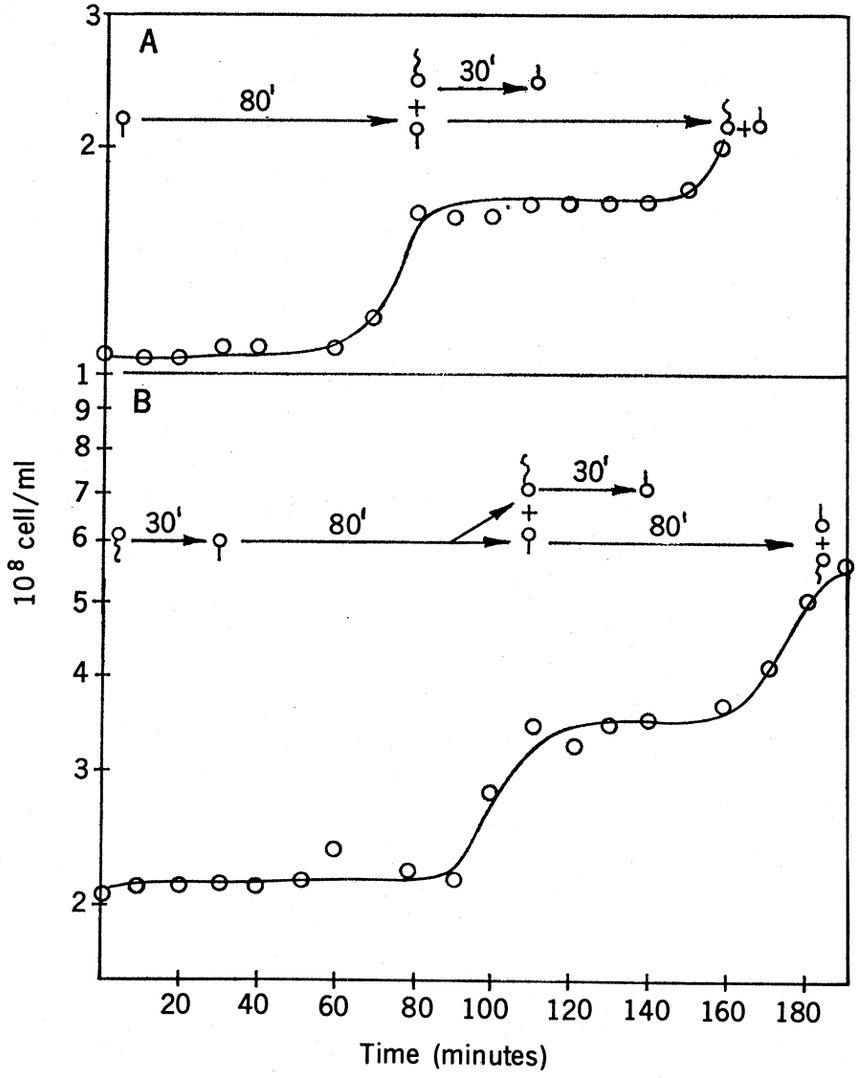

Fig. 5. Synchronous growth of *Caulobacter crescentus*. Pure cultures of either stalked cells (A) or swarmer cells (B) were prepared and then incubated at 30°C. Portions were withdrawn at the times indicated and assayed for viable bacteria [from (*34*)].

Table 4. Interspecific genetic exchange. The selective medium was minimal, str for Cross I; and minimal, pro for Cross II. CFU, colony-forming units.

Strains or crosses	Complete media (CFU)	Selective media (CFU)	Recombination or spontaneous mutation frequency
	Cross I		
CB15Str^{S}Bio$^+$	3.8×10^{10}	1.0×10^2	$1–2 \times 10^{-8}$
CV115Str^{R}Bio$^-$	8.6×10^{10}	0	0
CB15Str^{S}Bio$^+$ × CV115Str^{R}Bio$^-$	1.1×10^{11}	1.9×10^5	1.7×10^{-6}
	Cross II		
CV163Str^{R}Pro$^-$Vit$^-$ yellow	1.0×10^9	0	0
CB13Str^{S}Pro$^+$Vit$^+$ white	5.8×10^9	4.2×10^1	1×10^{-8}
CV163Str^{R}Pro$^-$Vit$^-$ yellow × CB13Str^{S}Pro$^+$Vit$^+$ white	1.0×10^{11}	1.5×10^5	1.5×10^{-6}

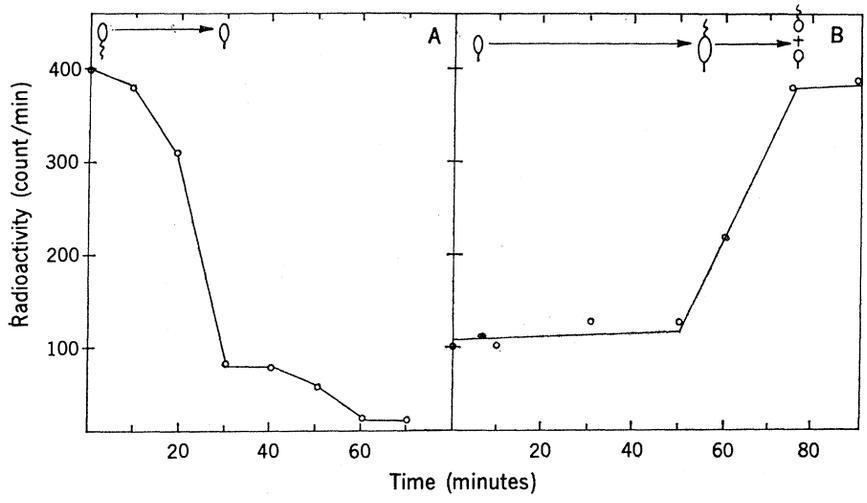

Fig. 6. Technique to monitor the transition between *Caulobacter* cell types in synchronous cultures. (A) Selective adsorption of [^{3}H]RNA bacteriophage ϕCb5 [data from (*34*)]. (B) Selective injection of bacteriophage ϕCbK [^{3}H]DNA.

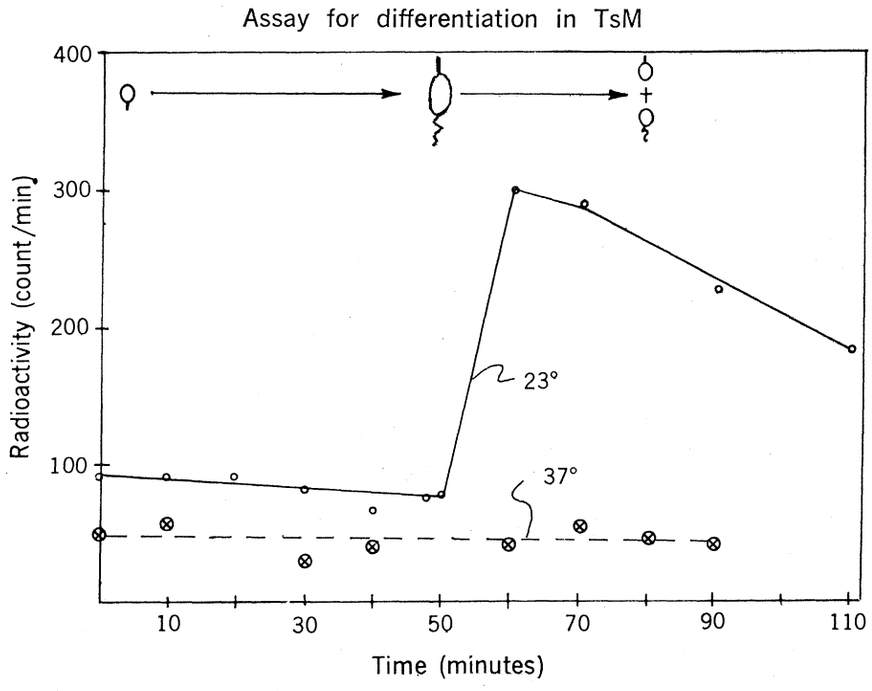

Fig. 7. Injection of bacteriophage ϕCbK [^{3}H]DNA during synchronous growth of TsM at 23° and 37°C.

ability to support bacteriophage ϕCbK DNA injection (Fig. 7). Synchronized populations of TsM were grown at both 23° and 37°C and challenged with radioactive phage at 10-minute intervals. The phage was unable to inject DNA at 37°C, whereas normal injection occurred at 23°C. With wild-type strains, phage DNA injection occurs normally at both temperatures. It appears, therefore, that TsM is unable to synthesize a phage receptor site at the restricted temperature, in addition to being unable to divide normally. Since normal *Caulobacter* cell division is dependent on cellular differentiation and one of the measurable determinants of the differentiation process is lacking at the restrictive temperature, TsM appears to represent a class of mutants blocked at some stage of differentiation.

Essential for the study of molecular events in differentiation is the demonstration of a mechanism of genetic exchange in the *Caulobacter* genus. Such a demonstration would permit us (i) to show that the apparent Ts mutants are a result of genetic alterations, (ii) to evaluate and localize different gene loci responsible for normal or aberrant differentiation phenotypes, and (iii) to ultimately map the gene loci controlling the cyclic differentiation process in *Caulobacter*. We have succeeded in mixing two populations of cells with different drug resistance and growth requirements and have obtained phenotypes showing markers from both parents (*40*; Table 4). Crossing was accomplished by mixing the cultures on the surface of solid, glucose-supplemented medium. Presumed mating types were grown together on solid media for 48 hours, eluted from the "mating plate," resuspended in distilled water, and then diluted and plated onto selective media. In one such cross, streptomycin-sensitive biotin-prototrophic (Str^{S}Bio$^+$) and Str^{R}Bio$^-$ genotypes were mixed, and Str^{R}Bio$^+$ progeny were selected. The exchange of genetic information occurred with an apparent frequency of 1.7×10^{-6}, while the frequency of spontaneous mutation of Str^{S}Bio$^+$ to Str^{R}Bio$^+$ was 2×10^{-8} and that of Str^{R}Bio$^-$ to Str^{R}Bio$^+$ could not be detected. In another cross, a strain resistant to streptomycin (Str^R), which cannot use proline (Pro$^-$) as a carbon source, requires vitamins (Vit$^-$) and is yellow (Str^{R}Pro$^-$Vit$^-$) was mixed with a strain sensitive to streptomycin (Str^S) which was proline and vitamin proto-

trophic (Pro+Vit+) and white (Str^S-Pro+,Vit+). "Recombinants" (Pro+-Str^R) were selected for ability to utilize proline as a carbon source in the presence of *Streptomycin* medium containing a source of vitamins, and occurred with a frequency of 1.5×10^{-6}, while the spontaneous rate of mutation of Pro+Str^S to Pro+Str^R was 1×10^{-8}. In addition, the "recombinant," progeny of this cross were analyzed for the distribution of unselected vitamin (Vit) utilization and pigmentation (yellow or white) markers. Out of a total of 391 colonies examined the genotypes were distributed as follows: 23, Pro+Vit−Str^R, yellow; 334, Pro+-Vit+Str^R, yellow; 30, Pro+Vit+Str^R, white; indicating either a strong linkage between Pro and Vit markers or the presence of a partial diploid.

It appears that the genetic transfer may be mediated by cell conjugation, because filtrates or cell extracts prepared by sonic treatment, substituted reciprocally for each of the parents in these crosses, fail to yield any recombinant progeny. Although the mechanism of genetic exchange is still under investigation, it seems apparent that cell contact is required. It is possible that the *Caulobacter* swarmer cell, analogous to the piliated male *E. coli* cell, transfers genetic information.

Conclusions

The foregoing studies are intended to define a differentiation process and to permit genetic access to the mechanisms that control this process. In order to elucidate the basic mechanisms whereby a cell dictates its own defined morphogenic changes, we have found it helpful to study an organism that can be manipulated both biochemically and genetically. We have attempted to develop the studies initiated by Poindexter, Stove and Stanier, and Schmidt and Stanier (*16, 17, 20*) with the *Caulobacter* genus so that these bacteria can serve as a model system for prokaryotic differentiation.

The *Caulobacter* life cycle, defined in synchronously growing cultures, includes a sequential series of morphological changes that occur at specific times in the cycle and at specific locations in the cell. Six distinct cellular characteristics, which are peculiar to these bacteria, have been defined and include (i) the synthesis of a polar organelle which may be membranous (*21–23*), (ii) a satellite DNA in the stalked cell (*26*), (iii) pili to which RNA bacteriophage specifically adsorb (*16, 33*), (iv) a single polar flagellum (*17*), (v) a lipopolysaccharide phage receptor site (*27*), and (vi) new cell wall material at the flagellated pole of the cell giving rise to a stalk (*19, 20*). Cell division, essential for the viability of the organism, is dependent on the irreversible differentiation of a flagellated swarmer cell to a mature stalked cell.

The specific features of the *Caulobacter* system which make it a system of choice for studies of the control of sequential events resulting in cellular differentiation can be summarized as follows.

1) Cell populations can be synchronized, and homogeneous populations at each stage in the differentiation cycle can thus be obtained.

2) A specific technique has been developed whereby the progress of the differentiation cycle can be accurately measured by adsorption of labeled RNA phage or penetration of labeled phage DNA into specific cell forms. This technique can be used to select for mutants blocked in the various stages of morphogenesis.

3) Temperature-sensitive mutants of *Caulobacter* that are restricted in macromolecular synthesis and development at elevated temperatures have been isolated.

4) Genetic exchange in the *Caulobacter* genus has been demonstrated and is now being defined.

Two questions related to control processes can now readily be approached experimentally. (i) Is the temporal progression of events occurring during bacterial differentiation controlled by regulator gene products? (ii) Is the differentiation cycle like a biosynthetic pathway where one event must follow another? The availability of temperature-sensitive mutants blocked at various stages of development permits access to both questions. An interesting feature of the differentiation cycle is that the polar organelle may represent a special segregated unit which is operative in the control of the differentiation process. Perhaps the sequential morphogenic changes exhibited by *Caulobacter* are dependent on the initial synthesis of this organelle. Because the ultimate expression of cell changes are dependent on selective protein synthesis, specific messenger RNA production—either from DNA present in an organelle or from the chromosome—may prove to be a controlling factor in cell differentiation. We have begun studies with RNA polymerase purified from *Caulobacter crescentus* to determine whether cell factors or alterations in the enzyme structure serve to change the specificity of transcription during the cell cycle. Control of sequential cell changes at the level of transcription has long been postulated and has recently been substantiated in the case of *Bacillus* sporulation (*6*). The *Caulobacter* bacteria now present another system in which direct analysis of these control mechanisms is feasible.

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Women in Academia

A study of the hiring decision in departments of physical science.

Arie Y. Lewin and Linda Duchan

Recently, there has been a great deal of concern over the role of women in today's society. There have been numerous allegations of discrimination against women in many areas, especially in certain occupational and professional fields. A significant part of the controversy has involved alleged bias against women in academia.

Charges of discrimination against women in universities have been made both formally and informally. In the former category, Gruchow (*1*) cites 43 colleges and universities which have been charged with discriminatory policies against women under laws which call for the cancellation of federal contracts held by the schools. Bikman (*2*) investigated charges of discrimination at Columbia University and noted that women are admitted to the school and trained, but not hired; the few positions they do obtain are at levels relatively lower than those offered to men. Harvard University has been particularly singled out by women's rights groups for discriminatory practices (*3*). Informal or subjective evidence has also been widely publicized. Alexander (*4*) obtained evaluations of bias against women by female faculty members at Johns Hopkins University: they described the discrimination they experienced as being in the form of "unseen pressure." There are numerous other anecdotal accounts highlighting the alleged existence of discrimination against female faculty members (*5*).

Perhaps the most conspicuous discrimination is evident from salary differentials between men and women. Alexander reports that salaries for women employed in higher education are generally lower (*3*). Bayer and Astin concluded that salary disparities between men and women exist in academia across all ranks, work settings, and fields of specialization (*6*). Furthermore, a recent report in the field of chemistry indicates that "obvious differences in the earnings of men and women chemists" exist; these differences seem to carry over time, rather than being clustered in the earlier years of employment (*7*).

Even more illuminating are the statistics which show that women are being discriminated against in the awarding of research grants. In the Senior Postdoctoral Fellowship competition recently held by the National Science Foundation, 14 of the 395 applicants were women. Fifty-four grants were awarded—none went to women. In a pilot study of grants and awards given by the National Science Foundation to university researchers in a physical science discipline for the years 1964–1968, women were only awarded less than 0.03 percent of the grants although they comprise 5 to 8 percent of the scientists in the discipline. Furthermore, the mean dollar value of the awards received by women was smaller than that of those received by men. For the same discipline, however, no similar discrimination was observed in the awarding of grants by the National Institutes of Health (*8*). The American Chemical Society does not have a much better record: although no national award prohibits female applicants, the only one which has gone to a woman (with the exception of the Garvan Medal which is restricted to women) is an award in high school chemistry teaching (*9*). Similarly, out of the 827 Sloan Research Fellowships that have been awarded by the Alfred P. Sloan Foundation over the past 16 years, only one or two women were among the recipients.

Other inequalities facing women in academia have also been charged. Lack of job opportunities and fewer advancements are often cited (*10*). Other research has indicated that psychological and social barriers exist for women, especially in science. Specifically, White (*11*) concluded that it is difficult for women to attain "challenging interaction" with male colleagues, that men are hesitant about sponsoring women as protégés, and that women are denied the informal signs of recognition and belonging.

Perhaps the key to the problem may be found in the analysis of cultural influences. The dominant attitudes and norms concerning the role of the woman in our society are perceived to be incompatible with the concept of a successful professional woman. In the socialization process, children are conditioned as to which traits and professions

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