

# Development of *Dictyostelium discoideum*: Chemotaxis, Cell-cell Adhesion, and Gene Expression

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## INTRODUCTION AND OVERVIEW

The cellular slime mold *Dictyostelium discoideum* has attracted the interest of developmental biologists for many years. Starvation of this unicellular, eukaryotic organism triggers a program of differentiation during which cells undergo chemotaxis to form a multicellular aggregate, followed by differentiation of two new cell types: spores and stalks. This developmental program exhibits many features seen during development of higher eukaryotic organisms: A homogeneous population of cells differentiate into two new

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cell types; developing cells communicate with each other via extracellular hormones; specific cell-cell contacts are formed during the multicellular stage of development; and a specific arrangement of spore and stalk cells form during morphogenesis. Yet *Dictyostelium* is haploid and amenable to mutational analysis; stocks of developmental lethal mutations can be propagated vegetatively and the developmental defects studied in detail. As differentiation occurs only when growth and DNA replication have ceased, one can concentrate on the developmental process in the absence of cell growth. Additionally, the two differentiated cell types and their precursors can be isolated in quantity, allowing biochemical analysis.

Here we review our current understanding of the developmental program of *Dictyostelium*, focusing on the signals that regulate differentiation, the changes in gene expression, and the molecular mechanisms used by *Dictyostelium* to regulate the levels of gene products during development. Morphogenesis and pattern formation are considered in a separate paper in this volume (MacWilliams and David). Several excellent reviews consider other aspects of *Dictyostelium* development and, in particular, two books edited by Loomis provide an excellent starting point for further reading (Loomis 1975, 1982).

*Dictyostelium* grows as single cells, feeding on bacteria, or axenically in a medium composed of glucose, salts, yeast extracts, and peptone. *Dictyostelium* is a true eukaryotic cell, containing a nucleus and a nuclear membrane. The haploid genome contains about three times the DNA of a yeast, or about 2% of a typical mammalian cell (Loomis 1975). There are seven chromosomes (Robson and Williams 1977) totaling roughly 50,000 kb of DNA. About 30,000 of these are single-copy sequences that encode a majority of the mRNAs. Its cytoplasmic mRNAs contain polyadenylic acid sequences at the 3' end and a m<sup>7</sup>G(5')ppp "cap" sequence at the 5' end (Firtel et al. 1972; Dottin et al. 1976). Most isolates are haploid, although diploids can be produced and, in fact, provide the basis for "parasexual" genetics (Brody and Williams 1974). Large quantities of growing cells can easily be obtained, as cells will grow to densities as high as 10<sup>7</sup>/ml. A defined medium permits the isolation of auxotrophic and other types of mutants (Franke and Kessin 1977).

The familiar life cycle of *Dictyostelium* is shown in Figure 1. Development is initiated by exhaustion or removal of the food source. Starvation for amino acids, in particular, triggers the developmental program (Marin 1976). Development is fueled by endogenous energy reserves, and there is extensive turnover of RNA, carbohydrate, and protein as differentiation proceeds (Coccucci and Sussman 1970; Hames and Ashworth 1974; Mangiarotti et al. 1981). Cells at the terminal spore or stalk stage contain only about half the RNA and protein found in a growing cell (Bonner and Frascella 1953). Most DNA synthesis ceases during the first few hours (Katz and Bourguignon 1974); thus, there is not a coupling between DNA replication and differentiation. However, there may be some synthesis of DNA in a subset of cells at the late stage of aggregation (Zada-Hames and Ashworth 1978).

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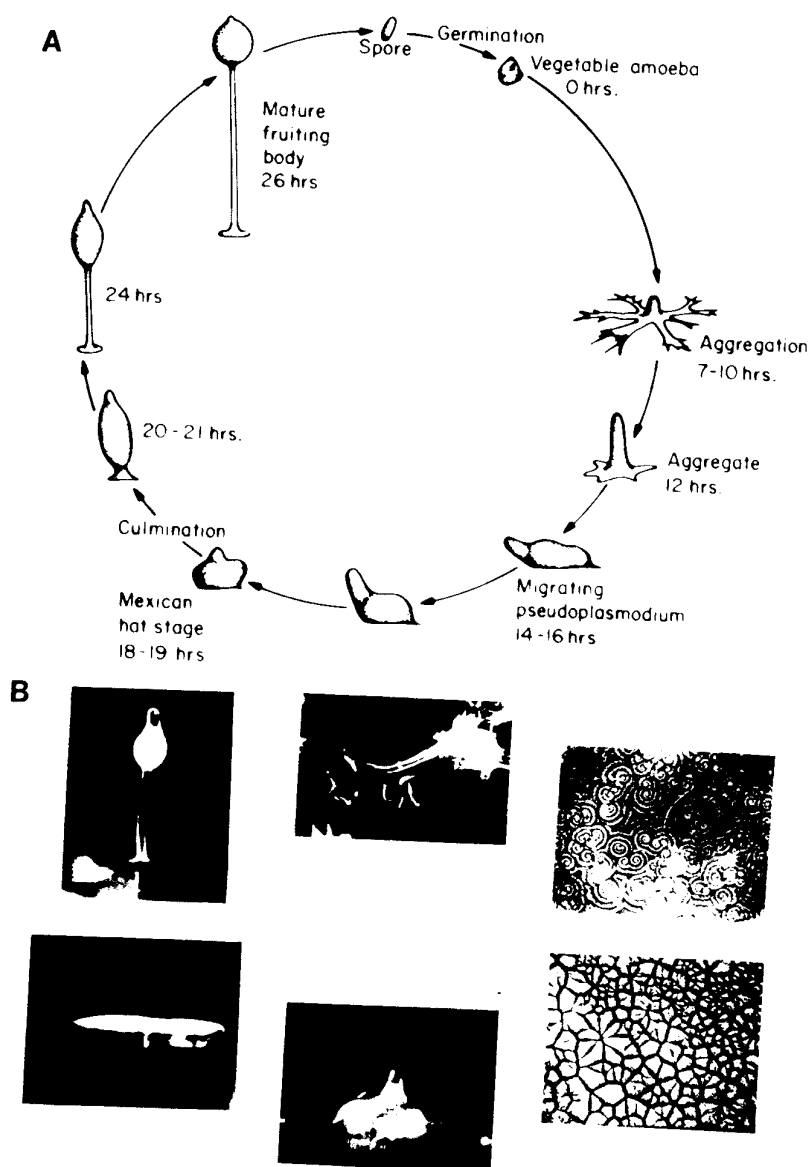


Figure 1 Life cycle of *D. discoideum*. (A) Schematic diagram of the developmental program. (B) Examples of several sequential stages in the developmental cycle are shown (clockwise, starting at the top). Scanning electron micrograph of individual amoeba ( $\sim 8 \mu\text{m}$  long); dark-field photograph of early aggregation ( $\sim 5-7$  hr) showing coordinated waves of cell motion (territories are  $\sim 1$  cm and contain  $\sim 10^6$  cells); dark-field photograph of streaming patterns in late aggregation ( $\sim 9-12$  hr); mound aggregate with apical tip (dia.  $\sim 1$  mm); tip elongates to form migrating slug ( $\sim 1.5$  mm long); terminal differentiation step forms fruiting body (1-2 mm high) consisting of stalk and spore cells; spores germinate to produce amoebae.

Soon after starvation, the cells begin a process of cell-cell signaling. A subset of the cells spontaneously begin emitting cAMP pulses to which the surrounding cells respond by moving chemotactically toward the source of the pulses (Konjin et al. 1969; Alcantara and Monk 1974). In addition, the responding cells relay the cAMP signal by synthesizing and secreting cAMP. This results in the establishment of an aggregation field, which can be several millimeters in size and can encompass a million cells. This phase of development, "aggregation," ends around 10–12 hours after starvation, with the formation of multicellular aggregates of roughly  $10^5$  cells. By 12 hours of differentiation, the mounds begin to develop a well-defined "tip" at the top. At this stage, the mounds begin synthesis of an extracellular protein-carbohydrate complex, which coats the organism (Freeze and Loomis 1977). The mounds undergo a series of morphogenetic movements that transform them into "pseudoplasmodia" or "slugs," with the tip now at the leading edge. Depending on environmental conditions, these slugs can migrate for several days. They are both phototactic and thermotactic (Poff and Loomis 1973; Poff and Skokut 1977; Fisher et al. 1981). Fate mapping studies indicate that the anterior one-third will become stalk cells and the posterior two-thirds are destined to become spores (Raper 1940). However, this commitment to a specific cell type is reversible; certain treatments can cause "prespore" cells to become "prestalk" cells and vice versa (Bonner and Frascella 1952; Bonner et al. 1955; Gregg 1965, 1968).

Culmination, the terminal stage of morphogenesis, is initiated by overhead light in conjunction with a suitable ionic environment (Slifkin and Bonner 1952; Newell et al. 1969; Schindler and Sussman 1977). The slug rights itself, so that the tip or prestalk cells are on top. These cells elongate and vacuolate, pushing down through the mass of differentiating spore cells (George et al. 1972). This results in the mass of spore cells being elevated on the elongating stalk. By 24 hours of differentiation the spores, now dehydrated, surmount the dead, vacuolated stalk to form the "fruiting body."

## CHEMOTAXIS AND ADHESION

### Organization of Aggregation by Signal Relay

A few hours after starvation, monolayers of amoebae on an agar surface divide into 1–2-cm territories encompassing about  $10^6$  cells. Amoebae move toward the center of the territories in steps, advancing for about 2 minutes and then stopping for about 5 minutes before moving again. In dark-field photographs, the coordinated movements appear as white bands that form spiral or concentric circles. The white bands delineate the zones of cells where, at that moment, amoebae are advancing chemotactically toward the center. In time-lapse films, each band can be seen spreading outwardly from a center as an enlarging ring. The period for initiation of successive rings is about 7–10 minutes. The band maintains a constant width of about 100

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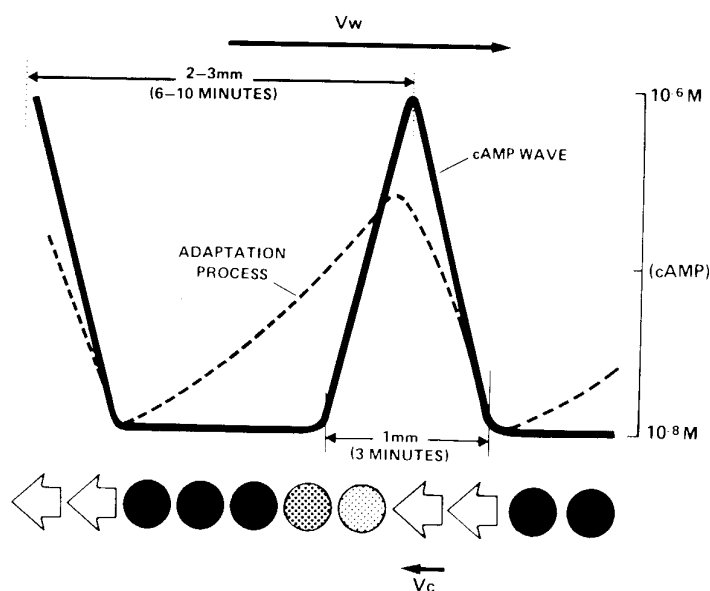
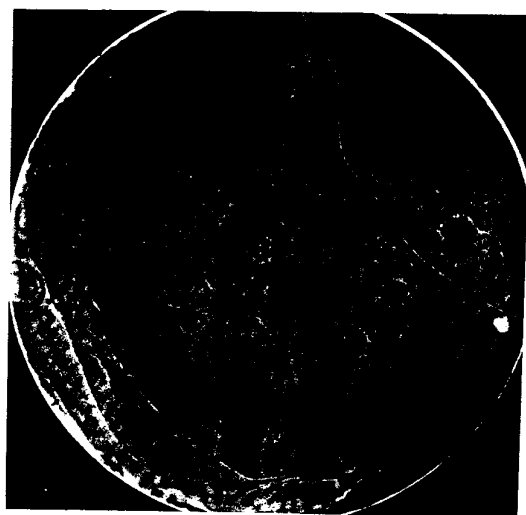
cells since, as a cell at the distal edge begins its inward movement step and elongates, one at the proximal edge ceases and becomes more rounded. These organized movements result in aggregation (for review, see Devreotes 1982).

There is little doubt that these organized movements are directed by cAMP. Cells are chemotactically attracted to cAMP, secrete cAMP in response to cAMP stimuli (see below), and uniform background levels of nonhydrolyzable cAMP analogs block aggregation (Konijn et al. 1969; Roos et al. 1975; Shaffer 1975; Rossier et al. 1979). The distribution of cAMP within the monolayer was determined by an isotope dilution-fluorographic technique. Figure 2 shows that regions of high cAMP concentrations form spiral or concentric circular patterns resembling the patterns of cell morphology seen in dark-field photographs. To determine the relative positions of the cAMP waves and chemotactic movement steps, the dark-field photographs were aligned with fluorographs. The movement step begins just as the leading edge of the cAMP wave reaches the position of the cell and ends as the peak of the wave arrives (Tomchik and Devreotes 1981).

The stable propagated cAMP waves result from the amoebae's capacity to relay cAMP signals. When stimulated by extracellular cAMP, cells synthesize and secrete additional cAMP, which serves as a signal for more distal cells. This response will be referred to as the "cAMP signaling response." In a synchronized cell suspension the response can be elicited by addition of exogenous cAMP or can occur in response to cAMP secreted by cells within the suspension. Much evidence suggests that the response of synchronized cell populations to exogenous cAMP corresponds to the cAMP waves that direct morphogenesis *in situ*. For instance, the concentrations and kinetics of cAMP synthesis are consistent with the dimensions of the cAMP waves detected in the monolayer. Mutants defective in the generation of cAMP signaling responses do not aggregate, and specific inhibitors of the response block aggregation (for review, see Devreotes 1982).

The cAMP signaling response is elicited by a change in the occupancy of the surface cAMP receptors, which leads to activation of adenylate cyclase. This results in a transient increase in intracellular cAMP; the cAMP secretion rate is directly proportional to the intracellular cAMP level. After several minutes, the rate of cAMP synthesis slows down (see below), and the cAMP present is degraded by extracellular and membrane-bound cAMP phosphodiesterases (PDEs). The cAMP secretion rate, intracellular and extracellular PDE activity, and receptor affinity or number are not observed to change during the transient response. Thus, the single control point determining the magnitude and duration of the cAMP signaling response appears to be at the level of synthesis of cAMP (for review, see Devreotes 1982).

In cell suspensions (or *in situ* within the monolayer), secreted cAMP binds to surface cAMP receptors creating a positive feedback loop. Extracellular PDE counteracts this feedback loop by decreasing extracellular cAMP. The two opposing activities acting on extracellular cAMP cause the system to be



**Figure 2** (Top) Fluorographic image of cAMP waves. Amoebae were developed to an early aggregation stage (see Fig. 1). Waves were detected by an isotope dilution-fluorography technique described in the text. The fluorograph was used as a negative to make this print. (Black circles are artifacts caused by air bubbles trapped between the two filters.) (Bottom) Dynamics of signal relay and chemotaxis. The heavy line representing the cAMP concentration is drawn from analyses of scans of the optical density of fluorographic images of the cAMP waves. Symbols in the lower part of the diagram represent a single radial line of cells: (Open arrows) Cells moving toward center; (shaded circles) randomly oriented cells; (arrow vectors) the speed and direction of motion of the cAMP wave ( $V_w = 300 \mu\text{m}/\text{min}$ ) and the moving cells ( $V_c = 20 \mu\text{m}/\text{min}$ ).

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#### Adenylate Cyclase

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excitable, which accounts for the maximal response to super-threshold stimuli ( $> 5 \times 10^{-9}$  M) and spontaneous oscillations in cAMP levels. The effects of both the positive-feedback loop and extracellular PDE are minimized when cells (labeled with [ $^3$ H]adenosine) are stimulated with exogenous cAMP under conditions of rapid perfusion. Secreted [ $^3$ H]cAMP is rapidly removed, and degraded cAMP is rapidly replaced. In this way extracellular cAMP is "clamped" at the concentration of the applied stimulus (Devreotes et al. 1979).

Under these conditions, an increment in the concentration of the cAMP stimulus elicits a response that subsides after several minutes, although a further increment in stimulus will elicit another response. The sum of the magnitudes of the two responses elicited by the two increments equals the magnitude of the response elicited by a single increment to the highest concentration (Fig. 3). The magnitude of the response saturates at  $10^{-6}$  M cAMP, and increments originating at  $10^{-6}$  M cAMP elicit no response. These properties suggest that cells respond to increases in the fractional occupancy of surface cAMP-binding sites. The extinction of each response by the adjustment of cellular sensitivity to the level of the current stimulus is referred to as adaptation (Fig. 3) (Devreotes and Steck 1979).

There is a rapid recovery of responsiveness (deadaptation) after the removal of the cAMP stimulus. The level of adaptation can be determined by an initial stimulus of defined magnitude and duration and, after a variable recovery period, it can be determined by the magnitude of the response to a second identical stimulus. The attenuation to the second stimulus is taken as a measure of residual adaptation to the first stimulus. The magnitude of the response to the second stimulus increases with the recovery time in a first-order fashion, with  $t_{1/2} = 3-4$  minutes for paired stimuli of  $10^{-8}$  M or  $10^{-5}$  M cAMP. Recovery is complete in 12-15 minutes (Fig. 3) (Dinauer et al. 1980).

Does adaptation depend on activation of the adenylate cyclase or the resultant increase in intracellular cAMP? This question was answered by the use of caffeine, which rapidly inhibits activation of the adenylate cyclase by exogenous cAMP. Cells pretreated with caffeine for as long as 22 minutes respond normally to cAMP when the drug is removed. However, cells pretreated with caffeine plus cAMP do not respond to cAMP when caffeine is removed, indicating that activation of adenylate cyclase is inhibited but its adaptation is not. Attenuation of subsequent responsiveness depends on both the concentration and duration of cAMP applied with caffeine regardless of whether the initial response was blocked. Thus, adaptation proceeds to the same extent and at the same rate whether or not adenylate cyclase is activated (Theibert and Devreotes 1983). Clearly, adaptation does not depend on the level of cAMP.

#### Adenylate Cyclase

The activation and subsequent reversible adaptation of adenylate cyclase via surface cAMP receptors in *D. discoideum* is analogous to the activation/de-

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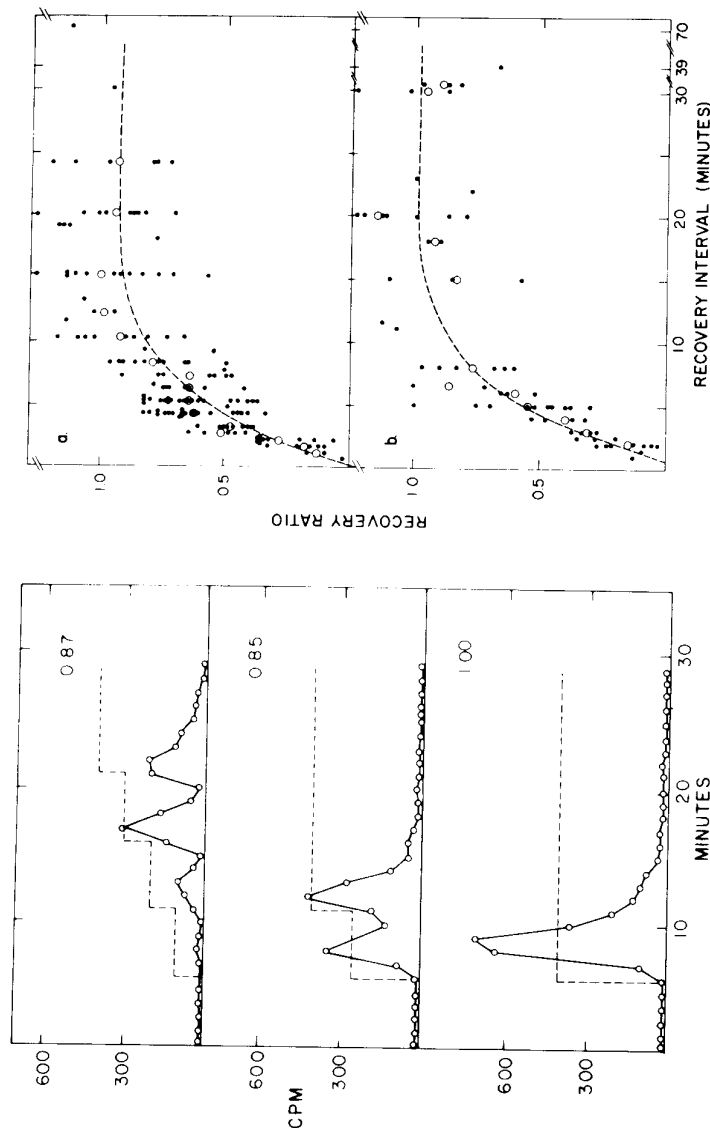


Figure 3 (Left) Response of amoebae to sequential increments in cAMP stimulus concentration. In the lower panel the stimulus was  $0 \rightarrow 10^{-6}$  M cAMP; in the middle panel it was  $0 \rightarrow 5 \times 10^{-8}$  M  $\rightarrow 10^{-6}$  M cAMP; in the upper panel it was  $0 \rightarrow 10^{-9}$  M  $\rightarrow 10^{-8}$  M  $\rightarrow 10^{-7}$  M  $\rightarrow 10^{-6}$  M cAMP. (-----) Approximate changes in receptor occupancy; (—) the rate of  $[^3\text{H}]\text{cAMP}$  secretion measured by the perfusion technique described in the text. Numbers at upper right indicate the total amount of  $[^3\text{H}]\text{cAMP}$  secreted in each case, normalized to that secreted in response to the  $0 \rightarrow 10^{-6}$  M stimulus. (Right) Recovery of the cAMP signaling response after adaptation to cAMP. Two identical stimuli were applied, separated by the recovery interval indicated. The magnitude of the second response normalized to that of the first is plotted. (a)  $10^{-8}$  M cAMP; (b)  $10^{-5}$  M cAMP.

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**Figure 3** (Left) Response of amoebae to sequential increments in cAMP stimulus concentration. In the lower panel the stimulus was  $0 \rightarrow 10^{-6}$  M cAMP; in the middle panel it was  $0 \rightarrow 5 \times 10^{-8}$  M  $\rightarrow 10^{-6}$  M cAMP; in the upper panel it was  $0 \rightarrow 10^{-9}$  M  $\rightarrow 10^{-8}$  M  $\rightarrow 10^{-7}$  M  $\rightarrow 10^{-6}$  M cAMP. (-----) Approximate changes in receptor occupancy; (—) the rate of  $[^3\text{H}]\text{cAMP}$  secretion measured by the perfusion technique described in the text. Numbers at upper right indicate the total amount of  $[^3\text{H}]\text{cAMP}$  secreted in each case, normalized to that secreted in response to the  $0 \rightarrow 10^{-6}$  M stimulus. (Right) Recovery of the cAMP signaling response after adaptation to cAMP. Two identical stimuli were applied, separated by the recovery interval indicated. The magnitude of the second response normalized to that of the first is plotted. (a)  $10^{-8}$  M cAMP; (b)  $10^{-6}$  M cAMP.

sensitization of the enzyme by cell-surface receptors for hormones and neurotransmitters in vertebrates. In these systems the hormone receptor is coupled to the catalytic unit via a regulatory component, designated Ns. Ns is a GTP-binding protein with inherent GTPase activity. Persistent activation of adenylate cyclase requires GTP bound to Ns; it is attained in the presence of nonhydrolyzable GTP analogs GMP-PNP or GTP- $\gamma$ -S, or when membranes are treated with cholera toxin and  $\text{NAD}^+$ , wherein ADP ribosylates Ns and inactivates its GTPase (Ross and Gilman 1980).

In *D. discoideum*, sonicates prepared from cells at the peak of the cAMP signaling response synthesize  $[^{32}\text{P}]\text{cAMP}$  5–20 times faster than those prepared from unstimulated cells. The activation of adenylate cyclase decays rapidly following cell lysis ( $t_{1/2} = 20\text{--}30$  sec at  $22^\circ\text{C}$ ;  $t_{1/2} = 5$  min at  $0^\circ\text{C}$ ). Subsequently, the basal activity is relatively stable but unresponsive to cAMP (i.e., the hormone in this case), GTP (or GMP-PNP or GTP- $\gamma$ -S), and cholera toxin- $\text{NAD}^+$  (Roos and Gerisch 1976). Thus, although the response of adenylate cyclase in *Dictyostelium* to extracellular hormones (i.e., cAMP) is very similar to that seen in vertebrates, the in vitro system differs and, at present, the mechanism of regulation of the enzyme is unknown. Recently, one of us (A.T.) reproduced the activation experiment described above by extraction of the cells with the zwitterionic detergent CHAPS. This opens the possibility of fractionating the extract from activated cells and mixing detergent extracts from activated cells with those of unstimulated cells or mutants unable to activate adenylate cyclase.

Most attempts to study the adenylate cyclase have been hindered by the enzyme's extreme stability. One of us (P.D.) has recently developed methods to purify the enzyme about 300-fold and greatly increase its stability ( $t_{1/2} = 2$  hr vs.  $t_{1/2} = 1$  day at  $0^\circ\text{C}$ ). All of the activity is membrane-bound. Although extraction of membranes in 16 mM CHAPS solubilizes over 95% of the membrane protein, nearly all of the adenylate cyclase activity remains particulate. Several CHAPS extractions followed by washing leads to approximately 200-fold purification; further treatment with CHAPS containing 2 M NaCl and 1 M KCl/1 M urea, followed by washing, increases the purification and markedly increases the stability. Electron microscopy of this enriched material revealed a homogenous preparation of vesicles about  $0.2 \mu\text{m}$  in diameter (see Fig. 4). There is probably lipid present, since a definite bilayer is seen. SDS-polyacrylamide gel electrophoresis (PAGE) shows two major protein bands of 30 kD and 33 kD and about 20 minor bands from 105–15 kD. The two major bands are highly enriched over the starting membranes and may maintain the structure of vesicles. The nearly quantitative retention of adenylate cyclase activity in the purified vesicle preparation suggests that it is in a specialized membrane domain resistant to detergent extraction. Triton-resistant bilayer structures, with similar SDS-PAGE patterns, have been reported previously (Luna et al. 1981; Spudich and Spudich 1982). These developments should allow identification of the enzyme and facilitate further studies of its regulation.



**Figure 4** Vesicular adenylate cyclase preparation. Transmission electron micrograph (magnification, 12,000 $\times$ ) of CHAPS-extracted, salt-washed membranes. This fraction contains an adenylate cyclase activity enriched 500-fold over crude cell lysates.

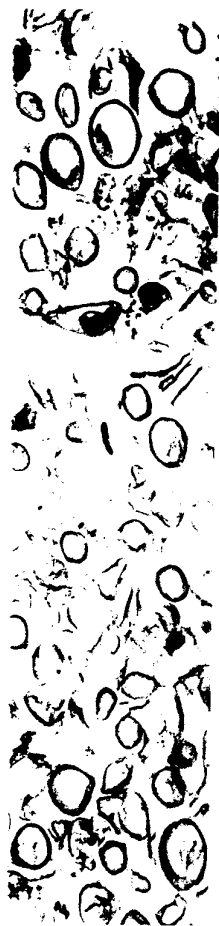
### Chemotaxis

Differentiated amoebae orient and move in gradients of low concentrations of cAMP ( $10^{-9}$  M). Growing amoebae are insensitive to cAMP but are attracted to folic acid (Pan et al. 1972). These responses were first demonstrated with a small population assay in which about 100 amoebae are placed near  $0.1 \mu\text{l}$  of chemoattractant on an agar surface. After about 30 minutes, by

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extension of pseudopodia, the amoebae redistribute toward the chemoattractant (Konijn 1970). When cAMP-containing microneedles are brought in close apposition to sensitive amoebae, a pseudopodium is extended in that direction after about 5–15 seconds (Swanson and Taylor 1981). When the needle is touched to the agar surface near an amoeba, it initially retracts—a response referred to as “cringing”—and after about 15–30 seconds, begins to move toward the needle (Futrelle et al. 1983).

We (D.F. and P.D.) have recently developed a perfusion assay to monitor cell-shape changes in response to temporal increases in extracellular cAMP. The initial response to a sudden jump in the cAMP concentration is a retraction (probably the cringe response). After about 15–30 seconds pseudopodia are extended in all directions, and the cell flattens and remains immobilized (Fig. 5). After 2–3 minutes it begins again to extend pseudopodia in random directions and move about in spite of the continuous presence of cAMP. The flattening response presumably reflects the chemotactic response as it is elicited by folic acid in growing cells and appropriate concentrations of cAMP in differentiated cells. Since the stimulus is applied uniformly across the cell, pseudopodia are extended in all directions causing the cell to flatten rather than moving off in a given direction as it does in response to a gradient. It appears that the chemotactic machinery adapts to the continuous presence of chemoattractant, since the cells resume motion after 2–3 minutes. An adaptation process for chemotaxis is also suggested by the observation that cells will orient in a gradient of  $10^{-6}$  M cAMP above a background of  $10^{-5}$  M (van Haastert 1983a). The adaptation process explains why, as described above, cells do not reorient as the cAMP wave passes and the gradient reverses. It may have a greater significance, as yet not understood, in allowing the cells to sense small gradients above high backgrounds.

The mechanism by which binding of cAMP to surface receptors leads to the flattening response is unknown, although there are two biochemical reactions that correlate in time with the cringe response. Pseudopodial extension could involve actin-myosin cytoskeleton interactions.  $[\gamma\text{-}^{32}\text{P}]\text{ATP}$  added to extracts prepared from stimulated cells is incorporated rapidly into a band that coelectrophoreses with myosin. The response is transient and lasts only about 60 seconds at  $11^\circ\text{C}$  (Rahmsdorf et al. 1978). Chemotactic stimulation also leads to a rapid increase in intracellular cGMP. Levels increase about tenfold in 10 seconds and return to 50% of the peak value after about 30 seconds (Mato et al. 1977a). The levels fall because guanylate cyclase is only transiently activated (i.e., it also adapts), and a cGMP-specific PDE removes the cGMP present. cGMP changes are correlated with the chemotactic response for numerous cAMP analogs and folic acid (Mato et al. 1977b). The strongest evidence for the involvement of cGMP in chemotaxis is the existence of “streamer” mutants. In streamers, the white bands described above are wider, occupying nearly the entire wavelength. This means that the cells either move longer or remain elongated after the gradient has reversed direc-



*Figure 5* cAMP-elicited shape change. Cells adhering to glass coverslips were perfused rapidly with buffer. The perfusion buffer was rapidly (<5 sec) switched to one containing  $10^{-4}$  M folic acid. Photomicrographs were taken 60 sec before, 60 sec after, and 4 min after application of the stimulus (top to bottom).

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#### Receptor

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tion. The defect in the streamers has been traced to the absence of the cGMP-specific PDE, causing the cGMP levels to remain elevated (Ross and Newell 1981).

### Receptors

Each of the reactions activated by extracellular cAMP could be linked to separate sets of cell-surface receptors or, perhaps, could be coupled to a single receptor class. By comparing the effectiveness of a set of cAMP analogs, it has been shown that cGMP accumulation and chemotaxis are mediated by the same receptor (Mato et al. 1977b; van Haastert 1983b). We have tested these same analogs for their ability to elicit the cAMP signaling response. As shown in Figure 6, a strong correlation exists between the order of effectiveness of these analogs in eliciting the cGMP (or chemotactic) response and the cAMP signaling response (A. Theibert et al., in prep.). This result suggests that cAMP receptors of very similar specificity, perhaps the same receptors, mediated these three processes.

Characterization of the surface cAMP receptor has been hindered by the rapid dissociation rate of the cAMP receptor complex, the absence of a slowly dissociating ligand, the low affinity of available photoactivatable analogs, the presence of very active PDE, and the presence of other cAMP-binding proteins (King and Frazier 1977; Mullens and Newell 1978). Most studies have measured equilibrium binding in which [ $^3\text{H}$ ]cAMP and PDE inhibitors are mixed with intact cells in the absence or presence of a saturating concentration of unlabeled cAMP. To remove unbound cAMP, cells were collected on filters, centrifuged through silicone, or collected in pellets (Malchow and Gerisch 1974; Green and Newell 1975; Henderson 1975). Recently, however, van Haastert and Kien (1983) have reported that ammonium sulfate appears to stabilize the cAMP receptor complex, and this technique allows a washing step to be performed.

Scatchard analysis of binding data yields plots that are curvilinear upward indicating either heterogeneity in binding affinities (10,000 sites with a  $K_d$  of 5 nM and 200,000 sites with a  $K_d$  of 100 nM) or negative cooperative interactions between binding sites. The specificity of this range of receptors is the same for different cAMP analogs (van Haastert and Kien 1983), and these affinities are consistent with the cAMP concentration over which chemotaxis, cGMP stimulation, and cAMP stimulation are seen. Receptors are developmentally induced, and the shape of the Scatchard plot does not change during early development (Green and Newell 1975).

A model of the cAMP receptor interaction has been proposed by testing the effectiveness of cAMP analogs with substitutions at various positions in eliciting a chemotactic and a cGMP response. In this model, the cAMP molecule binds to the receptor in the *anticonfiguration* with two hydrogen bonds at  $\text{N}^6\text{H}_2$  and 3' oxygen, and the adenine moiety is bound in a hydrophobic cleft of the receptor (Mato and Konijn 1977; Jastorff 1978; Jastorff et al.

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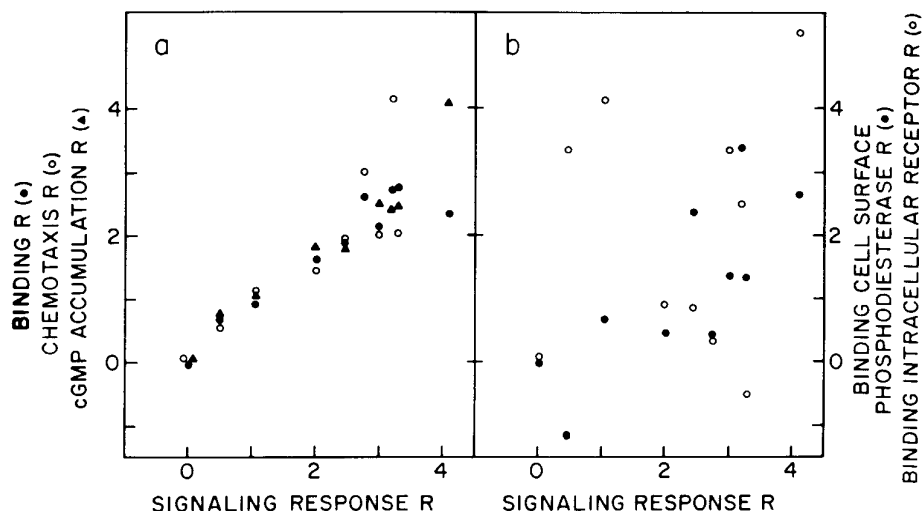


Figure 6 Comparison of responses elicited by cAMP analogs. The concentration of cAMP analog that elicited a half-maximal signaling response was determined (see legend to Fig. 3 and text). (R) Log (concentration of analog eliciting half-maximal response/concentration of cAMP eliciting half-maximal response). (a) R values for signaling response are plotted against R values for  $[^3\text{H}]\text{cAMP}$  binding (●), chemotaxis (○), and cGMP (▲) accumulation. (b) R values for signaling response are plotted against R values for membrane PDE (●) and intracellular cAMP-binding protein (○).

1978; Mato et al. 1978). In addition, a model for the activation mechanism has also been proposed (van Haastert and Kien 1983) in which, after binding, there is a nucleophilic attack by the receptor at the phosphorus atom of cAMP. The result is a pentacovalent phosphorus atom transiently but covalently bound to the receptor, which subsequently induces a conformational change by which the receptor is activated.

Desensitization of the receptor has been observed (Klein and Juliani 1977; Klein 1979). Although no apparent change in binding during the short time of the signaling response is observed (i.e., this is probably not responsible for adaptation), cells that are preincubated with unlabeled cAMP, washed, and rechallenge with  $[^3\text{H}]\text{cAMP}$  show a gradual decrease in the level of binding, which depends on the concentration and duration of pretreatment with cAMP.

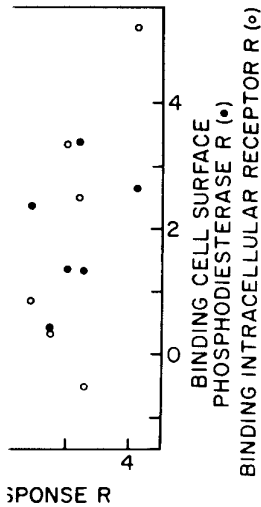
Several investigators have reported photoaffinity labeling of surface receptors by 8-azido  $[^{32}\text{P}]\text{cAMP}$  (Hahn et al. 1977; Juliani and Klein 1981; Wallace and Frazier 1979). Due to the low affinity of this analog, covalently labeled material runs as many bands on SDS gels, most of which are also photola-

beled in the presence of (1979) and Juliani (1979). A 45,000-m.w. band, labeled cAMP. Receptor (70,000-m.w. protein) separates of material purified this putative Receptor identification cAMP binding such (1982; van Haastert

### Specific Cell Adhesion

*Dictyostelium discoideum* cell adhesion requires a cell surface receptor system (csB), EDTA-sensitive, and to end adhesion system (csA) mediated through contact site B (csB) developing cells and blocks the end-to-end adhesion system (csA) prepared from protein, gp80, which was isolated (Muller

This protein, believed to mediate cell adhesion, is able to aggregate (Muller and the cell surface component (Parish et al. 1978; Frazier et al. 1982a). However, of gp80 do not bind to clonal antibodies that block csA-mediated adhesion; clonal anticarbohydrate block cell-cell adhesion; gp80 is involved in cell adhesion; molecule plays no role in cell adhesion; csB mediates adhesion; such as bacteria in the growth medium



The concentration of was determined (see eliciting half-maximal nse). (a) R values for binding (●), chemotaxis response are plot-AMP-binding protein

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beled in the presence of unlabeled cAMP. However, Wallace and Frazier (1979) and Juliani and Klein (1981) report that a 40,000-m.w. band and a 45,000-m.w. band, respectively, are protected from photolabeling by unlabeled cAMP. Recently, Meyers-Hutchins and Frazier (1984) report that a 70,000-m.w. protein, which cannot be photolabeled in intact cells or membranes (presumably due to the presence of an inhibitor), can be labeled after separation of material by anionic exchange chromatography. They have also purified this putative surface receptor (Meyers-Hutchins and Frazier 1984). Receptor identification may be aided by the recent finding of antagonists of cAMP binding such as adenosine and some cAMP analogs (Newell and Ross 1982; van Haastert 1983b; A. Theibert and P. Devreotes, in prep.).

### Specific Cell Adhesion

*Dictyostelium discoideum* is an excellent system for studying the specific cell-cell adhesion necessary for the development of multicellular organisms. Immunological and biochemical approaches have elucidated two specific cell-cell adhesion systems (Beug et al. 1970, 1973a,b). One system, mediated by contact site B (csB), is present in both vegetative and developed amoebae, is EDTA-sensitive, and causes side-to-side adhesion. An EDTA-resistant, end-to-end adhesion system is only present in aggregating amoebae and is mediated through contact sites A (csA). A polyclonal antiserum, directed against developing cells and extensively adsorbed with growing cells, specifically blocks the end-to-end, EDTA-resistant adhesion of differentiating cells. A Fab preparation from such sera can be used to define csA. An 80K glycoprotein, gp80, which blocks the Fab-induced inhibition of cell-cell adhesion, was isolated (Muller and Gerisch 1978; Muller et al. 1979).

This protein, believed to be at least partially responsible for csA-mediated adhesion, is able to reverse the Fab-induced dissociation of multicellular aggregates (Muller and Gerisch 1978). The appearance of this 80K protein on the cell surface corresponds to the acquisition of end-to-end adhesiveness (Parish et al. 1978; Parish and Schmidlin 1979; Murray et al. 1981; Ochiai et al. 1982a). However, monoclonal antibodies that bind to the protein portion of gp80 do not bind to intact amoebae (Ochiai et al. 1982b). Anti-gp80 monoclonal antibodies that bind to amoebae via the carbohydrate portion fail to block csA-mediated adhesion (Ochiai et al. 1982b; Murray et al. 1983). Polyclonal anticarbohydrate Fab, which reacts strongly with gp80, also fails to block cell-cell adhesion (Ochiai et al. 1982b). These studies suggest that if gp80 is involved in csA-mediated adhesion, the carbohydrate portion of the molecule plays no role.

csB mediates adhesion between amoebae and between amoebae and particles such as bacteria and latex beads (Vogel et al. 1980). An inhibitor found in the growth medium of a stationary-phase culture blocks adhesion between

vegetative cells and weakens amoebal adherence to glass (Swan et al. 1977; Jaffe et al. 1979). The effectiveness of this inhibitor corresponds to the time in development where csB mediates these adhesive processes. On the basis of these observations, it has been proposed that csB is part of a ligand-receptor system. Preliminary evidence suggests that the inhibitor is a carbohydrate (Jaffe et al. 1979) that may become detached from the csB receptor on the surface of stationary-phase amoebae. This proposal explains why stationary-phase amoebae, even in the absence of inhibitor will not cluster (there is no ligand) (Jaffe et al. 1979).

An immunological approach was used to identify a protein possibly involved in the csB system (Chadwick and Garrod 1983). Fab preparations from four polyclonal antibodies blocked csB-mediated adhesion. After iodination of the amoebal surface, detergent extraction, and immunoprecipitation with the antiserum, four labeled proteins emerged as candidates for csB. When these proteins were eluted from the SDS gel, only the 126-kD protein was able to neutralize adhesion-blocking Fab. When a fraction enriched in this protein was used as an antigen, the resultant Fab blocked adhesion. Previous studies had shown that the amount of a 130-kD protein corresponded with csB activity (Parish et al. 1978).

After the amoebae have aggregated, another adhesion system replaces csA and csB (Steinemann and Parish 1980; Wilcox and Sussman 1981a,b; Saxe and Sussman 1982). Fab, which blocks this slug-stage adhesion system, can be neutralized by a 95-kD glycoprotein (Steinemann and Parish 1980) whose appearance coincides with the system's activity (Parish et al. 1978). A mutant that lacks this system can be rescued by addition of the 95-kD protein (Saxe and Sussman 1982).

Other adhesion-blocking antibodies have been reported. Fab from a monoclonal antibody binds to a 69-kD and a 73-kD antigen, blocks adhesion, and is not neutralized by purified gp80 (Brodie et al. 1983). These antigens are synthesized by starved amoebae, but their appearance does not depend upon developmental stage. Another adhesion-blocking Fab could be neutralized by a large polysaccharide found in the medium of starving cells (Springer and Barondes 1982). This antigen is present both in vegetative and developing amoebae. Four other adhesion-blocking monoclonal antibodies have been reported (Springer and Barondes 1982). Two of these also require EDTA to block cell-cell adhesion and they interact with the same developmentally regulated antigens. The other two do not require EDTA and react with many antigens, some of which may be established adhesion molecules.

The discovery of endogenous carbohydrate-binding proteins (discoidins) (Rosen et al. 1973; Frazier et al. 1975) led to the speculation that these proteins were involved in specific cell adhesion (Rosen et al. 1973). Much work followed, which described the carbohydrate specificity, time of synthesis, cellular location, and putative discoidin surface receptor (for review, see Bartles et al. 1982). However, mutant strains that have less than 2% of the normal

levels of discoidins are viable. These mutants, plus the mutants that lack cell-cell adhesion, have no effect on cell adhesion.

## REGULATION OF GENE EXPRESSION

### Number of Developmental Stages

Studies beginning in the 1960s showed that the number of enzymes increased over the cell cycle (Sussman and Osmond 1969; Loomis and Osmond 1972; Firtel and Brackley 1972). These were shown to be specific for each stage (Firtel et al. 1971). Subsequent studies using [<sup>35</sup>S]methionine at hourly intervals showed that the number of dimensional polyacrylamide gels increased exclusively or primarily during the cell cycle (1977). Surprisingly, new enzymes are induced around the time of the cell cycle. The synthesis of these molecular weight mRNAs in the cytoplasm could not define the total number of genes.

Solution hybridization studies showed that the number of different genes (Blumberg and Lodish 1973) increased during the cell cycle in growing *Dictyostelium* cells. These represent the transition from vegetative to developing. The number of mRNA species increased during differentiation. Formation of a multicellular organism is a change in gene expression. 3000 new species of mRNA are present in the cell cycle. This number remains relatively constant throughout the developmental program, and there are few proteins synthesized during the cell cycle (Coloma and Lodish 1975).

The ability to isolate and clone genes has provided a new tool for studying developmentally regulated mRNAs have been identified. Reverse transcription of



glass (Swan et al. 1977; corresponds to the time processes. On the basis of a part of a ligand-receptor is a carbohydrate the csB receptor on the explains why stationary- do not cluster (there is no

a protein possibly in- (1983). Fab preparations ed adhesion. After iodi- , and immunoprecipita- d as candidates for csB. only the 126-kD protein a fraction enriched in blocked adhesion. Pre- D protein corresponded

on system replaces csA Sussman 1981a,b; Saxe e adhesion system, can and Parish 1980) whose h et al. 1978). A mutant he 95-kD protein (Saxe

rted. Fab from a mono- 1, blocks adhesion, and 33). These antigens are e does not depend upon could be neutralized by ing cells (Springer and etative and developing antibodies have been e also require EDTA to e developmentally reg- A and react with many molecules.

g proteins (discoidins) ulation that these pro- t al. 1973). Much work time of synthesis, cel- for review, see Bartles han 2% of the normal

levels of discoidins are able to complete development (Alexander et al. 1983). These mutants, plus the lack of convincing data implicating discoidins in cell-cell adhesion, have suggested that discoidins may not be involved in specific cell adhesion.

## REGULATION OF GENE EXPRESSION DURING DEVELOPMENT

### Number of Developmentally Regulated mRNAs

Studies beginning in the late 1960s established that the activities of a number of enzymes increased or decreased at defined times during the developmental cycle (Sussman and Osborn 1964; Ashworth and Sussman 1967; Coston and Loomis 1969; Loomis 1969a,b; Telser and Sussman 1971; Firtel and Bonner 1972; Firtel and Brackenbury 1972). In addition, certain enzyme activities were shown to be specific for developing spore or stalk cells (Ellingson et al. 1971). Subsequent experiments, in which cells were pulse-labeled with [<sup>35</sup>S]methionine at hourly intervals and the protein products resolved on two-dimensional polyacrylamide gels, showed that about 100 proteins are synthesized exclusively or predominantly in developing cells (Alton and Lodish 1977). Surprisingly, nearly 50% of the developmentally regulated proteins are induced around the time of cell aggregate formation. Induction of synthesis of these molecules was correlated with the appearance of translatable mRNAs in the cytoplasm (Alton and Lodish 1977). However, such studies could not define the total number of developmentally regulated mRNAs.

Solution hybridization experiments have led to consistent estimates of the numbers of different genes expressed at different stages of differentiation (Blumberg and Lodish 1980; Jacquet et al. 1981). As summarized in Table 1, growing *Dictyostelium* cells contain about 4500 discrete mRNA species. These represent the transcription of about 19% of genomic single-copy DNA. The number of mRNA species does not change significantly by 6 hours of differentiation. Formation of cellular aggregates is accompanied by a dramatic change in gene expression—transcription and accumulation of 2500–3000 new species of mRNA. At the tip stage, there are roughly 7000 mRNA species present in the cytoplasm, including the 4500 growth-stage mRNAs. This number remains relatively constant throughout the remainder of the developmental program, although two-dimensional gel analysis has shown that there are few proteins specifically synthesized during the culmination stage (Coloma and Lodish 1981).

The ability to isolate and characterize specific message molecules by cDNA cloning has provided us with important information about the patterns of developmentally regulated gene expression. Over 50 developmentally regulated mRNAs have been isolated from a bank of cDNA clones made by reverse transcription of mRNA prepared from cells developed for 15 or 22

Table 1 Complexity of cytoplasmic polyadenylated RNA during development

Developmental stage	Single copy genome expressed as cytoplasmic mRNA (%)	No. of genes expressed
growth 6 hr	38.6	4800
(pre-aggregation) 13 hr	38.6	4800
(postaggregation) 22 hr	59.6	7420
(culmination)	62.0	7750

hours (Barklis and Lodish 1983). Clones that hybridized to labeled mRNA from differentiating cells, but not to mRNA from growing cells, were selected for further study. A rather surprising result emerged from a study of these cloned mRNAs: Most were expressed exclusively or preferentially in either prespore or prestalk cells (Barklis and Lodish 1983).

Prestalk and prespore cells can be readily separated by centrifugation in Percoll (Tsang and Bradbury 1981; Ratner and Borth 1983). When the developmentally regulated cDNA clones were hybridized to RNA prepared from isolated prespore or prestalk cells, nearly all of them preferentially hybridized to RNA from one cell type, implying that the majority of the developmentally regulated genes isolated to date are expressed in a cell-type-specific fashion (Barklis and Lodish 1983). In addition, as shown in Figure 7A, these cDNAs appear to fall into discrete, coregulated classes based upon the time course of their accumulation and their cell-type specificity (Chisholm et al. 1983, 1984; Mehdy et al. 1983).

Genes specifically expressed in prestalk cells fall into two classes. The first, called prestalk class-I genes, are expressed at very low levels in growing cells but are induced to 20–50-fold higher levels between 8 and 12 hours of differentiation (prior to aggregation). Messages transcribed from prestalk class-II genes are undetectable in growing cells and first accumulate around 12 hours of development, at the time of aggregation. Messages specifically found in prespore cells also fall into two classes. Prespore class-I genes are induced between 4 and 8 hours (during chemotaxis), whereas prespore class-II mRNAs are first seen around 12 hours of differentiation. Of 23 prespore mRNAs, 3 are in class I and 20 are in class II. Two classes of messages present in both cell types have been isolated, although they appear to be relatively rare. Messages encoded by these appear between 8 and 12 hours of development.

Although the relatively large cDNA clones developed, with the exception of actin (McKeown and Lodish 1981; those induced by aggregation) have been

## Extracellular S

Using suspension culture, a 24-hour cycle, and cAMP pulses, and cell aggregation (Chisholm et al. 1983), suspension culture of Dictyostelium is observed. In summary, the cAMP-induced normally of the other genes. Ax3 cells do not, nor do they spontaneously, per se, is cell-surface cAMP.

Experimental results in inducing mRNAs (Fig. 1) form the EDTA aggregates (Chisholm et al. 1983) classes express

The final class induced in cAMP genes requires cAMP pulses brought into in begins within induced if the signal formation of the subsequent to cAMP. Figure 9 is a

Although the number of different cDNA clones that has been examined is relatively large, it is important to note that mRNAs used in constructing the cDNA clones have been obtained from cells in the postaggregation stage of development. This may cause us to miss certain classes of mRNAs. With the exception of the discoidins (Williams et al. 1979, 1980; Poole et al. 1981), actin (McKeown and Firtel 1981a,b), and a family of transcripts encoded by an apparent transposable element, DIRS-1 (Chung et al. 1981; Zuker and Lodish 1981; Zuker et al. 1983), few preaggregation-specific transcripts (i.e., those induced during the preaggregation stage but lost by the time of aggregation) have been identified.

### Extracellular Signals Regulate Developmental Gene Expression

Using suspensions of starved amoebae, triggered to initiate the developmental cycle, we have been able to investigate the effects of starvation, cAMP pulses, and cell contact on the patterns of developmental gene expression (Chisholm et al. 1983, 1984). In axenically grown cultures transferred to suspension culture in starvation buffer, induction of two classes of gene products is observed. First, the cell-surface cAMP receptor (Fig. 8A) and, presumably, the other components of the cAMP signal relay apparatus are induced normally. Second, prestalk class-I mRNAs are induced (Fig. 7B). None of the other gene classes are induced by starvation in suspension. Our starved Ax3 cells do not develop the ability to form EDTA-stable cell aggregates, nor do they spontaneously begin the cAMP signaling process. Thus starvation, per se, is sufficient for induction of the prestalk class-I genes and the cell-surface cAMP receptor.

Experimental addition of cAMP pulses to suspension-starved cultures results in induction of both prestalk class-II mRNAs and prespore class-I mRNAs (Fig. 7C), as well as the cell-surface molecules that enable cells to form the EDTA-stable interactions (Fig. 8B) characteristic of multicellular aggregates (Chisholm et al. 1983, 1984). In addition, one of the two gene classes expressed in both cell types is induced by cAMP.

The final class of prespore-specific mRNA, prespore class II, is not induced in cAMP-pulsed, suspension-starved cultures. Expression of these genes requires the formation of cell aggregates. If starved cells treated with cAMP pulses for 12 hours are plated into filter pads such that the cells are brought into immediate contact, accumulation of the prespore class-II genes begins within 1 hour (Fig. 7D). Similarly, this large class of mRNAs is induced if the speed of shaking the suspension culture is reduced, allowing formation of large aggregates. Thus, some aspect of cell-cell contact subsequent to cAMP pulses is required for induction of prespore class-II mRNAs.

Figure 9 is a schematic diagram representing our current understanding of

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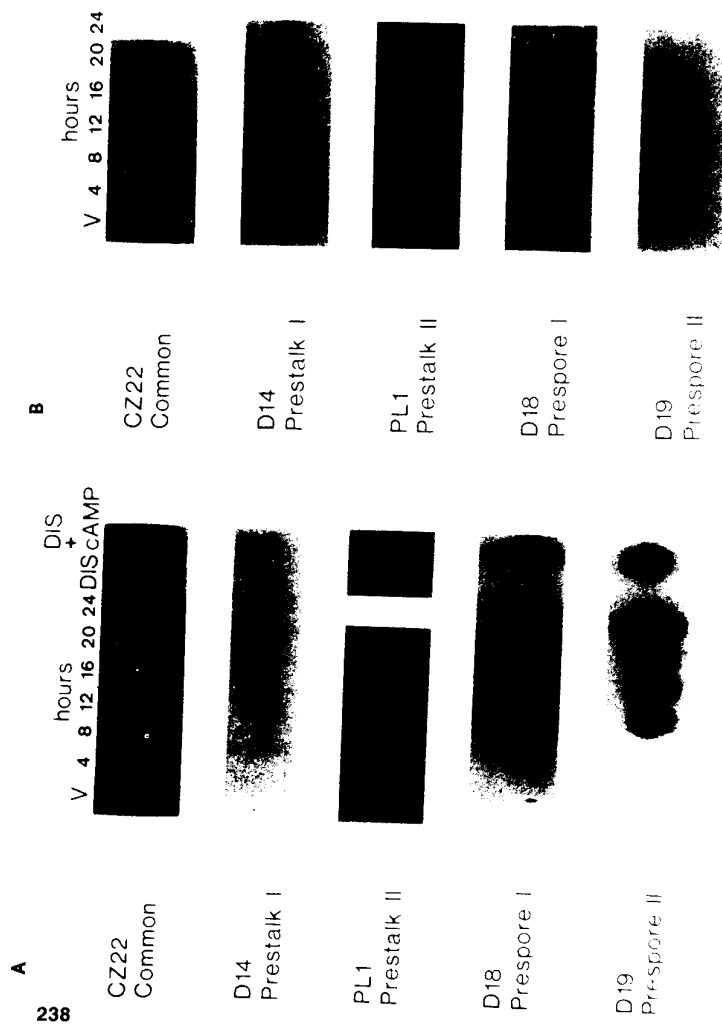
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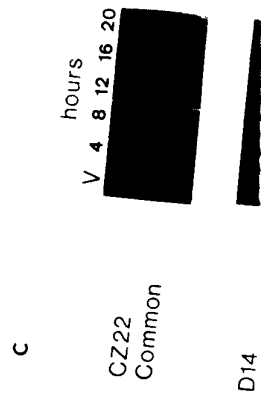
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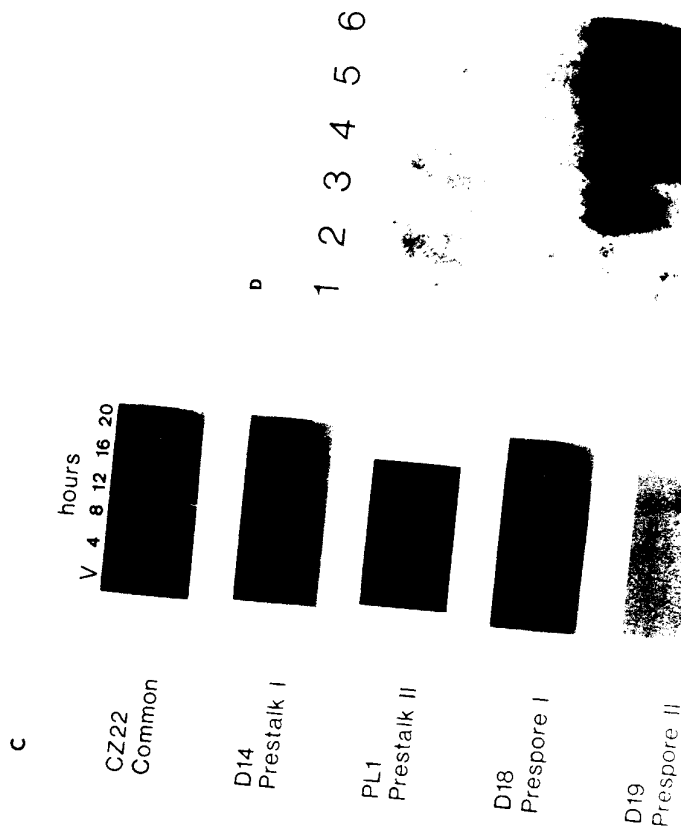
**Figure 7** Accumulation of prespore- and prestalk-specific mRNAs during normal development and as a result of starvation, cAMP pulses, and cell aggregation. RNA prepared from cells developed as described was size-fractionated, transferred to nitrocellulose, and hybridized to labeled cloned cDNAs representative of the gene classes described in the text. (A) Accumulation of prespore- and prestalk-specific mRNAs during normal development on filter pads. Lanes labeled DIS and DIS + cAMP contain total cytoplasmic RNA prepared from cells disaggregated after 15 hr of development, then shaken for 4 hr in the absence (DIS) or presence (DIS + cAMP) of 100  $\mu$ M cAMP. (B) Accumulation of prespore- and prestalk-specific mRNAs in cells starved in suspension.



D19  
Prespore II

D19  
Prespore II

**Figure 7** Accumulation of prespore- and prestalk-specific mRNAs during normal development and as a result of starvation, cAMP pulses, and cell aggregation. RNA prepared from cells developed as described was size-fractionated, transferred to nitrocellulose, and hybridized to labeled cloned cDNAs representative of the gene classes described in the text. (A) Accumulation of prespore- and prestalk-specific mRNAs during normal development on filter pads. Lanes labeled DIS and DIS + cAMP contain total cytoplasmic RNA prepared from cells disaggregated after 15 hr of development, then shaken for 4 hr in the absence (DIS) or presence (DIS + cAMP) of 100  $\mu$ M cAMP. (B) Accumulation of prespore- and prestalk-specific mRNAs in cells starved in suspension.



**Figure 7 (continued)** (C) Accumulation of prespore- and prestalk-specific mRNAs in cells starved in suspension and treated with 50-nM pulses of cAMP every 6 min beginning at 2 hr of starvation. (D) Induction of D19 (prespore class-II) mRNA following cell contact. Cells were starved in suspension and pulsed with cAMP as in C. At 12 hr of treatment they were harvested and deposited onto a filter pad. RNA was prepared from cells at the time of plating (lane 1) and after 1, 2, 3, 4, or 8 hr of further incubation (lanes 2-6, respectively).

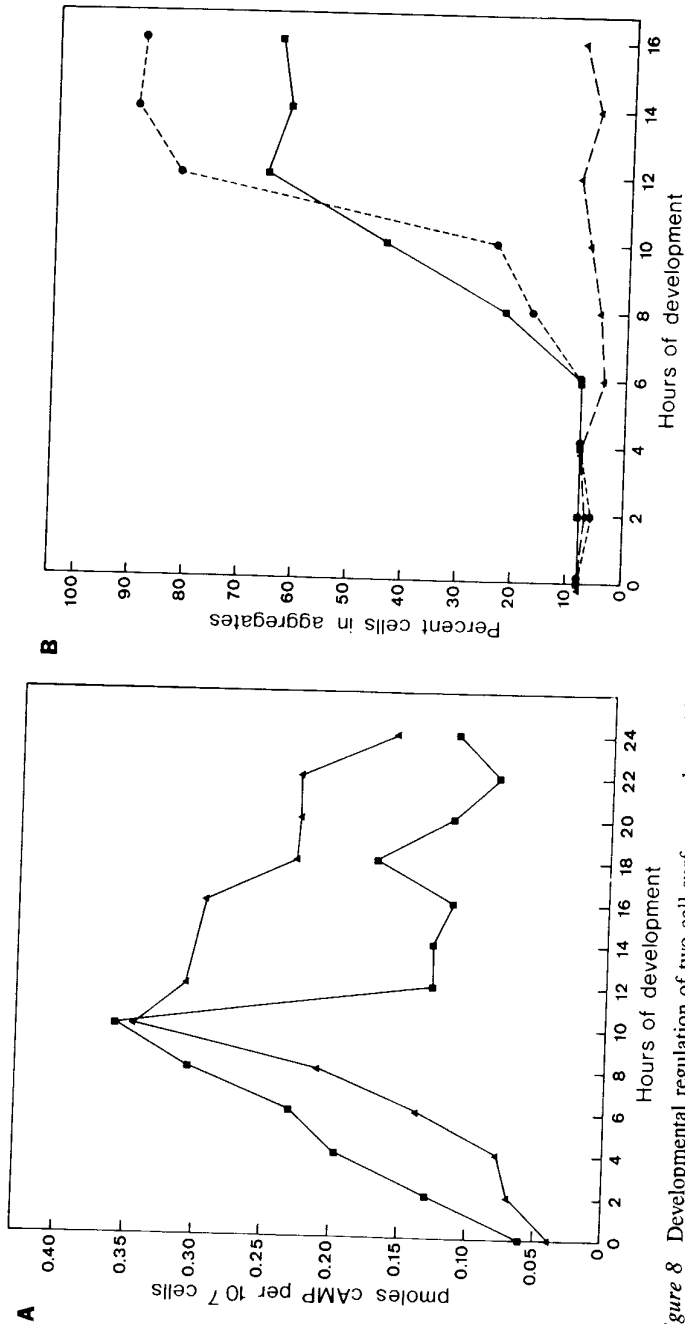


Figure 8 Developmental regulation of two cell-surface markers: (A) cAMP receptor; (B) molecules required for EDTA-stable aggregate formation. Cells were harvested at the indicated times during normal development (●), starvation in suspension (▲), or cAMP-pulsed suspension (■).

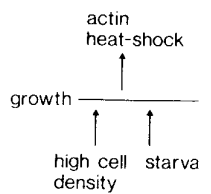


Figure 9 Schematic diagram and the factors responsible for the progression of growth, high cell density, starvation, and heat-shock, leading to actin.

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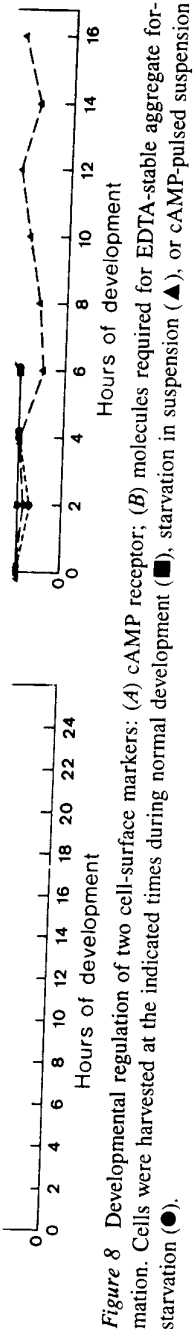


Figure 8 Developmental regulation of two cell-surface markers: (A) cAMP receptor; (B) molecules required for EDTA-stable aggregate formation. Cells were harvested at the indicated times during normal development (■), starvation in suspension (▲), or cAMP-pulsed suspension starvation (●).

gene regulation during *Dictyostelium* development. Upon exhaustion of a food source, growing cells enter a pathway that consists of a series of phases, each induced by an extracellular signal. Some of the products of genes induced at each phase of the developmental program are proteins that enable the cell to synthesize or detect the extracellular signal that induces progression into the next phase of the program. This view of the developmental program is reminiscent of the sequential pattern of gene expression during bacteriophage infection of bacteria.

Two factors are involved in initiating the developmental program: starvation for amino acids and a sufficiently high cell density. Induction of actin mRNA (Margolske and Lodish 1980a) and a set of mRNAs encoded by a family of heat-shock genes is induced solely by high cell density (Zuker et al. 1983), as is the enzyme *N*-acetylglucosaminidase (Grabel and Loomis 1978). Such gene induction may be mediated by secreted cell products that accumulate at high cell densities.

Two additional gene classes are induced by starvation of cells at high density: prestalk class-I genes and the cell-surface cAMP receptor. This represents the next step in the developmental cascade. The appearance of the cAMP receptor enables the cells to respond to extracellular cAMP signals. Detection of the cAMP signal results in progression into the next phase of development, characterized by the induction of prestalk class-II genes and prespore class-I genes, a class of genes expressed in both prespore and prestalk cells, and the accumulation of the cell-surface molecules necessary for the formation of multicellular aggregates. The next step in the cascade is the formation of multicellular aggregates. During this phase of development a large class of genes, prespore class II, is induced. Cells at this stage appear poised to proceed through the directed cell movement responsible for morphogenesis, culminating in the formation of the fruiting body. There must be other steps in the cascade, presently unknown, which occur as development progresses onward from the cellular aggregate stage.

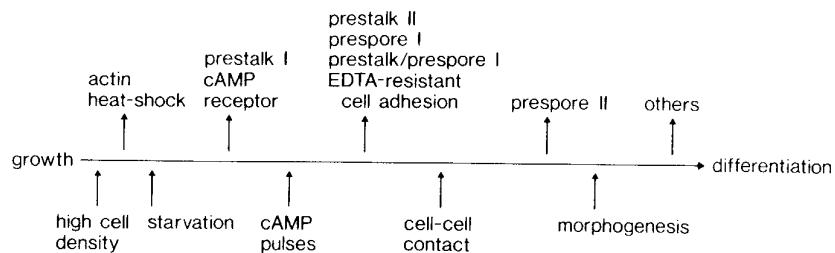


Figure 9 Schematic diagram of gene expression during *Dictyostelium* development and the factors responsible for induction of specific classes of genes.

### Removal of Extracellular Signals Results in the Loss of Specific Gene Products

Abundant evidence suggests that continued synthesis of aggregation-stage proteins and mRNAs is dependent on continued cell-cell interactions. Disaggregation of pseudoplasmodia results in immediate cessation of accumulation of several developmentally regulated enzymes, such as UDP-glucose pyrophosphorylase and UDP-galactose polysaccharide transferase (Newell and Sussman 1970; Newell et al. 1971, 1972). Likewise, synthesis of the predominant polypeptides, which initiates around the time of aggregation, ceases when the aggregates are dispersed and the cells are kept from reforming aggregates (Landfear and Lodish 1980).

Hybridization of mRNA to a cDNA probe specific for the population of 2500 developmentally regulated transcripts shows that the levels of these mRNAs are reduced in disaggregated cells (Chung et al. 1981). In contrast, the levels of mRNAs encoded by genes expressed throughout growth and development are unaffected by disaggregation. When cDNA clones representative of the cell-type-specific gene classes discussed above are used as probes for individual mRNA sequences, the levels of both prespore classes and the prestalk class-II mRNAs are reduced upon disaggregation, whereas those encoded by prestalk class-I mRNAs and "common" genes are unaffected (Fig. 7A; Barklis and Lodish 1983; Mehdy et al. 1983; Chisholm et al. 1984). Since disruption of the aggregates both eliminates the cell contact and, most likely, reduces the extracellular cAMP concentration, reductions in the levels of these three classes of mRNAs may result from removal of the signals that initially induced their accumulation.

Addition of 100  $\mu$ M cAMP to disaggregated cells restores normal accumulation of both classes of prespore-specific mRNAs (Barklis and Lodish 1983; see Fig. 7A). As the prespore class-I mRNAs are induced by nanomolar levels of cAMP, this result is not surprising. However, it is not clear why addition of millimolar cAMP also restores accumulation of the prespore class-II genes, since induction of these mRNAs requires cell aggregation. Although cell contact is necessary for primary induction of these genes, high levels of cAMP appear sufficient for maintaining their expression (Landfear and Lodish 1980).

Results to date concerning effects of cAMP on the prestalk class-II mRNAs are variable. The loss of mRNAs of this class varies from experiment to experiment, as does their restoration by the addition of cAMP to disaggregated cells.

### Expression of the Developmentally Regulated mRNAs Is Regulated Primarily at the Level of RNA Transcription

In most eukaryotic systems developmentally regulated gene expression is controlled at the level of transcription. Several lines of evidence suggest that this is also true for *Dictyostelium*. First, a cDNA probe specific for devel-

opmentally regulated from growing cells (number of these sequences must be unusual).

Second, in vitro transcription stages of development are being transcribed are incubated with labeled where initiation of new initiated by the cells produce only RNAs labeled with cells at the time the nuclei to a series of cell-type transcriptional activity of a series. Clones representing genes hybridize to labeled in the nuclei were isolated. Thus, these genes are there is no synthesis of genes, such as D18 and at 4 hours of development synthesize RNA that hybridize as well as to clone PL1.

Veg 4 hr



Figure 10 Transcription of in vitro transcription reactions: cells, 8-hr cells, 12-hr cells particular nucleic preparations and immobilized on nitrocellulose cDNAs, each spotted in triplicate.



sis of aggregation-stage cell-cell interactions. Dis-  
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opmentally regulated mRNAs does not hybridize to nuclear RNA prepared from growing cells (Blumberg and Lodish 1981). Thus, if any significant number of these sequences are transcribed in growing cells, the resulting RNAs must be unusually unstable.

Second, *in vitro* transcription by nuclei isolated from cells at different stages of development can be used to assess the rates at which different genes are being transcribed (Landfear et al. 1982). In such systems, isolated nuclei are incubated with labeled ribonucleoside triphosphates under conditions where initiation of new mRNAs is reduced, but transcriptional complexes initiated by the cells prior to isolation of the nuclei are completed. Thus, the only RNAs labeled will be those encoded by genes being transcribed in the cells at the time the nuclei were isolated. Hybridization of such labeled RNA to a series of cell-type-specific clones can be used to assess the transcriptional activity of a series of representative cell-type-specific genes (Fig. 10). Clones representing genes expressed throughout growth and development hybridize to labeled *in vitro* transcripts regardless of the stage from which the nuclei were isolated (see clones CZ5, CZ9, CZ22, SC29, and SC79). Thus, these genes are transcribed throughout differentiation. In contrast, there is no synthesis of RNA complementary to the developmentally regulated genes, such as D18 and D19, by nuclei isolated from growing cells or cells at 4 hours of development. Nuclei from cells at 8 hours of development synthesize RNA that hybridizes to clone D18 (a typical prespore class-I gene), as well as to clone PL1 (typical prestalk class II). No RNA complementary

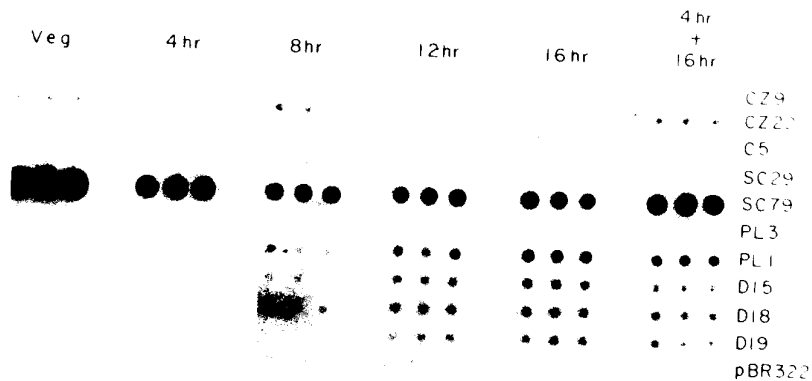


Figure 10 Transcription of regulated and common RNAs during development. *In vitro* transcription reactions were performed with nuclei from vegetative cells, 4-hr cells, 8-hr cells, 12-hr cells, and 16-hr cells. The labeled transcripts synthesized by a particular nucleic preparation were hybridized to DNAs prepared from cDNA clones and immobilized on nitrocellulose. The labels on the right refer to individual cloned cDNAs, each spotted in triplicate on each filter.

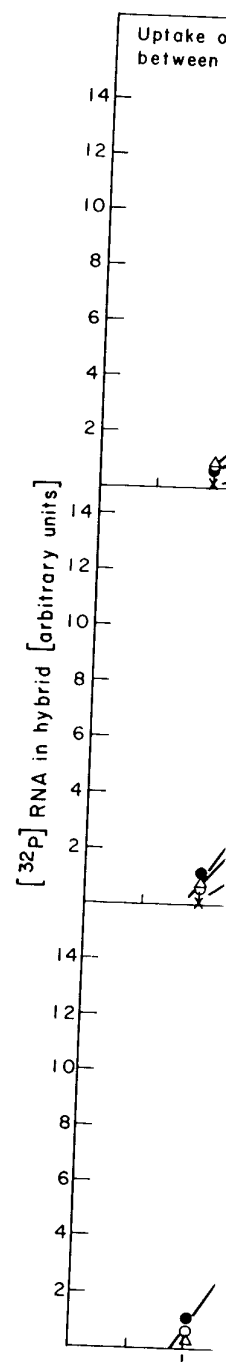
to the class-II prespore D19 gene is transcribed until 12 hours of differentiation. These results correlate well with the times at which transcripts accumulate (see Fig. 7A). Thus, transcription appears to be the principal mechanism that regulates the accumulation of developmentally induced mRNAs. However, our recent evidence suggests that the differential stability of mRNAs is also important in determining the levels of cytoplasmic messages. As *Dictyostelium* is one of the few systems where regulation at the level of mRNA stability has been documented, it is appropriate to review some of the key evidence here.

#### cAMP Stabilizes mRNA in Disaggregated Cells

Two techniques have been used to determine the stability of *Dictyostelium* mRNAs during normal differentiation: approach to steady-state labeling of mRNA (Mangiarotti et al. 1982) and addition of inhibitors of mRNA biosynthesis (Margolskee and Lodish 1980b; Chung et al. 1981). Both methods show that during growth and differentiation, the average half-life of all mRNA in the cell is about 4 hours. In particular, after aggregation both common and developmentally regulated mRNAs have the same half-life—4 hours or greater. The approach to a steady-state labeling experiment is shown in Figure 11, in which the incorporation of  $^{32}\text{P}$  into poly(A)<sup>+</sup> RNA corresponding to different cloned genes is measured in cells exposed to [ $^{32}\text{P}$ ]orthophosphate starting at 13 hours of development. The time at which incorporation of the  $^{32}\text{P}$  label into an mRNA reaches half of its steady-state value is an approximation of the half-life of that mRNA. The linear incorporation of  $^{32}\text{P}$  into all mRNAs for at least 3 hours shows that the half-life of all mRNAs tested is at least 3 hours. Note that both common genes such as SC29 and developmentally regulated genes like PL1 and SC253 show similar kinetics of labeling and, thus, similar half-lives. However, because the cells are starved for nutrients, a condition necessary for development, these experiments do not and could not meet the conditions of "balanced growth" necessary for achievement of steady state (Casey et al. 1983).

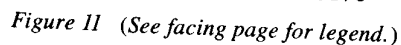
An alternative experimental approach is to follow the loss of radioactivity in RNA during a chase under conditions in which there is no new incorporation of label into RNA (Mangiarotti et al. 1982, 1983a). Messages decay with roughly a 4-hour half-life. These experiments were performed under conditions where there is no new incorporation of label into rRNA, suggesting that the chase is effective. Taken together, the results of these two exper-

**Figure 11** Uptake of  $^{32}\text{P}$  into cytoplasmic polyadenylated RNA during development. [ $^{32}\text{P}$ ]Orthophosphate was added to cells beginning at 13 hr of development. Polyadenylated RNA isolated at various times during the labeling was hybridized to a set of cloned cDNAs immobilized on a nitrocellulose filter. The amount of RNA hybridized to individual cloned cDNAs was determined by densitometric scanning of an autoradiogram of the hybridized filters.



**Figure 11** (See facing page)

NA during development. f development. Polyaden-  
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imental approaches are consistent, suggesting a 4-hour half-life for both developmentally induced and common mRNAs.

As described above, disaggregation of cellular aggregates after 12 hours of development results in loss of the prespore class-I, prespore class-II, and prestalk class-II mRNAs, without affecting the levels of common mRNAs or prestalk class-I mRNAs. The loss of these developmentally regulated messages occurs very rapidly – with a halftime of no more than 20–30 minutes – far too quickly to be explained on the basis of natural message turnover in the absence of active mRNA transcription (Chung et al. 1981; Mangiarotti et al. 1983a,b). Thus, the mRNAs that were rapidly lost upon disaggregation must be specifically degraded. Figure 12 shows an experiment in which cells are labeled with [ $^{32}$ P]orthophosphate from 13–17 hours and are then disaggregated, washed extensively, and shaken as single cells in the presence of an excess of unlabeled phosphate. There is no additional incorporation of label into RNA, indicating that the chase is effective. RNA was prepared from these cells at various times and hybridized to cloned DNA encoding various genes. The developmentally regulated messages such as D15, D18, and D19 degraded very rapidly – with a halftime of 30 minutes, whereas common mRNAs such as SC29 and C5 are stable. The half-lives of both prespore mRNA classes and the prestalk class-II messages are significantly less than that characteristic of the same messages in multicellular aggregates.

Figure 12 also shows that the addition of 100  $\mu$ M cAMP to the disaggregated cells prevents degradation of all the developmentally regulated mRNAs (Mangiarotti et al. 1983a). It is unclear how the cAMP brings about this specific stabilization of developmentally induced messages. However, this system is an excellent one in which one can study how message stability is regulated.

#### Transcripts of Developmentally Regulated Genes Are Processed at a Different Rate than Those of Common Genes

Mangiarotti et al. (1983b) have recently described another important difference between transcripts of developmentally regulated genes and those expressed throughout growth and development: Regulated mRNAs require a much longer time to exit from the nucleus. To show this cells were developed for 13 hours and then labeled with [ $^{32}$ P]orthophosphate. Cells were harvested at 45-minute intervals beginning 45 minutes after the addition of the radioactive phosphate, and RNA was isolated from separated nuclear and cytoplasmic fractions. The labeled RNA was then hybridized to a series of cloned cDNAs. The kinetics of the labeling of nuclear polyadenylated RNA was similar for all clones tested, at least for the first 90 minutes. However, labeled polyadenylated RNAs corresponding to common cDNAs appeared in the cytoplasm 45 minutes before labeled RNA corresponding to any developmentally regulated genes. One hypothetical explanation for the increased

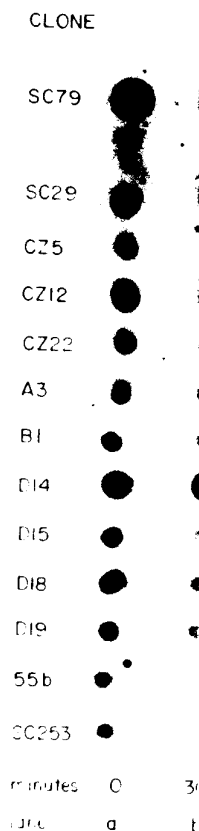


Figure 12 Effect of cAMP on the stability of RNA species following 17 hr of development. The presence or absence of cAMP at the times indicated and

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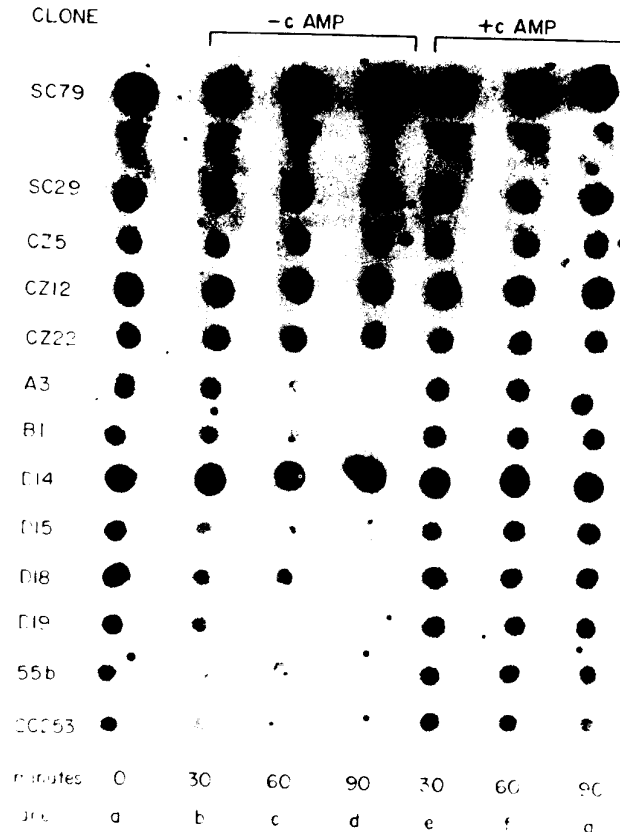


Figure 12 Effect of cAMP on the decay of  $^{32}\text{P}$  label incorporated into polyadenylated RNA species following disruption of cell aggregates. Cells were labeled from 13 to 17 hr of development, disaggregated, and shaken in suspension as single cells in the presence or absence of cAMP. RNA was prepared from disaggregated cells at the times indicated and hybridized to cloned cDNA immobilized on nitrocellulose.

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her important differ- genes and those ex- d mRNAs require a cells were developed . Cells were harvest- the addition of the :parated nuclear and idized to a series of polyadenylated RNA 0 minutes. However, 1 cDNAs appeared in onding to any devel- ion for the increased

transport time for the developmentally regulated messages is that these genes contain more intervening sequences than common genes. Little is known about the structure of the regulated genes, although discoidin and actin genes are known to lack introns. Another possible explanation is that the developmentally regulated messages are subjected to posttranscriptional modifications not required for exit of common mRNAs. It is tempting to consider the possibility that such a difference in modification between common and developmentally regulated genes might also account for their different stabilities following disaggregation of slug stage cells.

## CONCLUSIONS AND PROSPECTS FOR THE FUTURE

The value of *Dictyostelium* as a system for investigating several key problems of developmental biology at the molecular level is only now being appreciated. *Dictyostelium* is particularly valuable for studies of how cells communicate and sense extracellular signals. The use of cAMP pulses to orchestrate the transition from single cells into a multicellular aggregate is a model that may provide important clues as to how tissues in multicellular organisms are organized. It also provides one of the best opportunities to study chemotaxis in a eukaryotic organism. The ways in which extracellular signals are sensed and transduced by cells during chemotaxis can now be investigated at the molecular level. Additionally, the two key extracellular signals, cAMP and cell contact, are also crucial in regulating the program of developmental gene expression. It should be possible to learn both how cell-surface components function in sensing these signals and how the signal resulting from exposure to cAMP or the formation of cellular aggregates is transduced to the cytoplasm and nucleus to alter the pattern of gene expression, as well as the stability of individual mRNAs.

Several different mechanisms are employed by *Dictyostelium* to regulate the levels of specific gene products during development. As is the case for most eukaryotic systems, the primary control of gene expression appears to be transcriptional. Perhaps the relative simplicity of *Dictyostelium* compared with mammalian cells will simplify the difficult task of developing an in vitro transcription system that faithfully reproduces developmental gene regulation. *Dictyostelium* may also be a particularly valuable system in which the mechanisms that regulate stability of individual messages can be studied. The stability of specific classes of developmentally regulated messages can be manipulated experimentally by changes in the levels of cAMP in suspensions of disaggregated cells. Translational control of gene expression also occurs during early *Dictyostelium* differentiation, but virtually nothing is known about the mechanisms by which this is achieved. Finally, there is evidence that developmentally regulated mRNAs may be processed differently than common messages.

A major limitation of the *Dictyostelium* system is the limited availability of genetic tools. Many developmental mutants have been described, but relatively little is known about how gene expression has been altered by the mutations. Analysis of mutants defective in sensing the cAMP or cell-contact signals during development might provide important clues about signal transduction. The successful introduction of cloned DNA sequences into *Dictyostelium* offers many exciting possibilities for the cloning of important developmental genes by their ability to complement developmental mutants. If one can introduce cloned DNA segments and subsequently achieve normal regulated expression of those genes it will be possible to dissect the regulatory elements involved in developmental control of gene expression, as well as the sequences involved in regulating the stability of individual messages. All

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things considered, *Dictyostelium* seems likely to continue as an important system for the study of differentiation and development.

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Several key problems are now being appreciated: how cells communicate to orchestrate aggregate formation. A model that cellular organisms are able to study chemotaxis and how signals are sensed and investigated at the cell surface, cAMP and other developmental gene surface components are being investigated from exposure to the cytoplasm, as well as the

*Dictyostelium* to regulate. As is the case for other organisms, expression appears to be regulated in *Dictyostelium* compared to other organisms. Developing an in vitro system for developmental gene regulation in which the system in which the genes can be studied. Related messages can be studied. If cAMP in suspension expression also virtually nothing is. Finally, there is a processed differ-

limited availability of described, but related. Altered by the mutation or cell-contact about signal transductions into *Dictyostelium* of important developmental mutants. If one achieve normal regulate the regulatory expression, as well as dual messages. All

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