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Transduction of Chemical Signals In Dictyostelium Cells

G. Gerisch, A. Tsiomenko, J. Stadler, M. Claviez, D. Hülser, and C. Rossier

Max-Planck-Institut für Biochemie, D-8033 Martinsried bei München, Federal Republic of Germany

DEVELOPMENT OF DICTYOSTELIUM DISCOIDEUM FROM A UNICELLULAR INTO A MULTICELLULAR ORGANISM

Cells of D discoideum grow and multiply as single amoebae in the presence of nutrients, ie bacteria, or in laboratory strains, liquid medium. After the exhaustion of nutrients the cells undergo a sequence of changes exemplified by the expression of differentiation markers, which are characteristic of certain developmental stages. During the later stages of development, cell-type-specific antigenic markers become detectable. These changes of the cells are part of a developmental program manifested in a sequence of morphogenetic phenomena: (1) cell aggregation; (2) development of the aggregates into "slugs," fingerlike multicellular bodies equipped with a tip acting as an organizer; (3) differentiation of the anterior slug cells into prestalk cells and of the posterior slug cells into prespore cells; and (4) culmination as a process during which the fruiting body is shaped and terminal differentiation into spores and stalk cells occurs.

FUNCTIONS OF CYCLIC AMP IN DEVELOPMENT

Three different functions of cyclic AMP in D discoideum are known: (1) cAMP acts as a chemoattractant during cell aggregation, (2) it controls cell

A. Tsiomenko's permanent address is Institute of Biochemistry and Physiology of Microorganisms, USSR Academy of Sciences, Pushino (Moscow Region) 142292, USSR.

D. Hülser's present address is Biologisches Institut der Universität Stuttgart, Ulmer Strasse 227, 7000 Stuttgart 60, Federal Republic of Germany.

C. Rossier's present address is Laboratoire de Microbiologie Générale, Département de Biologie Végétale, Université de Genève, 3, Place de l'Université, 1211 Geneva, Switzerland.

development, particularly the acquisition of aggregation competence, and (3) it is involved in terminal cell differentiation. In this report we will concentrate on the functions 1 and 2 of cAMP. Chemotaxis requires the recognition of concentration gradients in the environment by attractant binding to cell surface receptors, the processing of signals from the receptors to the contractile system of the cells, extension of pseudopods at one part, and contraction at other parts of the cells in accord with the external gradient. One pathway of signal processing from the receptors to the contractile system involves the regulation of a myosin kinase.

The control of development up to aggregation competence is largely dependent on the temporal pattern of cAMP application: Only repetitive pulses enhance development. This effect has been studied using the expression of a membrane glycoprotein called contact site A as a differentiation marker.

SIGNAL PROCESSING IN CHEMOTAXIS

One response of chemotactically responsive D discoideum cells to cAMP is a change in myosin heavy chain phosphorylation [Rahmsdorf et al, 1978]. Cyclic AMP controls the influx of Ca^{2+} into chemotactically responsive cells [Wick et al, 1978]. Ca^{2+} and calmodulin inhibit a myosin heavy chain kinase [Malchow et al, 1981; Maruta et al, 1983]. Dephosphorylation of the myosin increases the actin-activated Mg^{2+}-ATPase activity and enhances the formation of myosin filaments [Kuczmarski and Spudich, 1980]. These effects appear to constitute steps in a signal processing pathway connecting the activation of cell surface receptors with responses in the contractile system, as it is a prerequisite for chemotactic orientation of an amoeboid cell (Fig. 1). The peculiarity of Dictyostelium cells or of granulocytes is the capability of these amoeboid cells to organize the intracellular transfer of chemotactic signals such that different responses in different parts of a cell become possible. It is an open question how at that part of the cell surface that is exposed to the highest attractant concentration pseudopods are induced, while on the opposite side the cell is retracting. In principle, granulocytes and Dictyostelium cells can protrude pseudopods from any part of their surface. But only transiently can two fronts with pseudopods exist on the same cell. A cell can be brought into this transient state by stimulation on opposite sides with two microcapillaries filled with cAMP (Fig. 2).

CONTROL OF EARLY DEVELOPMENT BY CYCLIC AMP

Cell development up to the acquisition of aggregation competence implies the increase of adenylate cyclase activity by more than one order of magni-

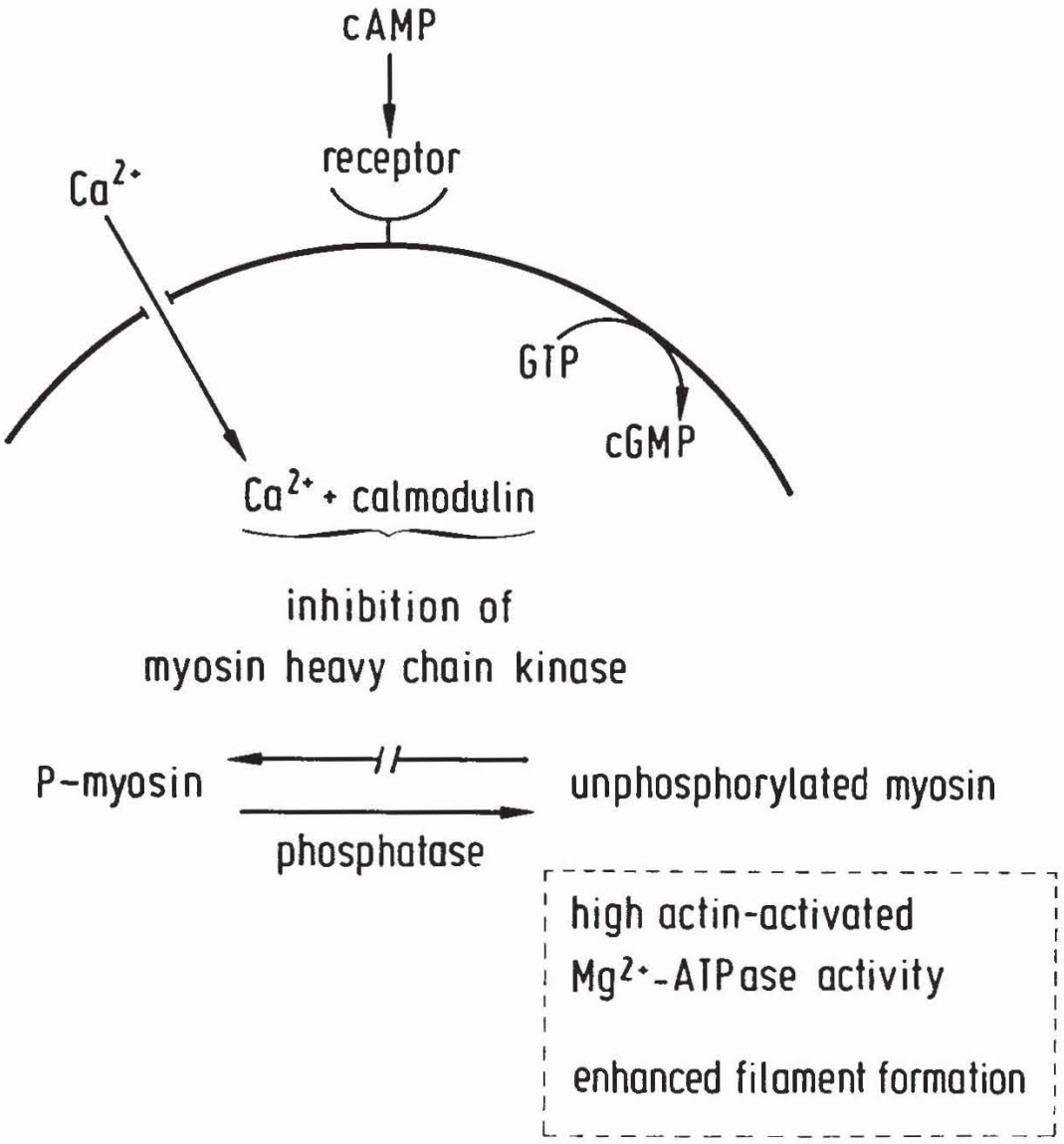

Fig. 1. Scheme of a proposed signal processing pathway involved in chemotaxis. Binding of cAMP to cell surface receptors is known to activate guanylate cyclase and to increase the influx of Ca^{2+} into the cells. The influx of Ca^{2+} can be linked, via the inactivation of a myosin heavy chain kinase, to an enhancement of myosin functions, which probably is responsible for local contraction of chemotactically responding cells.

tude above its low level during the growth phase [Klein, 1976]. Superimposed on what has been called the increase of "basal activity" are oscillatory changes through which the activity increases about seven fold from one min to the next, and falls down to basal activity within the next 1 or 2 min [Roos et al, 1977b]. These pulsatile activity changes are repeated every 6–8 minutes, and are followed by the release of the newly synthesized cAMP [Gerisch and Wick, 1975]. The cAMP acts as an intercellular signal that synchronizes development in a cell population. It is a characteristic feature

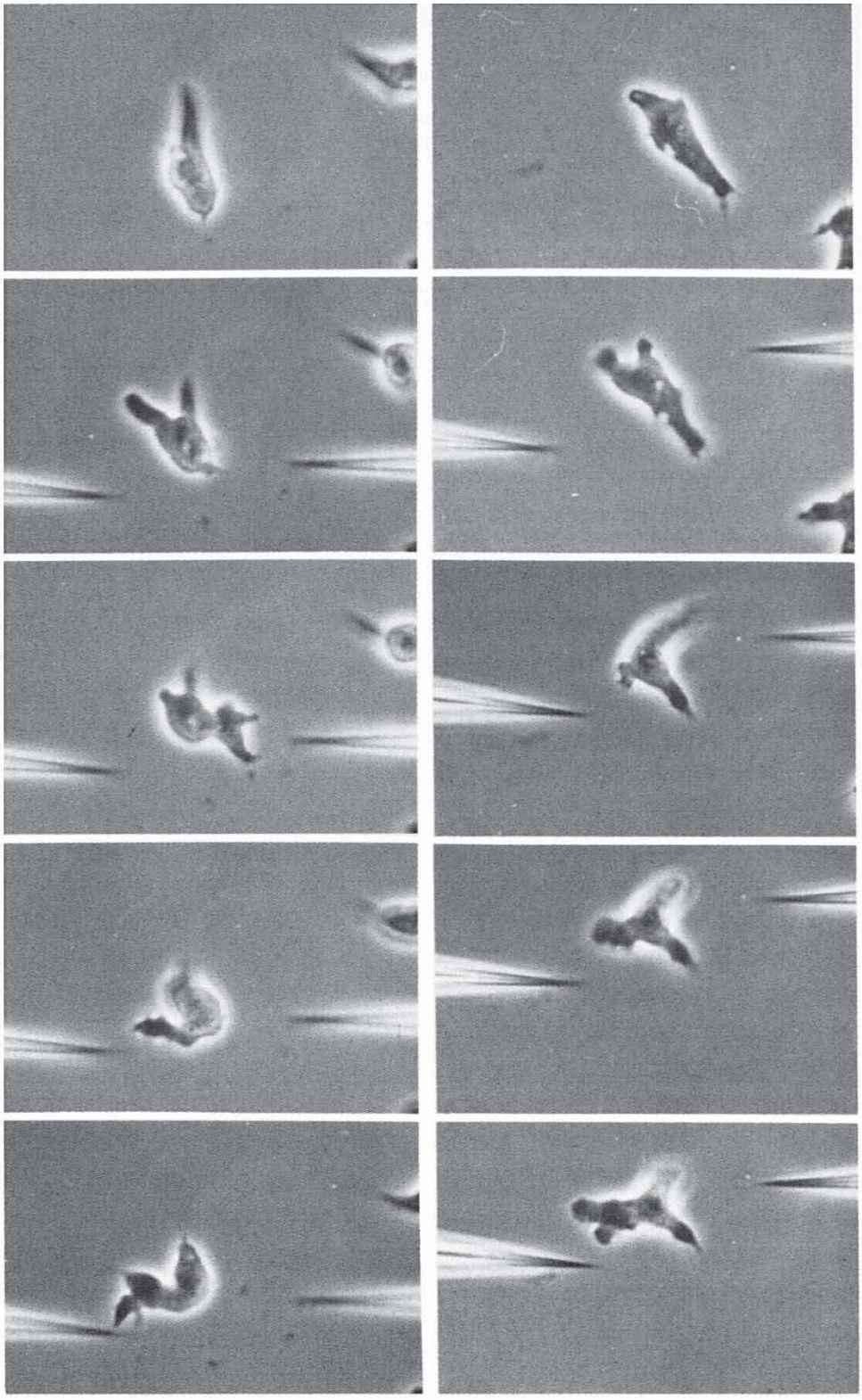

FIG. 2. Stimulation of D. discoideum cells from two microcapillaries filled with cAMP. A cell stimulated on opposite sides extends pseudopods into different directions, shows competition between these pseudopods, finally escapes from the unstable state, and moves along one of the gradients. Left cell from top to bottom: immediately before insertion of the capillaries, and 50, 75, 110, and 150 sec thereafter. Right cell: Immediately before insertion of the capillaries, and within 15 sec to about 1 min thereafter.

that opposite responses are obtained depending on whether cAMP is applied repetitively in form of pulses, or continuously to maintain elevated steady state concentrations. Pulses accelerate, continuous fluxes delay development. The opposite effects can be explained by adaptation of the response system that controls early development. Three fast responses, detectable within a few seconds after the stimulation of cells with cAMP, activation of guanylate and adenylate cyclase, and increased influx of Ca^{2+}, have been tested for adaptation. The first two responses are subject to rapid adaptation [Devreotes and Steck, 1979; Rossier et al, 1980; Wurster and Butz, 1983], whereas the accumulation of Ca^{2+} within the cells persists during the continuous presence of cAMP in the extracellular medium [Bumann et al, 1984].

Slow responses can also be classified into those subject to adaptation and others not subject to adaptation. To the first class belongs the developmental regulation of four membrane components, adenylate cyclase, cAMP receptors, cell surface cAMP-phosphodiesterase, and the contact site A glycoprotein. Expression of these components is enhanced only by cAMP pulses [Roos et al, 1977a]. The second type of responses is represented by the control of two glycoproteins released into the medium: extracellular phosphodiesterase and its inhibitor. The phosphodiesterase is induced and the inhibitor is suppressed by cAMP. In both cases pulses as well as continuous fluxes are effective [Tsang and Coukell, 1977]. The phosphodiesterase and its inhibitor are part of a negative-feedback system regulating extracellular cAMP. In this system short-term fluctuations of the signal amplitude are smoothed out as a result of nonadaptation of the response.

Because of the multiplicity of the responses elicited by cAMP, it is advantageous to concentrate on regulation of one specific protein whose expression is coupled to cAMP signals. The contact site A glycoprotein has proved to be of particular value as a differentiation marker. This integral membrane protein has been discovered as a target site of univalent antibody fragments that block the EDTA-stable type of cell adhesion that is characteristic of aggregation-competent cells [Müller and Gerisch, 1978]. The glycoprotein is strictly developmentally regulated [Murray et al, 1981]. Its appearance at the cell surface during the first 4 to 7 hr of development can be followed on the living cell by fluorescent antibody labeling [Ochiai et al, 1982a]. Specific monoclonal antibodies can be used also for detecting the glycoprotein by immunoblotting after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of total cellular proteins [Ochiai et al, 1982b]. The contact site A glycoprotein is distinguished from the bulk of other plasma membrane glycoproteins by its extensive sulfation [Stadler et al, 1983]. This property makes the 80 kilodalton (kd) glycoprotein easily detectable, even without antibodies, by in vivo labeling with ^{35}S-sulfate.

As an indicator of cell development, the 80-kd glycoprotein reflects the accelerating and delaying effects of cAMP on the acquisition of aggregation competence. These effects are particularly clear in AX3, a laboratory strain of D discoideum that in suspension cultures shows only rudimentary development to aggregation competence. Accordingly, the contact site A glycoprotein is only weakly expressed (Fig. 3). Stimulation with cAMP results in full aggregation competence, as judged by the formation of EDTA-stable contacts, and concomitantly in strong expression of the 80-kd glycoprotein. A continuous flow of the same average amount of cAMP per unit time, applied in form of pulses, suppresses contact site A expression as compared to the unstimulated controls.

BYPASS MUTANTS OF THE DEVELOPMENTAL CONTROL SYSTEM

Stimulation of the expression of cAMP receptors, adenylate cyclase, and membrane-bound phosphodiesterase by cAMP pulses means that a positive-feedback system is implemented in the control of development. The three components responsible for either production or recognition of cAMP signals, or for degradation of extracellular cAMP and thus for shaping of the

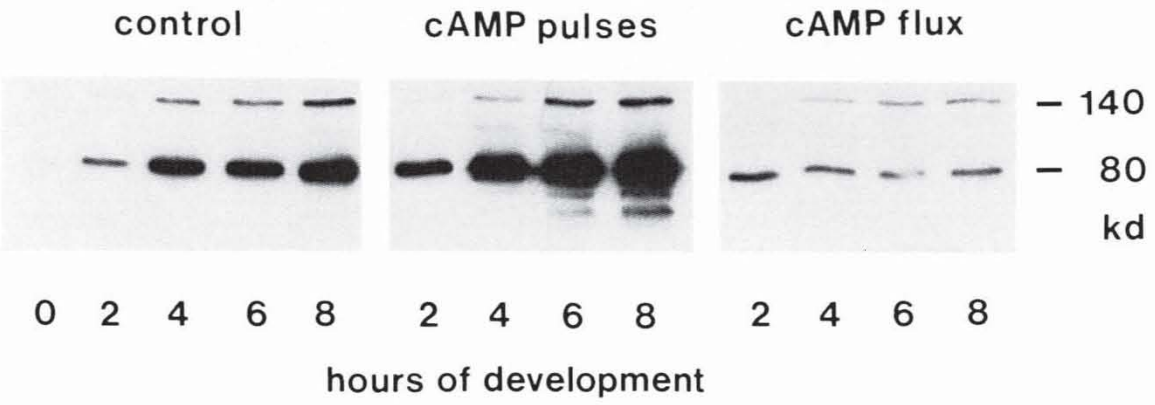

Fig. 3. Effects of cAMP on expression of the contact site A glycoprotein (gp 80) in AX3, a D discoideum strain showing only rudimentary development in suspension cultures. Cells were harvested from nutrient medium during exponential growth and shaken in 17 mM Soerensen phosphate buffer, pH 6.0, and proteins labeled with monoclonal antibody as described by Ochiai et al [1982a]. In accord with the weak developmental potency of the AX3 strain, the glycoprotein is only moderately expressed within an 8-hr period of starvation (left). Pulses of 2×10^{-8} M cAMP applied every 6 min strongly stimulate the expression (middle). The stimulation of gp 80 expression is accompanied by strong stimulation of EDTA-stable contact formation, and of the capacity of the cells to aggregate into streams of end-to-end associated cells, similar to what has been shown previously [Gerisch et al, 1975]. A continuous flux of 2×10^{-7} M cAMP per hour suppresses rather than stimulates gp 80 expression (right).

pulses, are fully expressed only in response to the function of the whole signal system. The connection of the elements of the signal system into a control circuit implies that the defect of any single element caused by mutation renders the whole system nonfunctional, resulting in rudimentary expression of all other elements of the system. Other developmentally regulated proteins, like the contact site A glycoprotein, whose expression is linked to the cAMP signal system, will be also not expressed in a mutant defective in the signal system. The cAMP control circuit is thus one reason for the pleiotropism of many developmental mutants in D discoideum. Mutants defective in one control element are blocked early in development, not expressing a whole series of developmentally regulated genes.

Because of the pleiotropism mutants defective in aggregation are only in special cases valuable tools for the analysis of the control system and for investigating the functions of specific developmentally regulated proteins. Such special cases are, for instance, mutants that can be supplemented by extracellular factors. Phosphodiesterase-negative mutants develop when the enzyme is added to the medium, such that pulsatile cAMP signals can be produced [Brachet et al, 1979]. Mutants defective in cAMP production develop when periodic cAMP pulses are applied [Darmon et al, 1977].

Investigation of the chemotactic response system is particularly complicated by the pleiotropism since cAMP receptors are required both for development to full chemotactic responsiveness, and for the chemotactic response itself. Structural gene mutants for the contact site A glycoprotein are hard to select because the majority of mutants defective in this protein are pleiotropic control mutants. For these reasons we focussed our attention on mutants in which developmental control mechanisms are bypassed.

Mutants in which the cAMP signal system is bypassed can be selected by cultivating cells on agar containing a cAMP analogue that is only slowly hydrolysed by the phosphodiesterases of D discoideum. A suitable analogue is cyclic 3′,5′-adenosine-phosphorothioate (cAMPS). At a concentration of 5×10^{-7} M cAMPS inhibits development of the AX2 wild-type strain, in the same way that a constant concentration of the rapidly degraded cyclic AMP would inhibit [Rossier et al, 1978]. Growth is not inhibited by cAMPS, which thus phenocopies nonaggregating mutants defective in pulsatile cAMP production. Mutants were selected that still aggregated and formed fruiting bodies in the presence of 1×10^{-5}M cAMPS. The inhibition of wild-type development by cAMPS and the resistance of mutants is reflected in the expression of the contact site A glycoprotein. In the mutants tested, the glycoprotein is still under strict developmental control, suggesting that the cAMP signal system is not the only control system of development.

A second control system becomes accessible to experimental analysis in cAMPS-resistant mutants in which the cAMP signal system is no longer required for the expression of developmentally regulated genes. In an attempt to bypass also the second control system, the cAMPS-resistant mutant cAMPS^R 302 has again been mutagenized. It was supposed that a double bypass mutant would express developmentally regulated genes already in growth medium where wild-type development is suppressed (Fig. 4). One mutant, cAMPS^R 302/18, has been obtained that aggregates, and accordingly expresses the contact site A glycoprotein, in the nutrient medium. Chemotactic stimulation of the mutant in growth medium causes the cells to orientate towards a cAMP capillary as it is typical of aggregation competent cells (Fig. 5).

Stepwise bypassing of developmental control systems appears to be not only a method for the analysis of these systems, but also for changing developmentally regulated genes into constitutive ones. Such mutants facilitate the investigation of cell functions that normally are restricted to certain developmental stages, as chemotaxis to cAMP and EDTA-stable cell adhesion is restricted to the aggregation stage of D discoideum. Bypass mutants

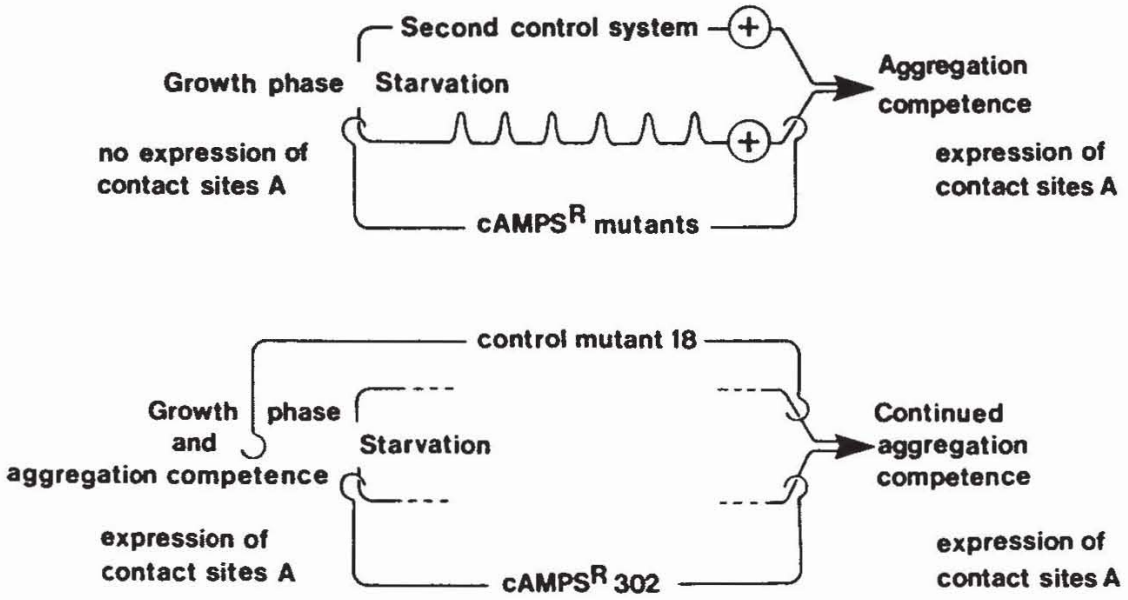

Fig. 4. Diagram summarizing control of early development as judged by expression of the contact site A glycoprotein, a marker of aggregation competence. Top: The parent strain, AX2, generates periodic cAMP pulses, and cAMPS or continuously applied cAMP retards development. In cAMPS^R mutants, eg, cAMPS^R 302, the requirement for cAMP pulses appears to be bypassed, but the glycoprotein is still under stringent developmental control. This result indicates a second control system still working in the mutants. Bottom: By mutagenesis of cAMPS^R 302 a mutant with a bypass in the second control system was obtained, as shown in Figure 5.

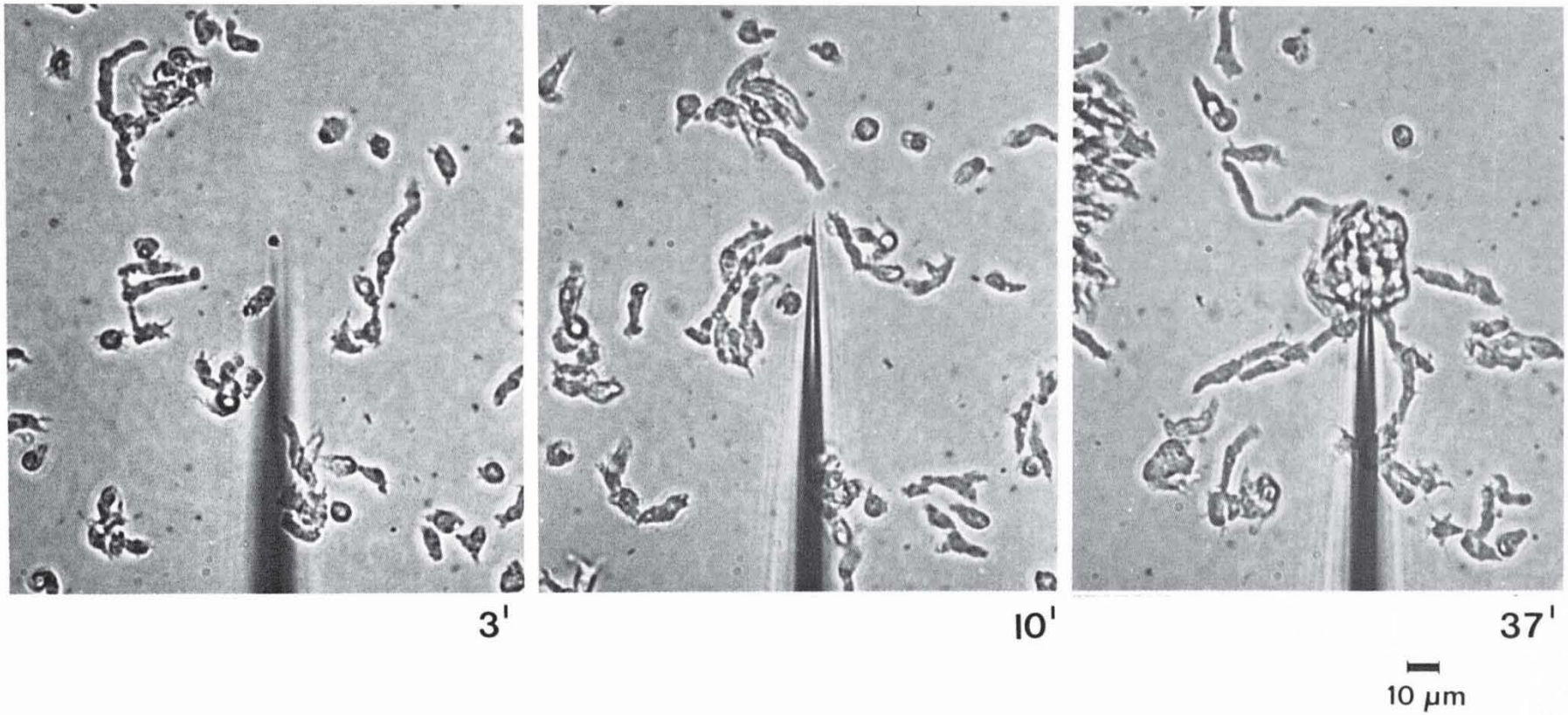

Fig. 5. Aggregation of the "double bypass mutant" cAMPS^R 302/18 in the presence of nutrient medium. In the axenic parent strain AX2, aggregation begins at about 6 hr of starvation in nonnutrient phosphate buffer, as indicated by end-to-end adhesion of elongated cells and strong chemotactic response to cAMP. In the mutant, aggregation occurs already in the nutrient medium. The cells are assembling end-to-end, and are attracted by a microcapllary filled with 1×10^{-3} M cAMP. Times are indicated in minutes after insertion of the capillary.

will be an adequate source for the production of mutants in genes coding for proteins essential for chemotactic sensing or for cohesion of aggregating cells.

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REFERENCES

Brachet P, Dicou EL, Klein C (1979): Inhibition of cell differentiation in a phosphodiesterase defective mutant of *Dictyostelium discoideum*. Cell Diff 8:255–265.

Bumann J, Wurster B, Malchow D (1984): Attractant-induced changes and oscillations of the extracellular Ca^{++} concentration in suspensions of differentiating *Dictyostelium* cells. J Cell Biol 98:173–178.

Darmon M, Barrand P, Brachet P, Klein C, Pereira da Silva L (1977): Phenotypic suppression of morphogenetic mutants of *Dictyostelium discoideum*. Dev Biol 58:174–184.

Devreotes P, Steck T (1979): Cyclic 3′,5′ AMP relay in *Dictyostelium discoideum*. II. Requirements for the initiation and termination on the response. J Cell Biol 80:300–309.

Gerisch G, Fromm H, Huesgen A, Wick U (1975): Control of cell-contact sites by cyclic AMP pulses in differentiating *Dictyostelium* cells. Nature 255:547–549.

Gerisch G, Wick U (1975): Intracellular oscillations and release of cyclic AMP from *Dictyostelium* cells. Biochem Biophys Res Commun 65:364–370.

Klein C (1976): Adenylate cyclase activity in *Dictyostelium discoideum* amoebae and its changes during differentiation. FEBS Lett 68:125–128.

Kuczmarski ER, Spudich JA (1980): Regulation of myosin self-assembly: Phosphorylation of *Dictyostelium* heavy chain inhibits formation of thick filaments. Proc Natl Acad Sci USA 77:7292–7296.

Malchow D, Böhme R, Rahmsdorf HJ (1981): Regulation of phosphorylation of myosin heavy chain during the chemotactic response of *Dictyostelium* cells. Eur J Biochem 117:213–218.

Maruta H, Baltes W, Dieter P, Marmé D, Gerisch G (1983): Myosin heavy chain kinase inactivated by Ca^{2+} calmodulin from aggregating cells of *Dictyostelium discoideum*. EMBO J 2:535–542.

Müller K, Gerisch G (1978): A specific glycoprotein as the target site of adhesion blocking Fab in aggregating *Dictyostelium* cells. Nature 274:445–449.

Murray BA, Yee LD, Loomis WF (1981): Immunological analysis of a glycoprotein (contact sites A) involved in intercellular adhesion of *Dictyostelium discoideum*. J Supramol Struct 17:197–211.

Ochiai H, Schwarz H, Merkl R, Wagle G, Gerisch G (1982a): Stage specific antigens reacting with monoclonal antibodies against contact site A, a cell-surface glycoprotein of *Dictyostelium discoideum*. Cell Diff 11:1–13.

Ochiai H, Stadler J, Westphal M, Wagle G, Merkl R, Gerisch G (1982b): Monoclonal antibodies against contact sites A of *Dictyostelium discoideum*: Detection of modifications of the glycoprotein in tunicamycin-treated cells. EMBO J 1:1011–1016.

Rahmsdorf HJ, Malchow D, Gerisch G (1978): Cyclic-AMP induced phosphorylation in *Dictyostelium* of a polypeptide comigrating with myosin heavy chains. FEBS Lett 88:322–326.

Roos W, Malchow D, Gerisch G (1977a): Adenylye cyclase and the control of cell differentiation in *Dictyostelium discoideum*. Cell Diff 6:299–239.

Roos W, Scheidegger C, Gerisch G (1977b): Adenylate cyclase activity oscillations as signals for cell aggregation in *Dictyostelium discoideum*. Nature 266:259–261.

Rossier C, Gerisch G, Malchow D, Eckstein F (1978): Action of a slowly hydrolysable cyclic AMP analogue on developing cells of *Dictyostelium discoideum*. J Cell Sci 35:321–338.

Rossier C, Eitle E, van Driel R, Gerisch G (1980): Biochemical regulation of cell development and aggregation in *Dictyostelium discoideum*. In Gooday GW, Lloyd D, Trinci APJ (eds): "The Eukaryotic Microbial Cell." Soc Gen Microbiol Symp 30. Cambridge University Press p. 405.

Stadler J, Gerisch G, Bauer G, Suchanek C, Huttner WB (1983): *In vivo* sulfation of the contact site A glycoprotein of *Dictyostelium discoideum*. EMBO J 2:1137–1143.

Tsang AS, Coukell MB (1977): The regulation of cyclic AMP-phosphodiesterase and its specific inhibitor by cyclic AMP in *Dictyostelium*. Cell Diff 6:75–84.

Wick U, Malchow D, Gerisch G (1978): Cyclic-AMP stimulated calcium influx into aggregating cells of *Dictyostelium discoideum*. Cell Biol Int Rep 2:71–79.

Wurster B, Butz U (1983): A study on sensing and adaptation in *Dictyostelium discoideum*: Guanosine 3′,5′-phosphate accumulation and light-scattering responses. J Cell Biol 96:1566–1570.