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INTER-RELATION OF CELL ADHESION AND DIFFERENTIATION IN *DICTYOSTELIUM DISCOIDEUM*

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INTRODUCTION

During the development of *Dictyostelium discoideum* single cells aggregate to form a multicellular organism. The change from the single cell state to multicellularity is mediated by chemotactic cell movement and intercellular adhesion. *Dictyostelium* represents a side branch in the evolution of multicellular organization in eukaryotes. Therefore, the general importance of any work done in *Dictyostelium* will be measured by its applicability to other biological systems. Similarities between the adhesion system of *D. discoideum* and those acting during embryonic development of vertebrates demonstrate that research in a 'simple' microorganism like *Dictyostelium* can provide a basis for studies in more complex organisms. In this survey, the adhesion system of aggregating cells and its regulation during development is taken as a platform from which to outline problems to be solved in the future.

TARGET SITES OF ADHESION BLOCKING Fab: CANDIDATES FOR CELL ADHESION MOLECULES

Several methods have been employed for the identification of cell surface components that mediate intercellular adhesion. One method is to deplete cells of certain components of the adhesion system, to purify the extracted components, and to reconstitute adhesiveness by readdition of these constituents to the non-adhesive cells (Lilien & Moscona, 1967; Noll *et al.* 1979). This method is easily applicable in cases in which an intercellular material forms a bridge between cells by interacting non-covalently with components anchored in the plasma membrane, and proved to be particularly useful in analysing the species-specific adhesion systems of the marine sponges *Haliclona* and *Microciona* (Humphreys, 1963; Misevic *et al.* 1982). A second principle by which an adhesion system can be analysed is to replace one of the interacting cells with a chemically defined surface. Thus cells will adhere *via* carbohydrate-binding sites on their surfaces to polyacrylamide gels derivatized with specific sugars (Schnaar *et al.* 1978). A third, more indirect method is to produce antisera against cell membrane antigens, to test the adhesion blocking activity of Fab prepared from these sera (Beug *et al.* 1970, 1973), and to purify membrane components that neutralize the blocking Fab (Huesgen & Gerisch, 1975; Müller &

Gerisch, 1978; for summary of the work on vertebrates, see Edelman, 1985). The target antigens of adhesion blocking Fab are candidates for molecules that mediate cell-to-cell adhesion.

The Fab neutralization method has limitations that relate to the size of the Fab molecule (which is roughly an ellipsoid of 6 nm length and 4 nm diameter), and to the possible cross-reactivity of target sites. Because of its size, Fab is hardly applicable as a probe for those substructures on cell-surface molecules whose interaction is responsible for cell adhesion. Thus it might not be feasible to determine whether oligosaccharide residues of a glycoprotein are directly involved in contact formation. Cross-reactivity between carbohydrate chains linked to different proteins causes another problem. When antisera are used that contain primarily anti-carbohydrate antibodies against a glycoprotein involved in cell adhesion, any other glycoprotein carrying carbohydrate residues with similar epitopes will neutralize the Fab from these antisera.

After having identified candidates for cell adhesion molecules by the Fab neutralization method it is necessary to prove their actual function in cell-to-cell adhesion. This can be done by incorporation of purified proteins into liposomes (Hoffman & Edelman, 1983) or by selecting mutants specifically defective in either these proteins or in carbohydrate residues linked to them. This is easily possible in *D. discoideum*, as will be shown in later sections.

DISTINCT EDTA-STABLE AND EDTA-SENSITIVE CELL ADHESION IN AGGREGATING *D. DISCOIDEUM* CELLS

Growth phase cells of *D. discoideum* form only EDTA-sensitive contacts, whereas aggregation-competent cells form EDTA-stable contacts in addition to the sensitive ones (Gerisch, 1961). At the time of this change in adhesiveness the cells acquire the capacity to aggregate into streams of end-to-end associated cells. Only cells capable of forming the EDTA-stable contacts are capable of sorting out by species-specific adhesion from cells of another species, *Polysphondylium pallidum* (Bozzaro & Gerisch, 1978; Gerisch *et al.* 1980). EDTA-labile as well as EDTA-stable contacts can be blocked independently by Fab of appropriate specificity (Beug *et al.* 1973). It is also possible to block cell adhesion of both types by the same Fab, which is the case if the cell surface components involved carry common epitopes such as carbohydrate structures (M. Yoshida & G. Gerisch, unpublished). From membranes of aggregation-competent cells an $80\times10^3 M_r$ glycoprotein, designated contact site A (csA), has been purified that is specifically expressed in aggregation-competent cells. This glycoprotein neutralizes the Fab species that block the EDTA-stable adhesion from polyspecific antisera against total membranes (Müller & Gerisch, 1978). In growth-phase cells a $126\times10^3 M_r$ glycoprotein has been identified as a target site of Fab that blocks the EDTA-labile type of cell adhesion (Chadwick & Garrod, 1983).

Results similar to those for *D. discoideum* have been obtained with cell adhesion systems involved in vertebrate embryogenesis. Mouse embryonic and teratocarcinoma cells can simultaneously operate two independent types of intercellular adhesion, a Ca^{2+} requiring and a Ca^{2+} independent one (Takeichi *et al.* 1979). As in

D. discoideum, the two types of cell adhesion differ in their specificity, and one can be blocked independently from the other by Fab of an adequate specificity (Takeichi *et al.* 1981; Shirayoshi *et al.* 1983).

A feature of cell aggregation in *D. discoideum* that is related to the dual nature of its adhesion system is the composite pattern of cell assembly. Specifically, if the EDTA-labile cell adhesion is blocked by Fab, the cells assemble preferentially end-to-end into chains or rosettes (Beug *et al.* 1973). Blockage of the EDTA-stable, csA-mediated cell adhesion results in a more irregular pattern of assembly since the EDTA-labile cell adhesion often causes the cells to associate through lateral extensions (Fig. 1). The restriction of a certain type of cell adhesion to a limited area of the cell surface is comparable to cell adhesion mediated by N-CAM in neurones of the chicken embryo. It is specifically the lateral association of outgrowing nerve fibres that is blocked by Fab against N-CAM (Rutishauser *et al.* 1978).

A problem left for future study is the relationship between cell shape and local cell adhesion. The end-to-end adhesion of the cylindrical aggregating cells is amenable to rapid changes when the polarity of a cell is changed by its reorientation in a cyclic-AMP gradient (Fig. 2). This observation suggests that cell adhesion sites interact, through the membrane, with the cytoskeleton, which determines cell shape, and are regulated in their activity by this interaction. The question of how adhesiveness is regulated is therefore linked to the question of how cell shape and oriented cell movement are controlled. Changes in the degree of myosin phosphorylation (Rahmsdorf *et al.* 1978; Berlot *et al.* 1985), which regulates polymerization of myosin into filaments (Kuczmarski & Spudich, 1980), in the localization of polymerized myosin within the cell (Yumura & Fukui, 1985), and in the quantity of membrane-associated actin (McRobbie & Newell, 1984), have been observed in cells stimulated with the chemoattractant, cyclic-AMP.

These observations need to be fitted into a consistent picture of how the cytoskeleton is organized and how its local activities are coordinately regulated in a moving amoeboid cell. In this approach mutants will be of great help in reducing complexity. This might be of importance because the cytoskeleton appears to consist of more components than are required for its actual function. In other words, there are non-essential proteins that, like the actin crosslinker α-actinin or the actin-severing protein severin, can be eliminated by mutation without severe effects on cell shape and motility (Wallraff *et al.* 1986). These proteins may serve as 'double insurance' components by supplementing other proteins of similar functions. For the experimental analysis of cell functions double insurance systems are a handicap, since two proteins have to be eliminated together before a functional defect is seen. It should be the aim to create 'minimal complexity' mutants that contain only the proteins absolutely necessary for functioning of the cells under standardized laboratory conditions.

CONSTITUENTS AND BIOSYNTHESIS OF THE CONTACT SITE A GLYCOPROTEIN

The csA glycoprotein with an apparent molecular weight of $80\times10^3 M_r$ is modified by phosphorylation at serine residues (Coffman *et al.* 1982; Schmidt & Loomis,

1982) and by fatty acid acylation (Stadler *et al.* 1984). Its carbohydrate moieties contain D-mannose, *N*-acetylglucosamine, L-fucose and probably D-glucose (Müller *et al.* 1979). Two types of carbohydrate residues can be distinguished. Type 2 carbohydrate reacts strongly with wheat germ agglutinin (Yoshida *et al.* 1984), type 1 carbohydrate with concanavalin A (H.-P. Hohmann, unpublished data). Furthermore, type 1 carbohydrate is distinguished from type 2 carbohydrate by its

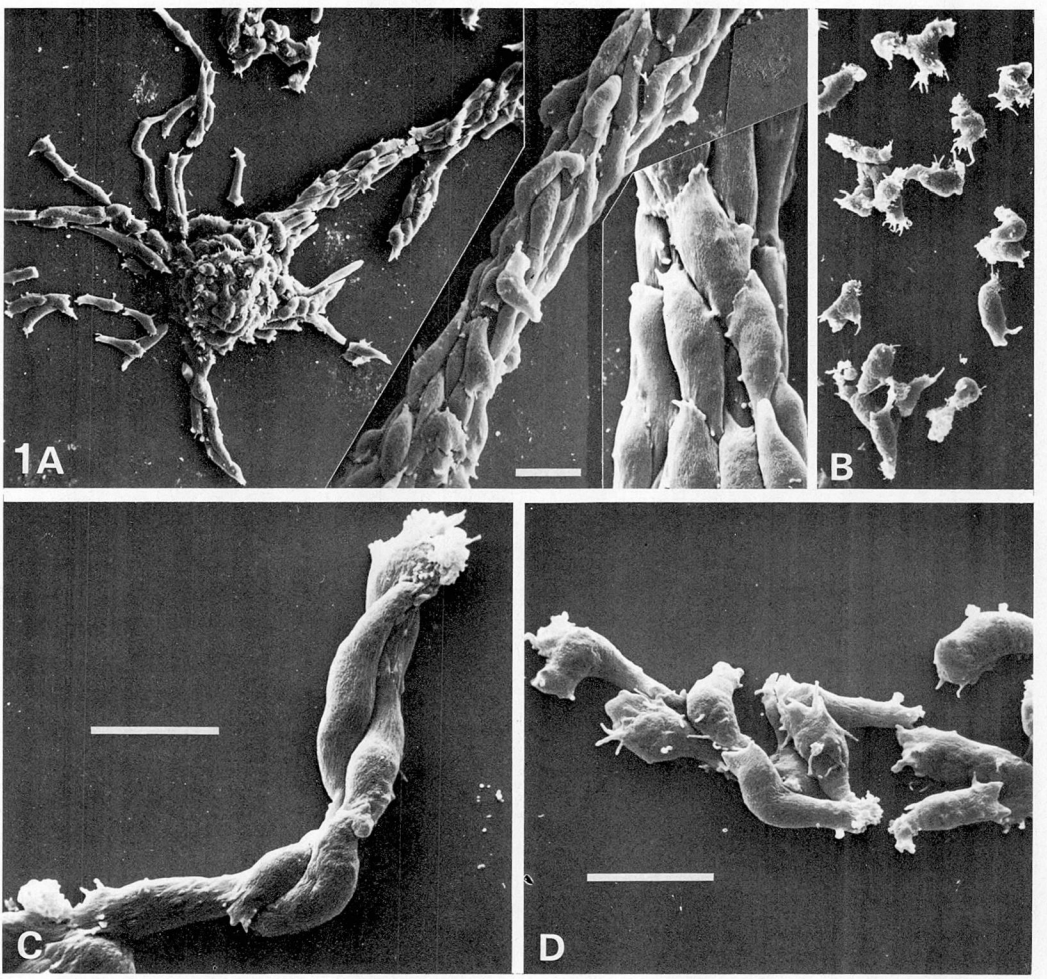

Fig. 1. A. Cells moving towards centres and forming streams during normal aggregation, shown at various powers of magnification. On the right, end-to-end adhesion and adhesion by lateral extensions is clearly seen. B. Cells of the aggregation stage dissociated by polyspecific Fab against total membranes of aggregation-competent cells. C. Cells forming end-to-end adhesions in the presence of Fab against membranes of growth phase cells. D. Cells forming irregular assemblies in the presence of Fab against total membranes of aggregation-competent cells, which has been absorbed with growth-phase cells. This Fab reacts primarily with the developmentally regulated csA glycoprotein (Gerisch *et al.* 1985*a*). Scanning EM photographs by R. Guggenheim, Basel; from Gerisch *et al.* (1980). Bars, 10 μm.

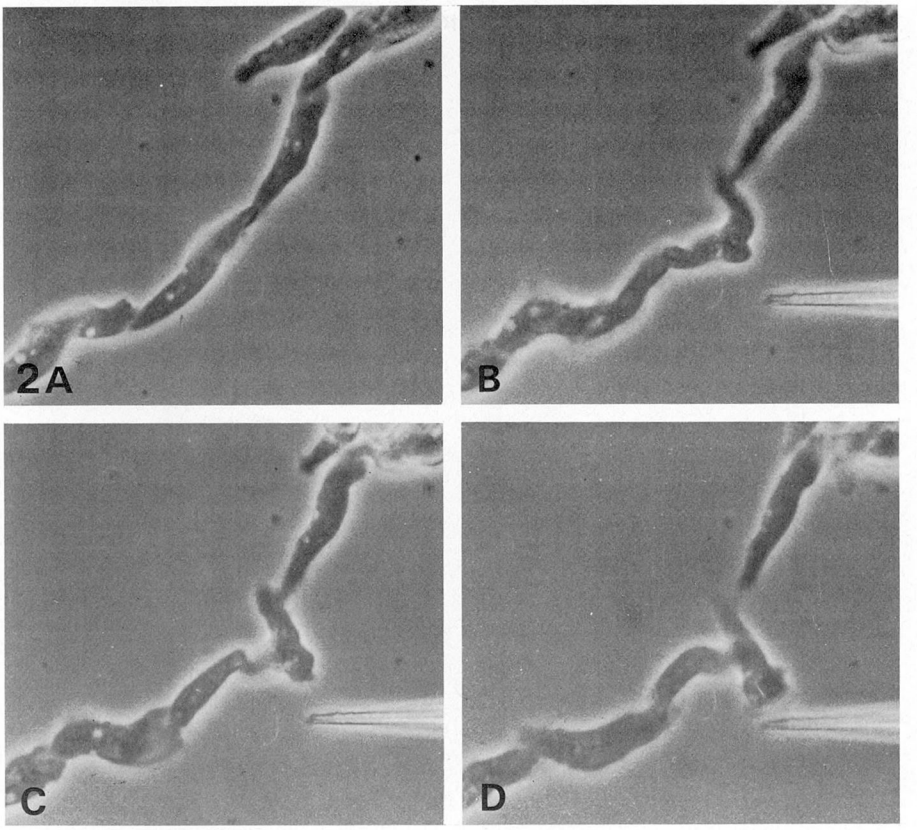

Fig. 2. End-to-end adhesion of aggregating *D. discoideum* cells (A) and subsequent stages of polarity change and disconnection of a cell exposed to a gradient of cyclic AMP (B–D). The chemoattractant has been applied through a micropipette filled with 1×10^{-4} M-cyclic-AMP, according to Gerisch *et al.* (1975*a*). Experiment performed together with D. Hülser, Stuttgart.

sulphation *in vivo* as well as in a cell-free system (Stadler *et al.* 1983; Hohmann *et al.* 1985). Type 2 carbohydrate is highly immunogenic in rabbits and mice. It is recognized by a series of monoclonal antibodies that cross-react to various extents with other glycoproteins. These different cross-reactivities mean that type 2 carbohydrate of the csA glycoprotein shares various epitopes with quite a number of other glycoproteins, whereas certain other epitopes of the type 2 oligosaccharide chains are more specific for the csA glycoprotein (Bertholdt *et al.* 1985).

In membrane fractions containing rough endoplasmic reticulum or Golgi membranes a glycoprotein that has an apparent molecular weight of $68\times10^3 M_r$ is enriched (Fig. 3). This glycoprotein is recognized by monoclonal antibodies against the polypeptide portion of the csA glycoprotein. It carries only type 1 carbohydrate and is degraded by glycosidases F or H into a $53\times10^3 M_r$ protein (Hohmann *et al.* 1985). The type 1 carbohydrate appears to be cotranslationally and *N*-glycosidically linked to the protein core. The $68\times10^3 M_r$ glycoprotein is a precursor that is converted

in the Golgi apparatus into the mature $80\times10^3 M_r$ glycoprotein by sulphation of its type 1 carbohydrate residues and by attachment of type 2 carbohydrate. Stability of its linkage to β-elimination suggests that type 2 carbohydrate is *N*-glycosidically linked, although the Golgi apparatus would be an unusual site for *N*-glycosylation (H.-P. Hohmann, unpublished data).

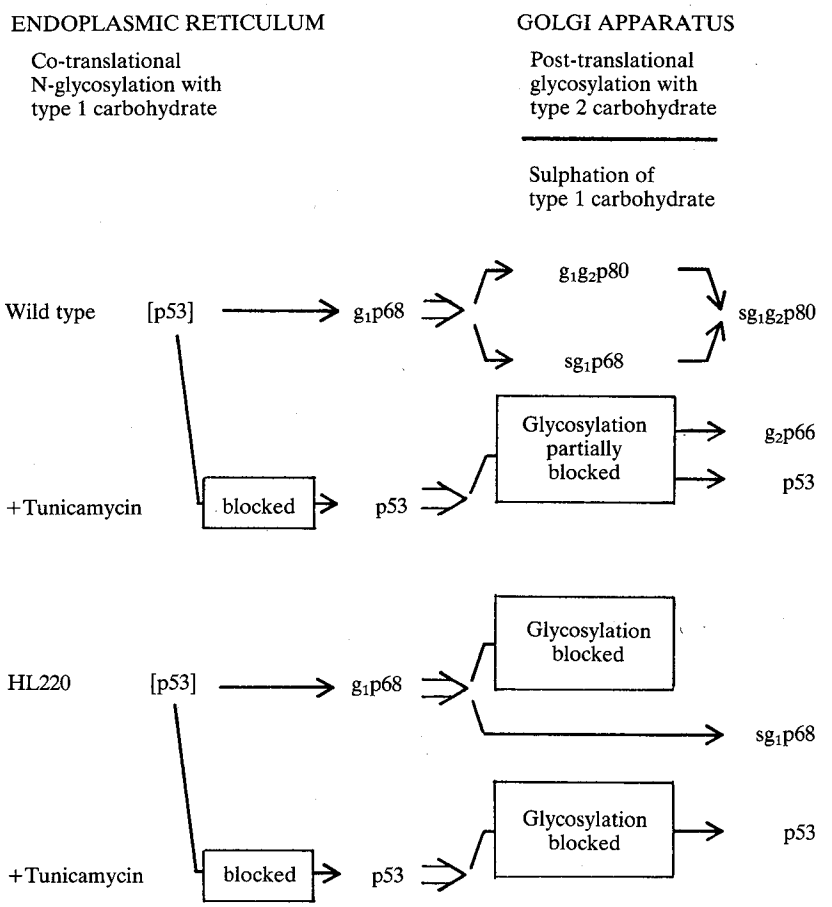

Fig. 3. Proposed steps of glycosylation and sulphation of the csA glycoprotein in wild-type and in mutant HL220 defective in type 2 glycosylation. The blocker of *N*-glycosylation, tunicamycin, inhibits preferentially type 1 glycosylation. Tunicamycin treated wild-type cells produce a $66\times10^3 M_r$ glycoprotein that carries only type 2 carbohydrate and a $53\times10^3 M_r$ protein that lacks both type 1 and type 2 carbohydrate. Tunicamycin-treated cells of the mutant produce only the $53\times10^3 M_r$ protein. The intermediates and the final product are designated by their apparent molecular masses ($\times10^{-3}$) with the prefix p for protein and by symbols for their modifications: g_1, glycosylated with type 1 carbohydrate; g_2, glycosylated with type 2 carbohydrate; s, sulphated (on type 1 carbohydrate). The mature contact site A glycoprotein carries all these modifications. The unmodified $53\times10^3 M_r$ protein of non-tunicamycin-treated cells is in brackets because it was not detectable by antibody labelling. Slightly modified from Hohmann *et al.* (1985).

Using monoclonal antibodies against type 2 carbohydrate as a probe, mutants defective in type 2 glycosylation have been selected (Murray *et al.* 1984; Loomis *et al.* 1985). In these mutants the $68\times10^3 M_r$ precursor is the end-product of csA biosynthesis (Yoshida *et al.* 1984; Gerisch *et al.* 1985*a*). These mutants produce relatively little of the $68\times10^3 M_r$ glycoprotein as compared to the production of the $80\times10^3 M_r$ glycoprotein in the wild type, and labelling of cells with antibodies shows that only a fraction of the $68\times10^3 M_r$ glycoprotein appears on the cell surface (H.-P. Hohmann & S. Bozzaro, unpublished data). These results suggest that type 2 carbohydrate is required for effective transport of the protein to the plasma membrane, and they suggest that $68\times10^3 M_r$ glycoprotein that has been led astray is degraded intracellularly (H.-P. Hohmann, S. Bozzaro & G. Gerisch, unpublished data). EDTA-stable cell adhesion has been reported to be detectable but weak under conditions in which some $68\times10^3 M_r$ glycoprotein is expressed in the mutants (Gerisch *et al.* 1985*a*), or to be lacking under conditions where the $68\times10^3 M_r$ glycoprotein remains undetectable (Loomis *et al.* 1985; West & Loomis, 1985). These results suggest that the protein equipped with type 1 carbohydrate can mediate EDTA-stable cell adhesion in the absence of type 2 carbohydrate, provided it is on the cell surface.

Similarities between the chemical nature of csA from *D. discoideum* and N-CAM from chicken embryos are obvious. Both are integral membrane proteins with negatively charged carbohydrate residues, and both proteins are further modified by acylation and phosphorylation. csA is the most prominently sulphated membrane protein of aggregating cells (Stadler *et al.* 1983). N-CAM is polysialylated (Rothbard *et al.* 1982) and also sulphated (Sorkin *et al.* 1984). The protein moieties of csA are phosphorylated at serine residues (Schmidt & Loomis, 1982), and those of N-CAM at serine and threonine residues (Sorkin *et al.* 1984). The csA glycoprotein is the most strongly labelled lipoprotein in membranes of developing cells that have been incubated with palmitic acid (Stadler *et al.* 1984), and also N-CAM incorporates palmitic acid (Edelman, 1985). Although none of these modifications is unique to cell adhesion molecules, their combination in two typical contact sites from unrelated organisms indicates their importance for the function of these molecules in cell adhesion or its regulation.

The precise functions of the various modifications of the csA glycoprotein remain to be elucidated. It is possible that the glycoprotein is designed not only for its interaction with other cells, but is also endowed with structures required for its transport to and its maintenance at the cell surface. Determining a clear distinction between these two functions should be the subject of future studies.

DEVELOPMENTAL REGULATION OF THE CONTACT SITE A GLYCOPROTEIN IN WILD-TYPE AND IN 'BYPASS' MUTANTS

In growth-phase cells the csA glycoprotein is not detectably expressed; it accumulates after 4–6 h of development. In some strains of *D. discoideum*, including the axenically growing laboratory strains AX2 and AX3, the expression of the

glycoprotein is regulated *via* cyclic AMP receptors on the cell surface. Periodic pulses of cyclic AMP are the optimal signals for stimulation of csA expression, apparently because the response to cyclic AMP undergoes adaptation. Pulsatile signals simulate the natural ones, since the cells themselves produce cyclic AMP rhythmically by periodic activation of adenylate cyclase (Roos *et al.* 1977). The pulsatile shape of the signals is maintained in the extracellular space by cell-surface and extracellular phosphodiesterases, which rapidly hydrolyse the cyclic AMP synthesized and released from the cells. The enhancement of csA expression and EDTA-stable cell adhesiveness is clearly seen when cyclic AMP is applied in pulses of 5–20 nM-amplitude every 6 min (Darmon *et al.* 1975; Gerisch *et al.* 1975*b*; Chisholm *et al.* 1984). In contrast to the stimulating effect of pulses, suppression of csA expression is observed when the same average amounts of cyclic AMP per time are applied in the form of a continuous flux to maintain non-fluctuating steady-state concentrations (Gerisch *et al.* 1984). A similar inhibition of csA expression is obtained in agar plate cultures to which a cyclic AMP analogue, 3′,5′-cyclic-adenosine phosphorothioate (cAMPS) is added (Rossier *et al.* 1978). This analogue acts as an agonist of the cyclic AMP receptors but is largely resistant to phosphodiesterases. The hydrolysis of cAMPS is slow enough to inhibit development completely in agar plate cultures for several days, while aggregation and fruiting body formation proceed in control cultures.

The inhibition of wild-type development by cAMPS can be used to select mutants that develop under conditions in which the normal pulsatile signals of cyclic AMP have been eliminated. Mutants that aggregate in the presence of cAMPS might not need cyclic AMP at all for development, or they might not need pulsatile signals. Mutants of the first class have been called 'bypass mutants' to indicate that the requirement for cyclic AMP as an inducer of development has been bypassed in these strains (Gerisch *et al.* 1985*b*). Among mutants of the second class non-adapting strains are expected that are stimulated by continuous signals in the same way as the wild type is stimulated by pulsatile signals.

HG302 is one of the strongly cAMPS-resistant mutants that form small but numerous aggregates and fruiting bodies in the presence of 10 μM-cAMPS (Wallraff *et al.* 1984). It is considered to be a bypass mutant and has been chosen for a detailed study and for further mutagenesis. The csA glycoprotein is still stringently regulated in HG302; it is undetectable in growth-phase cells and is expressed with the normal time course during development. This result indicates that cyclic AMP is not the only control factor in development; other control mechanisms exist that do not require cyclic AMP signals for their function. In wild-type cells, these mechanisms are obscured by the cyclic AMP signal system, which dominates the control of development in these cells.

In an attempt to isolate a double bypass mutant, HG302 has been mutagenized and progeny clones have been assayed for aggregation in the presence of nutrient medium. Neither wild-type nor HG302 cells will develop and aggregate when they reach the stationary phase as long as the nutrient medium is not replaced by a non-nutrient buffer. One mutant, HG592, has been selected from HG302 that aggregates

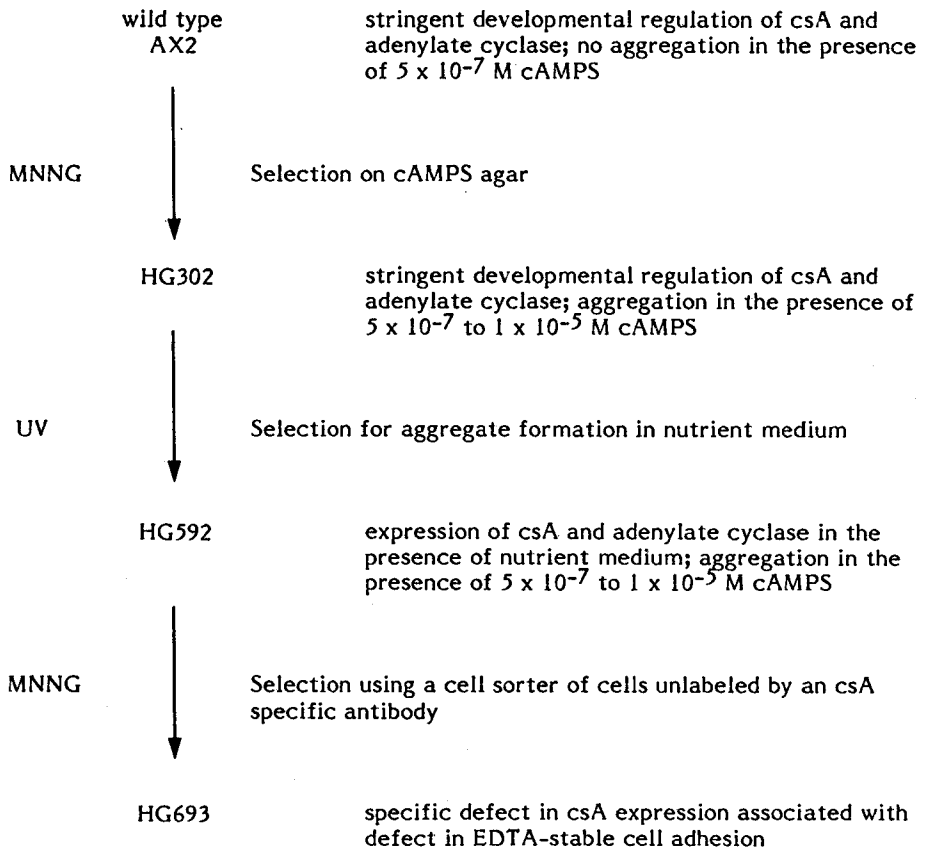

Fig. 4. Scheme of three steps of mutagenesis after which HG693, a mutant specifically defective in csA expression, has been selected. The cells have been mutagenized either with ultraviolet light (UV) or with 1-methyl-3-nitro-1-nitrosoguanidine (MNNG).

in the presence of nutrient medium (Gerisch *et al.* 1985*b*). In HG592 a second control mechanism of development appears to be bypassed in addition to the control by cyclic AMP. Simultaneously, a third control mechanism has been unmasked. This mechanism is responsible for the suppression of the csA glycoprotein during growth of HG592. This mutant expresses the csA glycoprotein when it enters the stationary phase, independent of whether or not nutrient medium is removed (Fig. 4).

Bypass mutants are of interest for two reasons. One is that these mutants permit a step-by-step analysis of the control mechanisms of development. The other reason is that bypass mutants provide a source for the selection of mutants specifically defective in certain developmentally regulated proteins. If wild type is used as a source, most developmental mutants are defective in regulatory genes. These mutants are usually pleiotropic, and the few mutants specifically defective in a single developmentally regulated protein are hard to separate from the pleiotropic ones. Selection of mutants specifically defective in a single protein is less tedious when

bypass mutants are used as a source, since defects in regulatory genes that have been bypassed will no longer result in developmental arrest. Consequently, among the mutants that do not express a certain protein, a higher proportion will be specifically defective in that particular protein.

In *D. discoideum* several membrane proteins of aggregating cells are under developmental control: cell-surface cyclic AMP receptors and phosphodiesterase, adenylate cyclase, and the csA glycoprotein. Bypass mutants may be applicable to the selection of structural gene mutants in all these proteins. In the following, the csA glycoprotein is taken as an example to test the usefulness of bypass mutants for mutant selection.

A MUTANT SPECIFICALLY DEFECTIVE IN CONTACT SITE A EXPRESSION

Since the csA glycoprotein is exposed on the cell surface and antibodies are available for fluorescence-activated cell sorting, it is no problem to enrich for mutants defective in the expression of this glycoprotein (Fig. 4). The double bypass mutant HG592 has been mutagenized and a highly specific monoclonal antibody has been used for the selection. This antibody recognizes a portion of the polypeptide chain that is exposed on the cell surface.

Out of more than 50 mutants that do not express the csA glycoprotein one mutant, HG693, has been found that appears to have specifically lost the capability of synthesizing the protein portion of the csA glycoprotein (Noegel *et al.* 1985). The mutant produces type 2 carbohydrate and modifies proteins other than the csA glycoprotein with this oligosaccharide. HG693 is also capable of sulphating carbohydrate residues since glycoproteins other than csA are sulphated in the mutant.

Evidence that HG693 specifically lacks csA has been provided by comparing developmentally regulated transcripts in wild-type and mutant cells by Northern blot analysis. Of the eight cDNA probes used in this study seven have been obtained from poly(A)$^+$ RNA that had been isolated from membrane-bound polysomes. All these seven cDNA probes recognize RNA species that are not or only weakly expressed during the growth phase of AX2 wild-type cells. Their expression increases strongly during the first 6 h of development and is enhanced to various degrees by stimulating wild-type AX2 cells with pulses of cyclic AMP. In mutant HG693 all seven RNA species are expressed, indicating that the mutant does not suffer from a general block in development.

The eighth DNA probe used has been obtained by screening a cDNA expression library with a monoclonal antibody that specifically recognizes the polypeptide moiety of the csA glycoprotein. This cDNA probe recognizes a $1{\cdot}9\times10^3$ base-pair transcript in the wild type, expression of which is strongly developmentally regulated and enhanced by cyclic AMP pulses. This transcript is the mRNA for the polypeptide moiety of the csA glycoprotein. In mutant HG693 it is expressed in only trace amounts (Noegel *et al.* 1985). The selective suppression of this transcript suggests that a regulatory region of the csA protein gene is changed in HG693.

Because of its specific defect in csA expression, mutant HG693 has been used to study the function of the csA glycoprotein. Cells of the mutant form almost no

EDTA-stable contacts after 8 h of starvation, the time at which EDTA-stable contact formation reaches its maximum in wild-type AX2 cells. EDTA-sensitive contacts, which are not developmentally regulated, are still observed in the mutant. These results are in accord with the blockage of EDTA-stable cell adhesion by Fab against the csA glycoprotein. In agar plate cultures cells of the mutant still aggregate and form fruiting bodies. The latter result indicates that the csA glycoprotein is not essential at the postaggregative stage of development, which is consistent with observations indicating down-regulation of the csA glycoprotein in the slug stage.

The aggregation of HG693 cells on agar plates shows that in the absence of shear forces the cells can accumulate into aggregates without requiring the csA glycoprotein for the strengthening of intercellular adhesion. It is conceivable that a double insurance system is implemented in the adhesion system of aggregating cells, similar to that discussed earlier for the cytoskeleton. In this connection a $130\times10^3 M_r$ protein is of interest; this is developmentally regulated in a similar way to the csA glycoprotein. The carbohydrate residues of the $130\times10^3 M_r$ glycoprotein resemble type 1 carbohydrate in being strongly sulphated (Stadler *et al.* 1983), but its polypeptide moiety is not recognized by antibodies against the polypeptide portion of the csA glycoprotein. Mutant HG693 may be used to find out whether or not the $130\times10^3 M_r$ glycoprotein can assist and partially replace the csA glycoprotein in mediating intercellular adhesion. The mutant will also help to reveal whether the csA glycoprotein is responsible for the species specificity of cell adhesion, as is observed when aggregating *D. discoideum* cells are mixed with those of a related species, *P. pallidum*.

CARBOHYDRATE MODIFICATION AND CELL AGGREGATION IN *P. PALLIDUM*

P. pallidum forms aggregates similar to those of *D. discoideum*. Sorting out between *D. discoideum* and *P. pallidum* cells is partly due to differences in the chemotactic systems; the attractant of *Polysphondylium* is a peptide rather than cyclic AMP (Shimomura *et al.* 1982). Involvement of cell adhesion in sorting out becomes evident when immobile cells of *D. discoideum* and *P. pallidum* are mixed (Gerisch *et al.* 1980). These cells form separate clusters in gently shaken suspensions, which indicates that the adhesion systems of the two species discriminate between self and non-self. This sorting out has been observed only with aggregation-competent cells of the two species (Bozzaro & Gerisch, 1978). Mixed growth-phase cells adhere indiscriminately to each other; they do not sort out before they reach the stage of aggregation competence. In accord with the proposed involvement of the csA glycoprotein in species-specific cell recognition, Fab specific for aggregation-competent cells of *D. discoideum* blocks the sorting out of the two species (Gerisch *et al.* 1980). This Fab does not block the non-species-specific type of cell adhesion of growth-phase cells.

Although the approach to identifying cell adhesion factors in *P. pallidum* has been similar to the method that has led to the isolation of the csA glycoprotein in *D. discoideum*, the outcome has not been the same. One reason is the strong

immunodominance of certain carbohydrate structures of *P. pallidum* glycoproteins. First, a $64\times10^3 M_r$ glycoprotein, designated contact site 1 (cs1), has been purified (Bozzaro *et al.* 1981). This single glycoprotein neutralizes most of the adhesion blocking activity of polyclonal Fab from rabbit antisera raised against aggregation-competent cells of *P. pallidum*. However, the adhesion blocking Fab that is neutralized is primarily directed against carbohydrate residues and cs1 is particularly abundant, though not the only protein that carries these carbohydrate chains. Polyclonal antisera and monoclonal antibodies raised against the purified cs1 glycoprotein cross-react with the carbohydrate residues of many other proteins, and anti-carbohydrate Fab prepared from the antisera blocks cell adhesion completely (Toda *et al.* 1984*a*). These results resemble those obtained by Springer & Barondes (1985) with *D. discoideum*. These authors have obtained adhesion blocking antibodies that are neutralized by a series of proteins of different molecular weight. As in the case of *P. pallidum*, the blocking antibodies are apparently directed against carbohydrate residues common to different glycoproteins.

If the target sites of adhesion blocking Fab are carbohydrate epitopes common to several glycoproteins, the Fab neutralization assay is obviously not the method of choice for attributing a function in cell adhesion to a specific protein. There might be a specific glycoprotein involved; however, it cannot be distinguished from other ones that neutralize the same adhesion blocking Fab, independent of whether or not they are involved in adhesion. Alternatively, the carbohydrate residues may play a role in cell adhesion independent of the particular protein to which they are linked. This latter possibility has been followed up in *P. pallidum*.

Fab of one monoclonal antibody, mAb293, has been found to block cell adhesion in growth-phase cells as well as in aggregation-competent cells of *P. pallidum* (Toda *et al.* 1984*a*). This antibody reacts with the non-reducing ends of oligosaccharide chains that contain L-fucose as the terminal sugar. Binding of the antibody to the cs1 glycoprotein is blocked by free L-fucose (Toda *et al.* 1984*b*). The epitope recognized by mAb293, termed ep293, is already detectable in exponentially growing cells. Nevertheless, it is not constitutively expressed. During the early exponential growth phase the cells lack ep293, and they acquire it one or two generations before the end of exponential growth (Toda *et al.* unpublished data). Aggregation competence is acquired much later, about 7 h after the beginning of starvation. Although ep293 expression is not immediately followed by cell aggregation, its presence on the cell surface might be a prerequisite for aggregation. Results obtained with mutants are in accord with this possibility, but they do not provide final proof.

Mutants defective in ep293 expression have been obtained by selection with a cell sorter (Francis *et al.* 1985). All of the mutants obtained show disturbed aggregation: most of them form rudimentary aggregates and aberrant fruiting bodies, some are almost non-aggregative. Recombination analysis has proved that the defects in ep293 expression and in morphogenesis are linked to each other, indicating that they are due to the same genetic defect. In most of the mutants, ep293 is not expressed at the normal time but after an extended period of starvation. Simultaneously with ep293 expression the mutant cells begin to aggregate. In a few mutant strains ep293 is

expressed only in trace amounts after prolonged starvation and aggregation is almost completely blocked. This coincidence of defects in ep293 expression and in cell aggregation could mean that ep293 is required for cell aggregation, but the possibility remains that ep293 expression and cell aggregation are caused by a common regulatory mechanism by which other cell surface changes are also controlled.

Glycoproteins that are later recognized by mAb293 are present in the cells before. A monoclonal antibody against the polypeptide moiety of the cs1 glycoprotein has been obtained by immunizing mice with antigen that had been treated with anhydrous HF (Mort & Lamport, 1977). This treatment removes the highly immunogenic carbohydrate residues and thus increases the probability of isolating hybridomas that produce antibodies against the polypeptide moiety of the glycoprotein. The anti-polypeptide antibody identifies the protein before ep293 is expressed, during early exponential growth in wild-type cells as well as in starving mutant cells (K. Toda, D. Francis & G. Gerisch, unpublished data). These and other results indicate that it is not the synthesis of the protein but the modification of its carbohydrate residues that is developmentally regulated and is retarded or suppressed in the mutants.

The modification of carbohydrate residues during development requires special attention as a means of modulating cell surfaces without changing the protein cores to which the carbohydrates are linked. Partial desialylation has been observed when the E-CAM of chicken embryos is changed into the A-CAM of adults (Rothbard *et al.* 1982). In *D. discoideum* binding sites for wheat germ agglutinin increase strongly during the later stages of development (Burridge & Jordan, 1979; West & McMahon, 1979). At least some of the newly appearing binding sites seem to be generated by modification of pre-existing glycoproteins (M. Yoshida & G. Gerisch, unpublished data). An increase in the proportion of fucosylated oligosaccharides in the later stages of *D. discoideum* development (Ivatt *et al.* 1984) may also be due to carbohydrate modification rather than to the induction of new specific proteins endowed with a unique carbohydrate structure.

CELL ADHESION AND THE REGULATION OF DEVELOPMENT IN THE *D. DISCOIDEUM* SLUG

In nuce, development of the multicellular *Dictyostelium* slug shows all facets of temporal and spatial cell differentiation and its regulation, of differential cell adhesiveness, and morphogenetic cell movements, as they are observed during embryogenesis of higher animals. Not only are the contact sites of aggregating cells subject to developmental regulation, as shown in previous sections, but cell adhesion is in turn a factor that controls development in the slug stage. In this stage differentiation of the cells into prespore and prestalk cells becomes manifest. Differentiation into these two cell types is embedded in a supercellular control system, which determines the correct position (prestalk cells in front and prespore cells behind), the precise proportioning of the two cell types, and also guarantees reversibility of the prespore/prestalk differentiation. Reversibility is a prerequisite

of the regulation observed in front and hind pieces of a slug, which, after their separation, readjust the proportions of prespore and prestalk cells (Raper, 1940).

In principle, there are two ways of investigating a system of that complexity. One is to take the system as a whole, to study its behaviour under various conditions, to test its regulatory responses after disturbing its homoeostasis, and to build mathematical models that reproduce the behaviour of the natural system (Meinhardt, 1983). Another way is to simplify the system step-by-step by replacing unknown factors with known experimentally controlled ones, and thus to sort out from the complex system single reactions that can be rigorously analysed down to the molecular level. To arrive at a complete picture of development in a multicellular system such as the *Dictyostelium* slug, a combination of both approaches seems to be indispensable.

In the following sections, recent results are compiled that focus on one facet or the other of the complex cell interactions going on in the slug, and provide starting points for future investigations.

Gene regulation during cell differentiation

As probes for cell-type-specific messages, cDNA clones have been isolated (Barklis & Lodish, 1983; Mehdy *et al.* 1983); and as probes for cell-type-specific proteins, polyclonal and monoclonal antibodies have been raised (Tasaka *et al.* 1983; Krefft *et al.* 1984; Wallace *et al.* 1984). These probes are being used to study gene regulation during and preceding differentiation into the final cell types, spores and stalk cells, and their precursors. One of the major purposes of these studies is to unravel the regulatory DNA sequences and to identify DNA binding proteins responsible for the concerted activation of sets of genes coding for cell-type-specific products. This work will take advantage of the techniques available for transformation of *D. discoideum* cells (Nellen *et al.* 1984) and for genetic engineering.

A group of genes that are activated after slug formation code for a specific class of prespore-specific transcripts, called prespore class II mRNAs (Chisholm *et al.* 1984). These genes are of interest because they are activated in cells kept in suspension cultures by a high concentration of cyclic AMP in combination with tight cell-to-cell adhesion. Several pieces of evidence indicate that cell adhesion in the slug is mediated by cell-surface molecules other than the csA glycoprotein, and that the cell adhesion molecules relevant for prespore differentiation are characteristic of the slug stage (Steinemann & Parish, 1980; Wilcox & Sussman, 1981; Siu *et al.* 1983; Kumagai & Okamoto, 1985).

Replacement or bypassing of cell-to-cell adhesion

The necessity of cell-to-cell adhesion for the activation of cell-type-specific genes limits experimental work to cell aggregates and thus restricts the accessibility of cells to external factors. These limitations have been overcome in different ways. One is simply to replace cell-to-cell contact by the adhesion of single cells to a plastic surface (Mehdy & Firtel, 1985). Expression of the class of prespore-specific mRNAs, which normally requires tight cell-to-cell contact, has been obtained in the presence of both

cyclic AMP and a conditioning factor, by plating cells into dishes where they adhere to the bottom. Another method has been used to study differentiation of single cells into spores. To this end mutants have been selected in which cell adhesion as a requirement for sporulation has been bypassed (Kay, 1982). In these sporogenous mutants cyclic AMP acts as non-pathway-specific inducer of differentiation into spores and stalk cells. The important point in this approach is the possibility of manipulating the fate of the cells. The decision whether a cell differentiates into a spore or a stalk cell is determined by a low molecular weight factor produced during the later stages of development. This differentiation-inducing factor (DIF) drives differentiation into the stalk-cell pathway (Kopachik *et al.* 1983). The direction of differentiation is also varied by the extracellular pH (Gross *et al.* 1983), indicating that by this method a variety of factors influencing cell differentiation can be investigated.

Starting cell differentiation from uniform cells

It is one of the peculiarities of *Dictyostelium* that multicellular development starts from an apparently homogeneous population of single cells. However, evidence has been provided that cells differ from each other already before they begin to aggregate, and that they sort out in the slug according to their pre-established differences. It is intriguing that an intrinsic variable is crucial for the fate of the cells. This variable is the cell cycle phase in a non-synchronized cell population (Weijer *et al.* 1984). Cells that are in early G_2 phase at the beginning of starvation tend to become prestalk cells, and cells in late G_2 phase prespore cells. In order to study other factors determining the fate of cells it will be important to start the experiments with synchronized cells. It is not known whether cells from different cell-cycle phases will differ in their adhesiveness, which could be a basis for their sorting-out behaviour.

Control of gene expression by attachment of cells to chemically defined surfaces

A step towards the definition of cell surface components involved in the control of cell differentiation is the replacement of cells by surfaces of known chemical composition as partners of contact interactions. A promising pilot experiment has been performed by attaching cells to polyacrylamide gel surfaces derivatized with specific sugars (Bozzaro *et al.* 1984). The cells interact with the immobilized sugars *via* carbohydrate binding sites on their surfaces. Cell attachment to glucoside-derivatized gels, on which the cells can move and aggregate, results in the blockage of development at a stage following aggregation. The binding to the glucoside-derivatized gel interferes with the expression of genes that are normally turned on when the slug is formed.

Potential use of antibodies to simulate morphogenetic signals

Tools of great potential value in the analysis of cell differentiation and its control by intercellular signals might be monoclonal antibodies against cell surface components that act as receptors for developmental signals. The value of antibodies that

bind to specific epitopes of receptors resides not only in their potential activity in blocking the response of a cell to a developmental signal but, more importantly, in their potential capacity to replace the natural signal in activating the receptors. It would be a great help in investigating the molecular mechanisms underlying signal processing from the cell surface to intracellular targets responsible for the activation of specific genes if signals that are normally mediated by intimate cell-to-cell contact could be replaced by an antibody. An observation indicating that cell differentiation in *D. discoideum* can be turned on by antibodies is the production of stalk cells when cells are incubated with a polyspecific Fab preparation that inhibits aggregation (Gerisch, 1980). It might be useful to immobilize the antibodies on a solid surface by a technique similar to the one described in the preceding paragraph, in order to simulate more closely the contact of a cell with the membrane of another cell.

It is to be hoped that in continuing work along the lines summarized above, the approaches originating from different points will merge to provide us with a comprehensive view of cell interactions underlying development in *Dictyostelium*, the simple model of more complicated developmental systems.

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