Regulation of slug size by the cell adhesion molecule gp80 in *Dictyostelium discoideum*

Rajender K. Kamboj*, Tak Yee Lam*,
and Chi-Hung Siu*†‡

*Banting and Best Department
of Medical Research

†Department of Biochemistry
University of Toronto

Toronto, Ontario, Canada M5G 1L6

We previously provided in vitro evidence that the cell surface glycoprotein of *M*, 80 000 (gp80) of *Dictyostelium discoideum* is capable of mediating EDTA-resistant cell-cell binding. Expression of gp80 is specific for the aggregation stage when cells form tight aggregates. To investigate the physiological role of gp80, *Dictyostelium* cells were transformed with a vector containing gp80 cDNA fused to an actin promoter. gp80 transcripts were detected in transformed cells in their vegetative growth phase. Transformants at this stage also exhibited EDTA-resistant cell cohesion, thus providing direct in vivo evidence that gp80 mediates cell-cell binding via homophilic interaction. While aggregates of the parental strain KAX3 had the tendency to break up to form small slugs, transformants expressing an increased amount of gp80 were able to maintain the integrity of aggregates, giving rise to larger slugs, resulting in the formation of bigger fruiting bodies. To further demonstrate that the increase in slug size could be correlated with the expression of gp80, cells of the parental strain were treated with exogenous cAMP pulses to stimulate an over-expression of gp80. The treated cells also gave rise to larger slugs, consistent with the notion that slug size is influenced by intercellular adhesiveness during development.

Introduction

Developmental processes in eukaryotes are often characterized by a complex series of cellular interactions, and it has become evident that cell-cell adhesion and cell-substratum adhesion play an important role in morphogenesis (for reviews, see Edelman, 1986; McClay and Etensohn,

1987). The cellular slime mold *Dictyostelium discoideum* offers a favorable experimental system to examine the relationship of cell-cell adhesion to morphogenesis. It has a simple and well-defined life cycle, which includes a developmental phase initiated upon the depletion of food source (Loomis, 1975). Cells undergo chemotactic migration in response to extracellular cAMP. Aggregates of cells are formed and develop into pseudoplasmodia or slugs, which after a period of migration will eventually give rise to mature fruiting bodies.

Multicellularity in *Dictyostelium* is maintained by the expression of at least two types of cell-cell adhesion sites on the cell surface (for reviews, see Gerisch, 1980; Siu *et al.*, 1988b). The EDTA-sensitive cell-cell adhesion sites are easily disrupted in the presence of 1 or 2 mM of EDTA, while the EDTA-resistant sites are stable in concentrations of EDTA up to 15 mM. The EDTA-sensitive sites appear soon after the initiation of development (Garrod, 1972), and the expression of EDTA-resistant sites corresponds to the onset of cell aggregation at ~6 h of development (Beug *et al.*, 1973). Serological evidence for these two types of cell-cell adhesion sites has been provided by the pioneering work of Gerisch and coworkers (Gerisch, 1980). A cell surface glycoprotein of *M*, 80 000 (gp80) has been found to neutralize the inhibitory activity of a polyspecific antibody preparation on cell aggregation (Muller and Gerisch, 1978). We have subsequently raised a monoclonal antibody (80L5C4), which is monospecific for gp80 and capable of blocking the EDTA-resistant cell-cell binding sites (Siu *et al.*, 1985; Kamboj and Siu, 1988). To provide direct evidence that gp80 is a cell adhesion molecule, gp80 has been purified to homogeneity and shown to have cell-binding activity in several in vitro assays (Siu *et al.*, 1987). Solubilized gp80 binds to cells in a dose-dependent and saturable manner. In addition, Covaspheres conjugated with gp80 bind to cells in a stage-specific manner and they can undergo selfaggregation, suggesting that gp80 may mediate cell-cell adhesion via homophilic interaction.

‡ Corresponding author.

Cells at the aggregation stage show rapid turnover and reorganization of the plasma membrane (De Silva and Siu, 1980, 1981). The cells become more elongated and the surface is covered with a large number of filopodia (De Chastellier and Ryter, 1980; Choi and Siu, 1987). Surface immunofluorescence labeling studies indicate that gp80 is preferentially associated with the polar ends of the cell, consistent with the notion that gp80 mediates end-to-end cell-cell adhesion (Beug *et al.*, 1973). At the electron microscopy level, gp80 has been shown to be highly enriched on filopodial structures (Choi and Siu, 1987). Since filopodial structures often appear to be in contact with similar structures of the cell surface of adjacent cells, it has been proposed that they are directly involved in the initial recognition process among cells and that gp80 plays an important role in the formation of stable contacts.

Recently, cDNAs for gp80 have been cloned and sequenced (Noegel *et al.*, 1986; Wong and Siu, 1986; Kamboj *et al.*, 1988). The structure of gp80 predicted from the deduced amino acid sequence consists of three major domains (Kamboj *et al.*, 1988; Siu and Kamboj, 1990). The amino-terminal domain contains three tandem homologous regions which are probably organized into three globular structures. This bulky domain is supported by a short-stalk region that contain sequences homologous to the hinge region of human IgA. This is followed by a membrane-anchor domain. gp80 does not have a cytoplasmic domain and its membrane anchor may be replaced by a lipid glycan moiety (Stadler *et al.*, 1989). The primary structure of gp80 also shows limited sequence homology with the neural cell adhesion molecule (Kamboj and Siu, 1990), suggesting that they may be distantly related and belong to the same superfamily of recognition molecules (Cunningham *et al.*, 1987; Williams and Barclay, 1988).

The availability of gp80 cDNA clones also permits us to examine the effects of expressing gp80 at a stage when gp80 is normally not expressed and the changes resulted from over-expressing gp80 during development. In this report, vectors containing gp80 cDNA under the control of the actin 6 promoter were introduced into *Dictyostelium* cells. EDTA-resistant cell-cell adhesion was observed at the vegetative growth stage of transformed cells, which also showed distinct phenotypic changes during morphogenesis. Both slugs and fruiting bodies were much larger than the parental cells or the control transformant, suggesting a direct correlation between slug size and cell-cell adhesion.

Results

Transformation of gp80 constructs into Dictyostelium cells

Two gp80 cDNA fragments were used to construct transformation vectors that would permit the expression of gp80 in the vegetative growth phase. They were cloned into the unique *EcoRI* site of the transformation vector pDNeoll (Witke *et al.*, 1987), which contains the neomycin-resistant gene driven by the actin 15 promoter and thus confers resistance to G418 in transformed cells. The expression of gp80 cDNA was under the control of the actin 6 promoter. Construct A contained a gp80 cDNA with 23 bp in the 5' nontranslated region, whereas construct B contained a gp80 cDNA with 100 bp in the same region. These two vectors were transfected into KAX3 cells using the calcium phosphate coprecipitation method (Nellen *et al.*, 1984), and cells resistant to G418 were selected. Single colonies were picked and further subcloned. DNA was isolated from the stabilized single-colony isolates and digested with *EcoRI*. DNA blots were hybridized with a gp80 cDNA probe. Quantitative analysis showed that clones derived from both constructs contained multiple copies of gp80 cDNA, averaging 20 to 40 copies per cell (data not shown). Since preliminary characterizations showed that clones derived from both constructs were fairly similar, only two transformants A-2 and B-4 were subjected to detailed analysis, A-2 being transfected with construct A and B-4 with construct B. As a control, cells were transformed with the vector pDNeoll without gp80 cDNA insert and a clone V-1 was stabilized for further characterization.

Expression of gp80 in transformants

The expression of gp80 is under stringent developmental regulation. Wild-type cells do not express gp80 mRNA during vegetative growth. Cells begin to express a relatively low level of gp80 mRNA at ~3 h of development, and the rate of gp80 transcription is greatly enhanced by low concentrations of exogenous cAMP (Siu *et al.*, 1988b; Mann and Firtel, 1989; Ma and Siu, 1990). To determine the levels of gp80 mRNA in transformed cells, RNA was extracted from transformants at the vegetative (0 h) stage and at 10 h of development, size fractionated on agarose gel, and blotted on nitrocellulose filter. RNA blots were probed with a ³²P-labeled gp80 cDNA fragment. A substantial level of gp80 mRNA was present in transformants at

the vegetative stage (Figure 1A). In contrast, gp80 mRNA was not detectable in KAX3 and V-1 cells during vegetative growth. Comparable levels of gp80 mRNA were expressed by both transformants A-2 and B-4. Estimations based on densitometric tracings showed that the level of mRNA at 0 h was ~30–35% of that observed at 10 h in the same transformant.

The expression of gp80 was also examined at the protein level by immunoblot analysis (Figure 1B). Protein blots were stained indirectly using the anti-gp80 monoclonal antibody 80L5C4. Both transformants A-2 and B-4 contained significant amounts of gp80 molecules during vegetative growth. The levels of gp80 were determined by densitometry and they corresponded closely to the relative levels of gp80 mRNA in these cells. At 10 h, levels of gp80 in A-2 and B-4 cells were ~30–40% higher than that in control cells.

To demonstrate that these gp80 molecules were expressed on the cell surface, live cells were labeled with the anti-gp80 monoclonal antibody 80L5C4 and a FITC-conjugated second antibody (Figure 2). Although the signal was relatively weak for the transformants at the vegetative stage, >75% of the cells showed fluorescent labeling on the cell surface. The control cells did not have any surface labeling at 0 h, but prominent surface fluorescence was observed at 10 h.

Acquisition of EDTA-resistant cell-cell adhesion in transformants during vegetative growth

To determine if the gp80 molecules expressed in transfected cells during vegetative growth were able to mediate EDTA-resistant cell-cell adhesion, cell reassociation assays were carried out in the presence of 5 mM EDTA (Figure 3). KAX3 cells and V-1 cells did not exhibit any appreciable amount of EDTA-resistant intercellular adhesion at 0 h. On the other hand, 30–35% of the cells of A-2 and B-4 were able to reform aggregates in the presence of 5 mM EDTA. Aggregates of 5–10 cells occurred frequently in these samples. At 10 h of development, the percentages of EDTA-resistant cell aggregation of transformants A-2 and B-4 were also significantly higher than those of the control samples (Figure 3). This might be a reflection of the higher amount of gp80 molecules expressed in the transformants.

Homophilic binding of 125 I-labeled gp80 to vegetative cells of transformants

The above results suggest that gp80 is the sole factor responsible for the appearance of EDTA-

Figure 1. Expression of gp80 in transformants. Transformants A-2 and B-4 were cultured axenically in liquid medium and were collected at their exponential growth phase. Development was carried out in 17 mM phosphate buffer, pH 6.4, and rotated on a platform shaker at 180 rpm at room temperature. Vegetative (0 h) cells and cells at 10 h of development were collected for RNA extraction and protein analysis. (A) RNA blots were hybridized with a 32 P-labeled gp80 cDNA probe (Wong and Siu, 1986) and (B) protein blots were immunostained with the anti-gp80 monoclonal antibody 80L5C4 (Siu *et al.*, 1985). Lanes a and b, 0 h and 10 h cells of strain KAX3; lanes c and d, 0 h and 10 h cells of transformant V-1; lanes e and f, 0 h and 10 h cells of transformant A-4; and lanes g and h, 0 h and 10 h cells of transformant B-4.

resistant cell-cell binding sites during vegetative growth. To demonstrate that gp80 is mediating cell-cell adhesion via homophilic binding, it was necessary to rule out the presence of any distinct gp80 receptor that might have been induced by the transformation process. The binding of 125 I-labeled gp80 to intact vegetative cells was carried out to address this issue (Table 1). Both A-2 and B-4 cells showed an ~3-fold higher binding than V-1 cells. The amount of labeled gp80 bound to V-1 cells was not significantly lowered in the presence of a 30-fold excess of unlabeled gp80, indicating nonspecific binding. After background subtraction, the

Figure 2. Phase contrast and epifluorescence micrographs of KAX3 cells and transformants. Cells were stained with the anti-gp80 monoclonal antibody 80L5C4 and FITC-conjugated goat anti-mouse-IgG second antibody. (a and b) KAX3 cells at 0 h of development; (c and d) transformant A-2 at 0 h; (e and f) transformant B-4 at 0 h; and (g and h) KAX3 cells at 10 h of development. a, c, e, and g are phase contrast micrographs; b, d, f, and h are epifluorescence micrographs. Bar = 20 μ m.

Figure 3. EDTA-resistant cell-cell adhesion expressed in transformants. The gp80 transformants (A-2 and B-4) and the control transformant (V-1) were cultured axenically in liquid medium and developed in 17 mM phosphate buffer, pH 6.4. Cells were collected at 0 h and 10 h of development and then suspended at 2.5×10^6 cells/ml in 17 mM phosphate buffer, pH 6.4, and 5 mM EDTA. Cells were dissociated mechanically and allowed to reassociate on a platform shaker rotating at 120 rpm. Cell aggregation was monitored at 15-min intervals. Values in the figure were taken from the 45-min point and represented the average of two determinations. (Hatched bar) 0 h cells; (stippled bar) 10 h cells.

amounts of gp80 bound to A-2 and B-4 cells were estimated to be 2.2×10^4 molecules/cell and 2.6×10^4 molecules/cell, respectively. The binding of gp80 was inhibited by 70–80% when cells were precoated with the anti-gp80 mono-

clonal antibody 80L5C4. These results indicate that the ^{125}I -labeled gp80 interacted homophilically with the cell surface gp80 of transformants and they are consistent with our previous observation that vegetative cells do not express a gp80 receptor (Siu *et al.*, 1987).

Phenotypic changes in transformants during development

In addition to the expression of a significant amount of gp80 in vegetative cells, gp80 transformants accumulated a higher level of gp80 during development (Figure 1B). To quantify the amount of gp80 molecules expressed on the cell surface at the aggregation stage, ^{125}I -labeled 80L5C4 IgG was used to bind to 10-h intact cells. The levels of gp80 on A-2 and B-4 cells were ~45% higher than that of KAX3 cells or V-1 cells (Table 2). It was therefore of interest to determine if the overexpression of gp80 had any effect on morphogenesis. Since KAX3 cells developed poorly on filter pads, cells were deposited on nonnutrient agar surfaces for development and the timing of cell migration, slug formation, and sporulation was monitored for both transformants A-2 and B-4. The amount of time required by the two transformants to complete the whole developmental sequence was similar to that of control cells, and significant acceleration of developmental events was not observed.

However, we noted that transformants grown on bacteria lawn uniformly gave rise to taller and bigger fruiting bodies than KAX3 cells. When cells were developed on a nonnutrient

Table 1. Binding of ^{125}I -labeled gp80 to vegetative cells of transformants

Transformant	80L5C4 IgG added ($\mu\text{g/ml}$)	Amount of gp80 bound (cpm/ 10^6 cells)	% Inhibition
V-1	0	1382	—
V-1 (+ cold gp80)	0	1128	18
A-2	0	4099	—
	1	1450	65
	4	1161	72
B-4	0	4568	—
	1	1511	67
	4	722	84

Vegetative cells were washed and resuspended at 1×10^7 cells/ml and 0.1 ml samples were incubated with ~50 ng ^{125}I -labeled gp80. Binding was carried out at 4°C for 45 min on a platform shaker. To determine background nonspecific binding, a 30-fold excess of unlabeled gp80 was added to a sample containing V-1 cells. In inhibition studies, cells were precoated with the anti-gp80 monoclonal antibody 80L5C4 for 15 min. After one wash, cells were resuspended in 0.25 mg/ml of goat anti-mouse-IgG Fab for 15 min before the addition of ^{125}I -labeled gp80. Values represent the average of duplicates.

Table 2. Binding of ^{125}I -labeled 80L5C4 IgG to intact cells

Strain	Amount of IgG bound (molecules/cell)	Relative amount
KAX3	7.8×10^4	1
KAX3 (+ cAMP)	22.0×10^4	2.82
V-1	8.1×10^4	1.04
A-2	11.4×10^4	1.46
B-4	11.6×10^4	1.48
NC4	12.5×10^4	1.60
AX2	15.1×10^4	1.94
HL216	3.8×10^4	0.49

Cells were developed in liquid culture for 10 h and collected for the binding assay as described in Materials and methods. Cells were resuspended at 10^7 cells/ml and 0.1 ml samples were incubated with a saturating amount of ^{125}I -labeled 80L5C4 IgG ($10 \mu\text{g/ml}$) (Siu *et al.*, 1985). Binding was carried out at 4°C for 45 min on a platform shaker. Values represent the average of triplicates.

agar surface, slugs formed from A-2 and B-4 were generally larger than those derived from V-1 or KAX3 cells. Interestingly, aggregates of V-1 and KAX3 cells had the tendency to break up into several smaller aggregates, each giving rise to a small slug (Figure 4). Fruiting bodies derived from KAX3 cells and V-1 cells were relatively small. On the other hand, fragmentation of aggregates rarely occurred in A-2 and B-4 aggregates, which gave rise to much bigger fruiting bodies (Figure 5). The sizes of slugs and fruiting bodies of A-2 and B-4 cells were similar to those of wild-type strains such as NC4.

To provide quantitative data for this observation, the length of slugs derived from the transformants were measured and compared. Figure 6 shows the size distribution of slugs prepared on nonnutrient agar surfaces. The size distribution patterns of the two gp80 transformants were quite similar, but they were distinctly different from that of the control transformant V-1. The differences in their distribution patterns were found to be statistically significant. The median for V-1 slugs was 0.3 mm and the mean length was 0.32 ± 0.14 mm. On the other hand, the medians for A-2 and B-4 slugs were ~ 0.43 and 0.45 mm, respectively, and the mean values were 0.44 ± 0.19 and 0.46 ± 0.18 mm, respectively. Although $>50\%$ of A-2 and B-4 slugs fell between 0.43 and 1 mm, only 15% of V-1 slugs were within this range. The median values also indicate that slugs derived from the gp80 transformed cells were $\sim 50\%$ larger than those derived from the control transformant.

Effect of gp80 overexpression induced by cAMP on slug size

Treatment of cells with exogenous cAMP pulses is known to induce a precocious and enhanced expression of gp80 (Siu *et al.*, 1988a). Therefore, KAX3 cells pulsed with cAMP are expected to accumulate more gp80 and to produce larger slugs. When such an experiment was performed, cAMP-pulsed KAX3 cells accumulated a much higher level of gp80 (Figure 7). At 10 h, the level of gp80 expressed on the cell surface was approximately threefold higher than that of control cells (Table 2). Cells at this stage formed large aggregates in solution and it was difficult to dissociate them. Therefore, cells were pulsed with cAMP for 4 h and then mechanically dissociated before plating on agar dishes for development. Cell streaming and slug formation occurred normally in both cAMP-pulsed cells and nonpulsed cells. In general, morphogenetic events happened 2–3 h earlier in the cAMP-pulsed cells and slug sizes were much larger than those in the control sample (Figure 8). The differences between the two samples were statistically significant. The median slug length for the pulsed cells was ~ 0.53 mm, which was

Figure 4. Micrographs of cell aggregates formed by transformants. Cells were plated on nonnutrient agars and photographs were taken at ~ 15 h of development. (a) Aggregates of B-4 cells and (b) aggregates of V-1 cells, showing fragmentation into smaller aggregates. Bar = 0.1 mm.

Figure 5. Micrographs of slugs and fruiting bodies of transformants. Cells were grown axenically and collected at their exponential growth phase. Cells were suspended in 17 mM phosphate buffer, pH 6.4, and deposited on 2% non-nutrient agar plates for development. Micrographs of slugs were taken at ~16 h of development and micrographs of fruiting bodies were taken at ~28 h. (a) Slugs formed from V-1 cells; (b) slugs of A-2 cells; (c) fruiting bodies of V-1 cells; and (d) fruiting body of A-2 cells. Bar = 0.2 mm.

identical to the mean (0.53 ± 0.22 mm), whereas the median for the nonpulsed control was 0.28 mm and the mean was 0.31 ± 0.16 mm. The

data thus indicate that slugs formed from cAMP-pulsed cells were almost twice as big as those of the control.

Slug size and gp80 expression in wild-type and mutant strains

The relationship between slug size and gp80 expression was further examined in three well-characterized strains: NC4, AX2, and HL216. The first two are wild-type strains, which accumulate substantially higher levels of gp80 than KAX3 cells (data not shown). On the other hand, HL216 carries a *modB* mutation which affects protein glycosylation (Murray *et al.*, 1984). We previously characterized the expression of gp80 in the two original *modB* mutants, HL220 and HL216, both of which accumulate, in much reduced amounts, a mutant gp80 of ~70 000 kDa which is still capable of mediating cell-cell binding (Siu *et al.*, 1985; Siu and Lam, 1988).

The relative amounts of gp80 expressed on the cell surface of these three strains were estimated by ^{125}I -labeled IgG binding to intact cells (Table 2). The levels of gp80 on NC4 cells and AX2 cells exceeded that of KAX3 cells by 60% and 94%, respectively. On the other hand, the level of gp80 on HL216 cells was about one-half of that on KAX3 cells. When slug size was examined, NC4 and AX2 shared a similar pattern of slug size distribution and the differences were not statistically significant (Figure 9). The median lengths were 0.47 and 0.49 mm and the mean values were 0.50 ± 2.6 and 0.52 ± 2.6 mm for NC4 and AX2, respectively. The average slug size of AX2 cells is similar to that of cAMP-treated KAX3 cells, although the latter had a substantially higher level of gp80, suggesting the presence of an upper limit in slug size. In contrary, HL216 cells gave rise to much smaller slugs. Aggregates of HL216 often broke up and formed minute slugs. The median slug size was 0.23 mm and the mean was 0.24 ± 0.11 mm. The differences in slug size between HL216 and NC4 or AX2 were statistically significant. To assess the relationship between slug size and gp80 content, these results and those derived from the transformants were subjected to linear regression analysis (Figure 10). A strong positive correlation was observed, with a correlation coefficient of 0.976, indicating that the mean slug size is dependent on the amount of gp80 expressed on the cell surface.

Discussion

We obtained and characterized stable transformants that express the cell adhesion molecule

Figure 6. Changes in slug size of gp80 transformants.

Transformed cells were grown and developed as described in the legend of Figure 5. The length of ~150 slugs were measured for each transformant. (A) Size distribution of V-1 slugs; (B) slugs of A-2 cells; and (C) slugs of B-4 cells. When the data were subjected to two-sample *t* test for independent samples, the differences in the size distribution of V-1 slugs and A-2 slugs or B-4 slugs were found to be statistically significant ($p < 0.001$ for both cases). However, the differences between A-2 and B-4 were not significant ($p > 0.5$).

gp80 during both vegetative growth and development. gp80 is normally expressed only during the period of cell aggregation (Lam and Siu, 1981; Murray *et al.*, 1981). Transcription of the gp80 gene is triggered during early development and subsequently augmented by cAMP pulses (Mann and Firtel, 1989; Ma and Siu, 1990). Thus cells in the growth phase and in the preaggregation stages cannot undergo cell-cell binding in the presence of 2 mM EDTA. When gp80 cDNA under the control of the actin 6 promoter is transfected into cells, gp80 expression can be observed during vegetative growth. Although the amount of gp80 accumulated during the

growth phase represented ~30% of the amount normally accumulated in these transformants at the aggregation stage, the gp80 molecules appear to be fully functional and a significant number of cells are capable of forming EDTA-resistant cell-cell contacts. The percentage of cell aggregation at 0 h is comparable to that observed in cells which accumulate a similar amount of gp80 during development (Siu and Lam, 1988). The actin 6 promoter is most active at 2.5 h of development (Romans *et al.*, 1985), and its activity decreases substantially by 10 h (Nellen *et al.*, 1986). Nevertheless, the amount of gp80 accumulated on the cell surface

Figure 7. Stimulation of gp80 expression by cAMP. KAX3 cells were developed in 17 mM phosphate buffer, pH 6.4, and shaken at 180 rpm. Beginning at 2 h of development, cells were given pulses of exogenous cAMP (2×10^{-8} M final concentration) at 7-min intervals. Cells were collected at 8 h and total cellular proteins were separated by gel electrophoresis. Protein immunoblots were stained with the anti-gp80 monoclonal antibody 80L5C4. Lane a, 0 h cells; lanes b and c, 4 h and 8 h of control cells; lanes d and e, 4 h and 8 h of cAMP-pulsed cells. The arrowhead indicates the position of gp80.

of 10 h transformants is $\sim 45\%$ higher than that of KAX3 cells, suggesting that gp80 molecules are relatively stable at this stage. The level of cell-cell adhesion exhibited by these transformants is also higher and it correlates closely with the level of gp80 expression in these transformants. Similar observations have been made when cDNAs of neural cell adhesion molecule and E-cadherin are introduced into L cells, resulting in the expression of specific cell-cell adhesion (Edelman *et al.*, 1987; Nagafuchi *et al.*, 1987). Both molecules have been implicated to mediate cell-cell adhesion by homophilic binding.

In vitro studies carried out previously in our laboratory have demonstrated that purified gp80 is capable of undergoing homophilic binding (Siu *et al.*, 1987; Kamboj *et al.*, 1988). The homophilic interacting site has been mapped to an octapeptide sequence YKLVNDS located near the amino-terminus (Kamboj *et al.*, 1989). The in vivo studies reported here indicate that the acquisition of EDTA-resistant cell-cell adhesion at the vegetative stage is a direct result of the expression of gp80. Experiments have been carried out to rule out the presence of a gp80 receptor that might be induced by the process of transformation or the precocious expression of gp80. Binding studies using

^{125}I -labeled gp80 show that gp80 binds to A-2 and B-4 vegetative cells but not V-1 vegetative cells (Table 1). The binding of gp80 to A-2 and B-4 cells are inhibited by precoating cells with an anti-gp80 monoclonal antibody, indicating that the labeled gp80 binds directly to gp80 associated with the cell surface and not to a different receptor. Our results thus provide direct in vivo demonstration that gp80 mediates cell-cell adhesion via homophilic interaction.

Morphogenesis of *Dictyostelium* cells is disrupted by the overexpression of certain gene products, such as cAMP phosphodiesterase (Faure *et al.*, 1988), the regulatory subunit of cAMP-dependent protein kinase (Simon *et al.*, 1989), and the $G_{\alpha 1}$ protein (Kumagai *et al.*, 1989). These proteins represent key components in cAMP signaling during differentiation and are therefore expected to have pleiotropic effects on morphogenesis. Unlike these developmentally regulated proteins, expression of gp80 at the wrong stage of development does not seem to affect morphogenesis. It is possible that the amount of gp80 overexpressed in these transformants is relatively low and deleterious effects may not be readily detectable. On the other hand, a 3-fold overaccumulation of gp80 molecules induced by exogenous cAMP pulsing does not seem to affect morphogenesis. It remains to be determined if a 10- to 20-fold overexpression of gp80 in vegetative cells has any effect on growth and development.

An unexpected observation from this study is the effect of gp80 on slug size. The average size of slugs derived from the two gp80 transformants are $\sim 50\%$ larger than those of control cells. While KAX3 cells develop poorly and usually give rise to relatively small fruiting bodies, fruiting bodies derived from the transformants are much larger, similar to the morphology of those derived from the wild-type strain NC4. Quantitative analysis shows that the differences in slug sizes are statistically significant. It should be noted that KAX3 cells were derived from the strain AX3, which is an axenic derivative of the wild-type strain NC4 (Poole and Firtel, 1984). Similar to AX3 cells, KAX3 cells express a suboptimal level of gp80, which is $\sim 60\%$ of that expressed in NC4 cells under identical developmental conditions. The transformants overexpress gp80 by $\sim 50\%$ of the amount normally found in KAX3 cells, thus reaching a level comparable to that of NC4 cells. It is remarkable that the restoration of the wild-type level of gp80 expression can lead to the restoration of the wild-type phenotype of slug and fruiting body development.

Figure 8. Effect of exogenous cAMP pulsing on slug size. KAX3 cells were developed and pulsed with cAMP for 4 h beginning at 2 h of development. Cells were collected and dissociated mechanically before plating on nonnutrient agar plates for development. Lengths of slugs were measured 15 h later. (A) Control cells without cAMP pulsing; (B) cells pulsed with cAMP. The data were subjected to *t* test and the differences between these two samples were found to be statistically significant ($p < 0.001$).

To further ascertain this relationship, KAX3 cells were pulsed with cAMP to stimulate the expression of gp80. KAX3 cells respond by accumulating gp80 on the cell surface to a level about threefold higher than that of the non-pulsed cells. Slugs derived from the cAMP-pulsed cells are about twice the size of the control. These results are consistent with the notion that cell-cell adhesion mediated by gp80 is a major factor involved in size control.

To determine whether the slug size is influenced by the amount of gp80 or by the premature expression of gp80, we have examined the patterns of slug size distribution for three other strains of *D. discoideum*. A linear-regression plot of these results together with those obtained with the transformants shows a positive correlation between the mean slug size and the amount of gp80 on the cell surface. Therefore, the amount of gp80, and not the timing of gp80 expression, has a direct effect on slug development.

Earlier studies suggest that the size of the fruiting body is regulated at two stages of development. At the aggregation stage, the cell density within an aggregation territory appears to be an important factor since, with certain limits, aggregation territories remain fixed in size regardless of cell density (Bonner and Dodd, 1962). The second moment of size con-

trol comes at the end of the aggregation period. Hohl and Raper (1964) suggested that there exists a critical mass that ultimately determines the size of the sorocarp. If an aggregate exceeds a certain critical mass or volume of cells, intercellular integration cannot be maintained. Multiple tips, and thus slugs, will be formed. In the case of KAX3 and HL216 cells, the sizes of slugs and fruiting bodies remain small whether they are developed under our experimental conditions or at high density on bacterial lawn. In addition, gp80 transformants derived from KAX3 cells give rise to larger slugs under identical conditions. Therefore, cell density does not seem to be a major cause for the differences in slug size observed here. It is more likely that these differences are due to a factor operating at the second moment of size control. Our results suggest that gp80 plays a crucial role in determining the critical mass that these strains can sustain in the aggregate. In this regard, it is of interest to note that aggregates formed by KAX3 cells and HL216 cells have a tendency to break up into smaller entities. The fragmentation of HL216 aggregates is consistent with the previous report of Loomis *et al.* (1987) on *modB* mutants. The lack of type 2 carbohydrate on the mutant gp80 expressed by *modB* cells renders the molecule highly susceptible to proteolytic cleavage on the cell surface (Hohmann *et al.*,

Figure 9. Distribution of slug sizes in three different strains. NC4, AX2, and HL216 were cultured on bacterial lawn and collected at their exponential growth phase. Cells were washed and deposited on nonnutrient agar for development. Lengths of slugs were measured between 16 and 18 h of development. Results were subjected to *t* test. The differences between NC4 and AX2 were not significant ($p > 0.5$), whereas the differences between HL216 and NC4 or AX2 were statistically significant, with $p < 0.001$ in both cases.

1987), resulting in a much lower level of accumulation during development (Gerisch *et al.*, 1985; Siu *et al.*, 1985; Siu and Lam, 1988). It is therefore evident that the adhesive forces operating among cells constitute a major regulator that determines whether a large aggregate would break up into smaller slugs.

Our results also indicate that the average size of the slug does not increase after reaching a certain limit regardless the amount of gp80 associated with the cell surface. Size regulation is obviously a complex phenomenon, which may involve a number of different factors. Kopachik (1982) has demonstrated that a slug tip has the ability to secrete a diffusible inhibitor to prevent cells within a certain area from forming another independent tip. The size of an aggregate also depends on the ability of the surrounding cells to resist the inhibitor coming from the newly arisen tip. The nature of the inhibitor and the mechanisms involved remain to be elucidated.

Proper cell-cell adhesion appears to be a prerequisite for morphogenesis. Inhibition of gp80 function by univalent antibodies or synthetic oligopeptides results in abnormal cell migration and blocks development (Siu and Kamboj, 1990). Similar observations have been reported when the EDTA-sensitive binding sites are blocked by antibodies (Loomis, 1988). However, Harloff *et al.* (1989) have shown that transformants with the gp80 gene selectively eliminated by homologous recombination are still capable of forming fruiting bodies. The apparent discrepancies between these observations have not been resolved. Previous studies have shown that the cell-binding activity of gp80 is replaced by other cell-adhesion molecules in the postaggregation stages (Siu *et al.*, 1988b) and the expression of these late-stage adhesion molecules overlaps with gp80 at the aggregation stage (Parish and Schmidlin, 1979; Geltosky *et al.*, 1979). It is therefore conceivable that the

Figure 10. Relationship between slug size and gp80 level. The mean slug size for the eight strains studied was plotted against the amount of gp80 associated with the cell surface (Table 2) by simple linear-regression analysis. The two variables were positively correlated with a correlation coefficient of 0.976. (▲), HL216; (●), KAX3; (○), V-1; (■), A-2; (□), B-4; (△), NC4; and (▼), AX2.

continued accumulation of these late-stage adhesion molecules eventually enables the gp80 null mutants to reform proper cell-cell contacts, thus permitting morphogenesis to proceed. In this regard, it is of interest to note that these null mutants still retain a significant degree of EDTA-resistant cell-cell adhesion at the aggregation stage.

Cell-cell binding has also been implicated in a number of differentiation and morphogenetic processes. Recent studies by Fontana and Price (1989) suggest that the formation of cell-cell contact may have subtle effects on cAMP secretion and signaling. In the postaggregation stages, differential cell adhesion is involved in the formation of the spatial pattern of prespore cells and prestalk cells in the slug (Feinberg *et al.*, 1979; Tasaka and Takeuchi, 1979; Lam *et al.*, 1981; Siu *et al.*, 1983). Cell-cell contact has been found to be important in sustaining the continued expression of certain mRNA species (Chung *et al.*, 1981; Chisholm *et al.*, 1984).

Although the mechanism by which gp80 mediates cell-cell binding is now known, the physiological effects of gp80-gp80 interaction remain to be elucidated. Results presented in this report suggest that a major factor involved in size control is the amount of gp80 expressed on the plasma membrane of aggregation-stage cells. Additional studies are necessary to determine whether the increase in gp80 concentration merely generates a higher adhesive force to maintain the integrity of an aggregate or it can give rise to additional developmental signals re-

quired for slug size regulation. If specific signals are involved, their nature and mechanism of transmission will be important subjects in our future studies.

Methods

Cell strain and culture conditions

Axenic strains of *D. discoideum* were cultured axenically in HL-5 medium as described by Cocucci and Sussman (1970). Growth phase cells ($2-4 \times 10^6$ cells/ml) were collected and washed free of medium for development. Nonaxenic strains were cultured on SM-agar plates in association with *Klebsiella aerogenes* as described by Sussman (1966). Cells in their exponential growth phase were collected and washed free of bacteria. For development, cells were suspended in 17 mM Na₂/K phosphate buffer, pH 6.4, and plated on 2% nonnutrient agar for development at a density of $4-5 \times 10^4$ cells/cm². Excess liquid was removed and plates were incubated in a moist chamber at room temperature. Temporal events of morphogenesis were monitored at regular intervals. Slug sizes were measured between 15 and 18 h of development.

Alternatively, KAX3 cells were suspended at 10^7 cells/ml in 17 mM Na₂/K phosphate buffer, pH 6.4, and shaken at 180 rpm for development at room temperature. To investigate the effect of cAMP on slug size, cells that had been developed in liquid medium for 2 h were pulsed with exogenous cyclic adenosine monophosphate [(cAMP) 2×10^{-8} M final concentration] at 7-min intervals for 4 h. At 6 h of development, cells were mechanically dissociated and then plated on nonnutrient agar plates. Migrating slugs were usually observed 15 h later and the lengths of 150 to 200 slugs were measured for each sample. All morphogenesis experiments were repeated 3–5 times.

Construction of vectors

Two constructs were prepared using the *D. discoideum* transformation vector pDNeoll (Witke *et al.*, 1987), which contains the neomycin-resistant gene of transposon TN 903 under the control of the actin 15 promoter and terminator sequences (Knecht *et al.*, 1986). Two different gp80 cDNAs covering the complete coding region were inserted separately into the EcoRI site of the pDNeoll vector, such that gp80 expression would be under the control of the actin 6 promoter. Construct A contained a 2 kb gp80 cDNA with 23 nucleotides in the 5' nontranslated region and construct B contained a 1.8 kb gp80 cDNA with 100 nucleotides in the 5' nontranslated region.

Transformation of Dictyostelium cells

Transformation of *D. discoideum* KAX3 cells was carried out using the calcium phosphate coprecipitation method as described by Nellen *et al.* (1984). Selection of transformants was carried out in the presence of the antibiotic G418 at 20 µg/ml. Transformants were picked and grown axenically in HL-5 medium. For further clonal selection, transformants were plated on SM-agar plates in association with *K. aerogenes* (Sussman, 1966). Spores from single colonies were picked and cultured in HL-5 medium again. After the initial selection phase, all axenic cultures contained G418 at 10 µg/ml.

RNA blots

Total cellular RNA was isolated from samples derived from different stages of development. Cells were resuspended

in 50 mM tris(hydroxymethyl)aminomethane (Tris)-HCl, pH 7.8, and then lysed in the presence of 1% sodium dodecyl sulfate (SDS). The lysate was subjected to phenol/chloroform extraction three times and the RNA was precipitated in ethanol. RNA samples were size fractionated in a 1% agarose gel containing 6.7% formaldehyde and then capillary-transferred to nitrocellulose filter. RNA filters were hybridized with a gp80 cDNA probe labeled with [³²P]dATP by the random priming method (Feinberg and Vogelstein, 1984). Hybridization was carried out at 42°C in the presence of 50% formamide and 5× SSC and then washed twice with 0.1× SSC at 60°C. Quantification was carried out by densitometric tracings of autoradiograms.

Immunoblots

Total cell protein was solubilized and reduced in 2% SDS, 8 M urea, and 2% β-mercaptoethanol by heating at 95°C for 5 min. Samples were electrophoresed in 10% polyacrylamide gel according to Laemmli (1970). Proteins were transferred electrophoretically from slab gels to nitrocellulose filter (Towbin *et al.*, 1979). Filters were first blocked with 5% skim milk, 0.05% Tween-20, and 0.02% sodium azide in phosphate-buffered saline, pH 7.4, and then incubated with the anti-gp80 monoclonal antibody 80L5C4 (Siu *et al.*, 1985), followed by alkaline phosphatase-conjugated goat anti-mouse-IgG antibodies. Color development was carried out using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates. The relative amounts of gp80 were estimated by densitometric tracings.

Immunofluorescence microscopy

Live cells taken from different developmental stages were suspended in cold 17 mM phosphate buffer, pH 6.4, containing 0.1% bovine serum albumin and 5 mM EDTA and then incubated with the monoclonal antibody 80L5C4 for 30 min at 4°C. Cell samples were washed five times with the same buffer and cell clumps were dispersed by gentle vortexing. Cells were incubated with FITC-conjugated goat anti-mouse-IgG antibodies for 30 min at 4°C. After several washes to remove the unbound antibodies, epifluorescence was observed using a Wild Leitz Orthoplan universal large-field microscope.

Cell cohesion assay

Cell-cell adhesion was assayed as described previously (Lam *et al.*, 1981). Cells were dissociated mechanically and suspended at 2.5×10^6 cells/ml in 17 mM phosphate buffer, pH 6.4, containing 5 mM EDTA. Samples were rotated vertically at 120 rpm. Cells were counted at different time intervals using a hemacytometer. The percentage of aggregated cells was calculated by dividing the difference of the total number of cells and the number of single cells by the total number of cells.

Binding of gp80 to intact cells

Purified gp80 was labeled with ¹²⁵I using chloramine T. Vegetative cells were collected, washed, and resuspended in 17 mM phosphate buffer, pH 6.4, at 1×10^7 cells/ml. ¹²⁵I-labeled gp80 was added to 0.1 ml cell samples and incubated at 4°C for 45 min on a platform shaker (Siu *et al.*, 1987). Cell samples were washed twice with cold phosphate buffer and then counted in a gamma counter. To estimate background binding, cells were incubated in the presence of a 30-fold excess of unlabeled gp80. In inhibition studies, cells were first incubated with 80L5C4 IgG for 15 min. Cells were washed once and then incubated with 0.25 mg/ml goat anti-

mouse-IgG Fab for 15 min. Cells were gently vortexed to dissociate aggregates before the addition of ¹²⁵I-labeled gp80.

Quantification of cell surface gp80

To quantify the relative amount of gp80 expressed on the cell surface, 10 h cells were washed and resuspended at 1×10^7 cells/ml in 17 mM phosphate buffer solution containing 5 mM EDTA and 1% bovine serum albumin. ¹²⁵I-labeled 80L5C4 was added to 0.1 ml cell samples and incubated at 4°C for 45 min on a platform shaker at 150 rpm (Siu *et al.*, 1975). Cells were washed twice with cold phosphate buffer and the samples were counted in a gamma counter.

Acknowledgments

We thank Dr. R.A. Firtel for the KAX3 strain, Dr. W.F. Loomis for the HL216 strain, W. Witke for the *Dictyostelium* transformation vector pDNeoll, and L. Desbarats for assistance and advice on cell transformation. This work was supported by an operating grant from the Medical Research Council of Canada.

Received: June 4, 1990.

Revised and accepted: August 15, 1990.

References

- Beug, H., Katz, F., and Gerisch, G. (1973). Dynamics of antigenic membrane sites relating to cell aggregation in *Dictyostelium discoideum*. *J. Cell Biol.* 56, 647–658.
- Bonner, J.T., and Dodd, M.R. (1962). Aggregation territories in the cellular slime molds. *Biol. Bull.* 122, 13–24.
- Chisholm, R.L., Barklis, E., and Lodish, H.F. (1984). Mechanism of sequential induction of cell-type specific mRNAs in *Dictyostelium* differentiation. *Nature (Lond.)* 310, 67–69.
- Choi, A.H.C. and Siu, C.-H. (1987). Filopodia are enriched in a cell cohesion molecule of Mr 80,000 and participate in cell-cell contact formation in *Dictyostelium discoideum*. *J. Cell Biol.* 104, 1375–1387.
- Chung, S., Landfear, S.M., Blumberg, D.D., Cohen, N.S., Lodish, H.F. (1981). Synthesis and stability of developmentally regulated *Dictyostelium* mRNAs are affected by cell-cell contact and cAMP. *Cell* 24, 785–797.
- Cocucci, S., and Sussman, M. (1970). RNA in cytoplasmic and nuclear fractions of cellular slime mold amoebae. *J. Cell Biol.* 45, 399–407.
- Cunningham, B.A., Hemperly, J.J., Murray, B.A., Prediger, E.A., Brackenbury, R., and Edelman, G.M. (1987). Neural cell adhesion molecule: structure, immunoglobulin-like domains, cell surface modulation, and alternative RNA splicing. *Science* 236, 799–806.
- De Chastellier, C. and Ryter, A. (1980). Characteristic ultrastructural transformations upon starvation of *Dictyostelium discoideum* and their relationship with aggregation: study of wild type amoebae and aggregation mutants. *Biol. Cell.* 38, 121–128.
- De Silva, N.S., and Siu, C.-H. (1980). Preferential incorporation of phospholipids into plasma membrane during cell aggregation of *Dictyostelium discoideum*. *J. Biol. Chem.* 255, 8489–8496.
- De Silva, N.S., and Siu, C.-H. (1981). Vesicle-mediated transfer of phospholipids to plasma membrane during cell

- aggregation of *Dictyostelium discoideum*. *J. Biol. Chem.* 256, 5845–5850.
- Edelman, G.M. (1986). Cell adhesion molecules in the regulation of animal form and tissue pattern. *Annu. Rev. Cell Biol.* 2, 81–116.
- Edelman, G.M., Murray, B.A., Mege, R.-M., Cunningham, B.A., and Gallin, W.J. (1987). Cellular expression of liver and neural cell adhesion molecules after transfection with their cDNAs results in specific cell-cell binding. *Proc. Natl. Acad. Sci. USA* 84, 8502–8506.
- Faure, M., Podgorski, G.J., Franke, J., and Kessin, R.H. (1988). Disruption of *Dictyostelium discoideum* morphogenesis by overproduction of cAMP phosphodiesterase. *Proc. Natl. Acad. Sci. USA* 85, 8078–8080.
- Feinberg, A.P., and Vogelstein, B. (1984). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137, 266–267.
- Feinberg, A.P., Springer, W.R., and Barondes, S.H. (1979). Segregating of prestalk and prespore cell of *Dictyostelium discoideum*: observations consistent with selective cell cohesion. *Proc. Natl. Acad. Sci. USA* 76, 3977–3981.
- Fontana, D.R., and Price, P.L. (1989). Cell-cell contact elicits cAMP secretion and alters cAMP signaling in *Dictyostelium discoideum*. *Differentiation* 41, 184–192.
- Garrod, D.R. (1972). Acquisition of cohesiveness by slime mould cells prior to morphogenesis. *Exp. Cell Res.* 72, 588–591.
- Geltosky, J.E., Weseman, J., Bakke, A., and Lerner, R.A. (1979). Identification of a cell surface glycoprotein involved in cell aggregation in *Dictyostelium discoideum* during development. *Cell* 18, 391–368.
- Gerisch, G. (1980). Univalent antibody fragments as tools for the analysis of cell interactions in *Dictyostelium*. *Curr. Top. Dev. Biol.* 14, 243–270.
- Gerisch, G., Weinhart, U., Bertholdt, G., Claviez, M., and Stadler, J. (1985). Incomplete contact site A glycoprotein in HL220, a modB mutant of *Dictyostelium discoideum*. *J. Cell Sci.* 73, 49–68.
- Harloff, C., Gerisch, G., and Noegel, A.A. (1989). Selective elimination of the contact site A protein of *Dictyostelium discoideum* by gene disruption. *Genes Dev.* 3, 2011–2019.
- Hohl, H., and Raper, K.B. (1964). Control of sorocarp size in the cellular slime molds. *Dev. Biol.* 9, 137–153.
- Hohmann, H.-P., Bozzaro, S., Merki, R., Wallraff, E., Yoshida, M., Weinhart, U., and Gerisch, G. (1987). Post-translational glycosylation of the contact site A protein of *Dictyostelium discoideum* is important for stability but not for its function in cell adhesion. *EMBO J.* 6, 3663–3671.
- Kamboj, R.K., and Siu, C.-H. (1988). Mapping of the monoclonal antibody 80L5C4 epitope on the cell adhesion molecule gp80 of *Dictyostelium discoideum*. *Biochim. Biophys. Acta* 951, 78–84.
- Kamboj, R.K., Wong, L.M., Lam, T.Y., and Siu, C.-H. (1988). Mapping of a cell-binding domain in the cell adhesion molecule gp80 of *Dictyostelium discoideum*. *J. Cell Biol.* 107, 1835–1843.
- Kamboj, R.K., Garipey, J., and Siu, C.-H. (1989). Identification of an octapeptide involved in homophilic interaction of the cell adhesion molecule gp80 of *Dictyostelium discoideum*. *Cell* 59, 615–625.
- Kopachik, W. (1982). Size regulation in *Dictyostelium*. *J. Embryol. Exp. Morphol.* 68, 23–35.
- Knecht, D.A., Cohen, S.M., Loomis, W.F., and Lodish, H.F. (1986). Developmental regulation of *Dictyostelium discoideum* actin gene fusions carried on low-copy and high-copy transformation vectors. *Mol. Cell. Biol.* 6, 3973–3983.
- Kumagai, A., Pupillo, M., Gundersen, R., Miake-Lye, R., Devreotes, P.N., and Firtel, R.A. (1989). Regulation and function of G_α protein subunits in *Dictyostelium*. *Cell* 57, 265–275.
- Laemmli, V.K. 1970. Cleavage of structural proteins during assembly of the bacteriophage T4. *Nature (Lond.)* 227, 680–685.
- Lam, T.Y. and Siu, C.-H. (1981). Synthesis of stage-specific glycoprotein in *Dictyostelium discoideum* during development. *Dev. Biol.* 83, 127–137.
- Lam, T.Y., Pickering, G., Geltosky, J., and Siu, C.-H. (1981). Differential cell cohesiveness expressed by prespore and prestalk cells of *Dictyostelium discoideum*. *Differentiation* 20, 22–28.
- Loomis, W.F. (1975). *Dictyostelium discoideum*: A Developmental System. New York Academic Press, 1–85.
- Loomis, W.F., Knecht, D.A., and Fuller, D.L. (1987). Adhesion mechanisms and multicellular control of cell-type divergence of *Dictyostelium discoideum*. In: *Molecular Approaches to Developmental Biology*, R.A., Firtel, and E.H. Davidson, eds, New York: Alan R. Liss, Inc., 339–349.
- Loomis, W.F. (1988). Cell-cell adhesion in *Dictyostelium discoideum*. *Dev. Genet.* 9, 549–559.
- Ma, P.C.C., and Siu, C.-H. (1990). A pharmacologically distinct cAMP receptor is responsible for the regulation of gp80 expression in *Dictyostelium discoideum*. *Mol. Cell. Biol.* 10, 3297–3306.
- Mann, S.K.O., and Firtel, R.A. (1989). Two-phase regulatory pathway controls cAMP receptor-mediated expression of early genes in *Dictyostelium*. *Proc. Natl. Acad. Sci. USA* 86, 1924–1928.
- McClay, D.R., and Etensohn, C.A. (1987). Cell adhesion in morphogenesis. *Annu. Rev. Cell Biol.* 3, 319–325.
- Muller, K., and Gerisch, G. (1978). A specific glycoproteins as the target site of adhesion blocking Fab in aggregating *Dictyostelium* cells. *Nature (Lond.)* 274, 445–449.
- Murray, B.A., Yee, L.D., and Loomis, W.F. (1981). Immunological analysis of a glycoprotein (contact sites A) involved in intercellular adhesion of *Dictyostelium discoideum*. *J. Supramol. Struc. Cell. Biochem.* 17, 197–211.
- Murray, B.A., Wheeler, S., Jongens, T., and Loomis, W.F. (1984). Mutations affecting a surface glycoprotein, gp80, of *Dictyostelium discoideum*. *Mol. Cell. Biol.* 4, 514–519.
- Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda, K., and Takeichi, M. (1987). Transformation of cell adhesion properties by exogenously introduced E-cadherin cDNA. *Nature Lond.* 329, 341–343.
- Nellen, W., Silan, C., and Firtel, R.A. (1984). DNA-mediated transformation in *Dictyostelium discoideum*: regulated expression of an actin gene fusion. *Mol. Cell. Biol.* 4, 2890–2898.
- Nellen, W., Silan, C., Saur, U., and Firtel, R.A. (1986). Regulatory sequences in the promoter of the *Dictyostelium* Actin 6 gene. *EMBO J.* 5, 3367–3372.
- Noegel, A., Gerisch, G., Stadler, J., and Westphal, M. (1986). Complete sequence and transcript regulation of a cell adhesion protein from aggregating *Dictyostelium* cells. *EMBO J.* 5, 1473–1476.

- Parish, R.W., and Schmidlin, S. (1979). Synthesis of plasma membrane proteins during development of *Dictyostelium discoideum*. *FEBS Lett.* 98, 251–256.
- Poole, S.J., and Firtel, R.A. (1984). Genomic instability and mobile genetic elements in regions surrounding two dis-coidin I genes of *Dictyostelium discoideum*. *Mol. Cell. Biol.* 4, 671–680.
- Romans, P., Firtel, R.A., and Saxe, C.L. III. (1985). Gene-specific expression of the actin multigene family of *Dictyostelium discoideum*. *J. Mol. Biol.* 186, 337–355.
- Simon, M.N., Driscoll, D., Mutzel, R., Part, D., Williams, J., and Veron, M. (1989). Overproduction of the regulatory subunit of the cAMP-dependent protein kinase blocks the differentiation of *Dictyostelium discoideum*. *EMBO J.* 8, 2039–2043.
- Siu, C.-H., Des Roches, B., and Lam, T.Y. (1983). Involvement of a cell-surface glycoprotein in the cell-sorting process of *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA* 80, 6596–6600.
- Siu, C.-H., Lam, T.Y., and Choi, A.H.C. (1985). Inhibition of cell-cell binding at the aggregation stage of *Dictyostelium* by monoclonal antibodies directed against the contact site A glycoprotein. *J. Biol. Chem.* 260, 16030–16036.
- Siu, C.-H., Cho, A., and Choi, A.H.C. (1987). The contact site A glycoprotein mediates cell-cell adhesion by homophilic binding in *Dictyostelium discoideum*. *J. Cell Biol.* 105, 2523–2533.
- Siu, C.-H., and Lam, T.Y. (1988). Mediation of cell-cell adhesion by the altered contact site A glycoprotein expressed in *modB* mutants of *Dictyostelium discoideum*. *Exp. Cell Res.* 117, 338–346.
- Siu, C.-H., Lam, T.Y., and Wong, L.M. (1988a). Expression of the contact site A glycoprotein in *Dictyostelium discoi-deum*: quantitation and developmental regulation. *Biochim. Biophys. Acta* 968, 283–290.
- Siu, C.-H., Wong, L.M., Lam, T.Y., Kamboj, R.K., Choi, A., and Cho, A. (1988b). Molecular mechanisms of cell-cell interaction in *Dictyostelium discoideum*. *Biochem. Cell Biol.* 66, 1089–1099.
- Siu, C.-H., and Kamboj, R.K. (1990). Cell-cell adhesion and morphogenesis. *Dev. Genet.* (In press).
- Stadler, J., Keenan, T.W., Bauer, G., and Gerisch, G. (1989). The contact site A glycoprotein of *Dictyostelium* carries a phospholipid of a novel type. *EMBO J.* 8, 371–377.
- Sussman, M. (1966). Biochemical and genetic methods in the study of cellular slime mold development. *Methods Cell Physiol.* 2, 397–409.
- Tasaka, M. and Takeuchi, I. (1979). Sorting out behavior of disaggregated cells in the absence of morphogenesis in *Dictyostelium discoideum*. *J. Embryol. Exp. Morphol.* 49, 89–102.
- Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and applications. *Proc. Natl. Acad. Sci. USA* 76, 4350–4354.
- Williams, A.F., and Barclay, A.N. (1988). The immunoglobulin superfamily—domains and cell surface recognition. *Annu. Rev. Immunol.* 6, 381–405.
- Witke, W., Nellen, W., and Noegel, A. (1987). Homologous recombination in the *Dictyostelium* α -actinin gene leads to an altered mRNA and lack of the protein. *EMBO J.* 6, 4143–4248.
- Wong, L.M. and Siu, C.-H. (1986). Cloning of cDNA for the contact site A glycoprotein of *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA* 83, 4248–4252.