Cyclic AMP Enhances the Sexual Agglutinability of *Chlamydomonas* Flagella

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Abstract. Sexual adhesion between *Chlamydomonas reinhardtii* gametes elicits a rise in intracellular cAMP levels, and exogenous elevation of intracellular cAMP levels in gametes of a single mating type induces such mating responses as cell wall loss, flagellar tip activation, and mating structure activation (Pasquale, S. M., and U. W. Goodenough. 1987. *J. Cell Biol.* 105:2279–2292). Here evidence is presented that sexual adhesion mobilizes agglutinin to the flagellar surface, and that this mobilization can be induced by exogenous presentation of cAMP to gametes of a single mating type. It is proposed that *Chlamydomonas* adhesion entails a positive feedback system—initial contacts stimulate the presentation of additional agglutinin—and that this feedback is mediated by adhesion-induced cAMP generation.

When *Chlamydomonas* gametes make sex-specific adhesive contacts via their flagellar surfaces, the cells respond with a rapid rise in intracellular cAMP (21, 23). In *C. reinhardtii* the elevated cAMP levels have been shown to induce four subsequent mating responses (21): a change in the morphology of the flagellar tips (flagellar tip activation); the liberation of an enzyme (lysin) that removes the cell wall; the activation of membrane-associated mating structures; and cell fusion itself.

The present paper focuses on a fifth mating response, that of gametic adhesivity. It has been shown for the *C. eugamatos* species that when gametes are mixed together, both mating types increase their adhesivity about eightfold within the first minutes of mating (5, 28). Moreover, an increase in adhesivity is also observed if gametes of one *mt* are stimulated by adhesion to flagellar membrane vesicles or glutaraldehyde-fixed cells of opposite *mt*, and an increase in agglutinin can be directly observed by immunolabeling (5). Normally, mating cells quickly go on to fuse at which time the flagella lose their adhesivity completely (18) by some unknown mechanism. If, however, cell fusion cannot occur because of gene mutation or experimental manipulation, then adhesivity remains at elevated levels (5, 10). During such protracted interactions, flagella are in fact continuously losing adhesivity (22, 26), again by an unknown mechanism, and simultaneously regaining it, first from a preexisting pool of proteins and then by protein synthesis (4, 24, 26). This loss/replacement cycle serves to maintain agglutinability at constant levels for many hours when cell fusion is inhibited.

Two features of this complex mating response are explored in the present study. First, what is the nature of "increased adhesivity"? Are more agglutinin proteins recruited to the flagellar surface, or are existing proteins "activated" or "rearranged" in some way such that they are more effective? And second, what is the relationship between cAMP levels and this response? Since adhesion is required for the elevation of cAMP levels (21, 23), is cAMP in turn required to elevate adhesivity, or is a separate regulatory circuit involved? Is adhesion itself necessary, or can the response be elicited by exogenous presentation of cAMP?

The results provide evidence that additional agglutinin proteins are indeed added to the flagellar surface, and that this recruitment can be elicited directly by cAMP. Therefore, the cAMP signal not only stimulates the mating responses attendant to cell fusion, but also stimulates an up-regulation of agglutinin presentation, thereby assuring that the agglutinated state is maintained until cell fusion is achieved.

Materials and Methods

Strains and Culture Conditions

Wild-type strains *C. reinhardtii* CC-620 (mt^+) and CC-621 (mt^-) and the mutants *imp-1* mt^+ (CC-462) (10, 12) and *gam*-1 (CC-1693) (8) were used, as well as *C. smithii* mt^+ (CC-1373) (2), all available from the *Chlamydomonas* Genetics Center, Duke University, Durham, NC. Plate gametes (19) were suspended in nitrogen-free high-salt minimal medium (NFHSM)[1] (19) for 1–2 h before use. Mating efficiency was determined by counting biflagellate cells (BFC) and quadriflagellate cells (QFC) in fixed samples after mixing equal numbers of mt^+ and mt^- gametes and applying the formula: % cell fusion = (2 QFC × 100)/2 QFC + BFC.

Corpse Inactivation Assay

This assay is based on the ability of living gametes to inactivate the agglutinins carried on the flagella of glutaraldehyde-fixed gametes ("corpses") of opposite mating type: the more abundant or active the agglutinins, the longer it takes for living cells to inactivate them. In a typical assay, 1-ml aliquots of gametes (1×10^7 cells/ml) at successive stages of cAMP stimulation are mixed with 0.2 ml of 2.5% glutaraldehyde, fixed for 10 min,

1. *Abbreviations used in this paper:* db-cAMP, dibutyryl-cAMP; IBMX, isobutylmethylxanthine; NFHSM, nitrogen-free high-salt minimal medium; QFC, quadriflagellate cells.

pelleted, washed twice in NFHSM, and suspended at a final concentration of 1×10^7 corpses/ml. Successive samples (100 μl) are aliquoted to individual wells of a microtiter dish, living gametes of opposite *mt* (6×10^7 cells/ml, 25 μl) are added, and agglutination in each well is monitored with a dissecting microscope. Large clumps of agglutinating corpse/gamete groups initially form in all the wells, but the clumps disperse far more rapidly in the low titer than in the high titer samples. The time taken for dispersion of all clumps in a sample is recorded. This end point can be accurate only within 1–2 min, but the ratio of gametes to corpses used is such that the stimulated samples agglutinate at least 1 h longer than the controls, rendering irrelevant the 1–2-min error.

Agglutinin Bioassay

Flagella were isolated from 2×10^9 cells by pH shock (32), harvested, and suspended in 0.8 ml of 30 mM octylglucoside in 30 mM Hepes pH 7.4, 25 mM KCl, 5 mM $MgSO_4$, and 4% sucrose (HKMS) for 10 min with pipetting. Extracted axonemes were pelleted and the supernatant dialyzed overnight against 3 liters of water. Dialyzed extract was serially diluted in microtiter wells, and 1 λ aliquots were allowed to dry on glass slides. The ability of a suspension of gametes to adhere to the dried material was then assayed by phase microscopy (see reference 1 for details of this assay).

For cell body–agglutinin assay, control cells were treated with lysin (9, 20) to remove their walls (walls are shed by gametes treated with dibutyryl-cAMP [db-cAMP] and isobutylmethylxanthine [IBMX]). After deflagellation, the pelleted control and treated cells were shaken in 25 ml of 100 mM octylglucoside in HKMS plus 1 mM $CaCl_2$ for 10 min at room temperature. Cell breakage, as judged by phase microscopy, was virutally complete and was equivalent for all samples. The extracts were centrifuged at 40,000 *g* for 20 min and the supernatants dialyzed for 12 h against four changes of 3 liters of water. Bioassay was performed as for flagellar extracts.

Quick-Freeze Deep-Etch Transmission Electron Microscopy

Flagella were obtained from control and db-cAMP/IBMX–treated cells by pH shock. They were mixed with polylysine-coated mica flakes (13) in 10 mM Pipes pH 7.4, and 7% sucrose, the sucrose being necessary to maintain their straight configuration (32). The flakes with absorbed flagella were then pelleted and suspended in 1% glutaraldehyde in Pipes/sucrose. After a 5-min fixation they were again pelleted and washed in NFHSM to remove the nonetchable sucrose. Finally they were pelleted and quick-frozen as described (13). This procedure yields a much higher density of flagella per mica flake than obtained in our previous study (11).

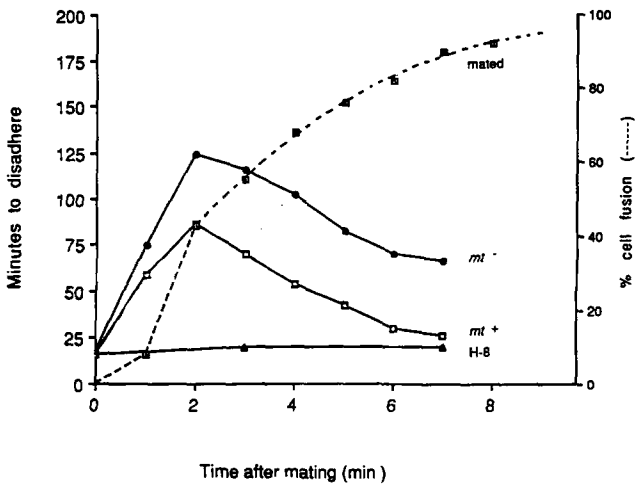


Figure 1. Gamete adhesivity during the native mating reaction. Wild type mt^+ and mt^- gametes were mixed at 1×10^7 cells/ml and, at 1-min intervals, 1-ml samples were withdrawn, mixed with 200 μl of 2.5% glutaraldehyde, fixed for 10 min, washed, and subjected to the corpse inactivation assay (see Materials and Methods). (□) *Plus* adhesive activity; (●) *minus* adhesive activity; (△) *plus* adhesive activity in gametes preincubated for 5 min in 1 mM H-8; (⊡) percent cell fusion.

Table I. Effect of db-cAMP/IBMX Incubation on Flagellar Agglutinability

Gamete type	Exposure to db-cAMP/IBMX in minutes 0	10	20	30	40	Fold increase
wt mt^+ gametes						
Experiment 1	12	30	93	93	93	7.8
Experiment 2	13	36	88	88	99	7.6
Experiment 3	15	15	65	72	87	5.8
Experiment 4	18	60	60	60	65	3.6
+ cycloheximide (10 μg/ml)					62	3.4
+ trifluoperizine (1 μg/ml)					59	3.3
imp-1 mt^+ gametes						
Experiment 1	12	19	46	48	48	4
Experiment 2	14	39	56	58	61	4.4

Gametes (wt mt^+ or *imp-1* mt^+) at 1×10^7 cells/ml were incubated for the indicated times in 10 mM db-cAMP + 1 mM IBMX in NFHSM. Each sample was fixed for 10 min, washed, and subjected to the corpse inactivation assay: the fixed cells were mixed with live mt^- testers and the time (in minutes) taken for all adhering clumps to disadhere was recorded.

Reagents

All drugs and chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) except H-8 (*N*-[2-(methylamino ethyl]-5-isoquinoline sulfonamine), (Seikagaku, Inc., St. Petersburg, FL) and octylglucoside (Calbiochem-Behring Corp., La Jolla, CA).

Results

Adhesivity During the C. reinhardtii Mating Reaction

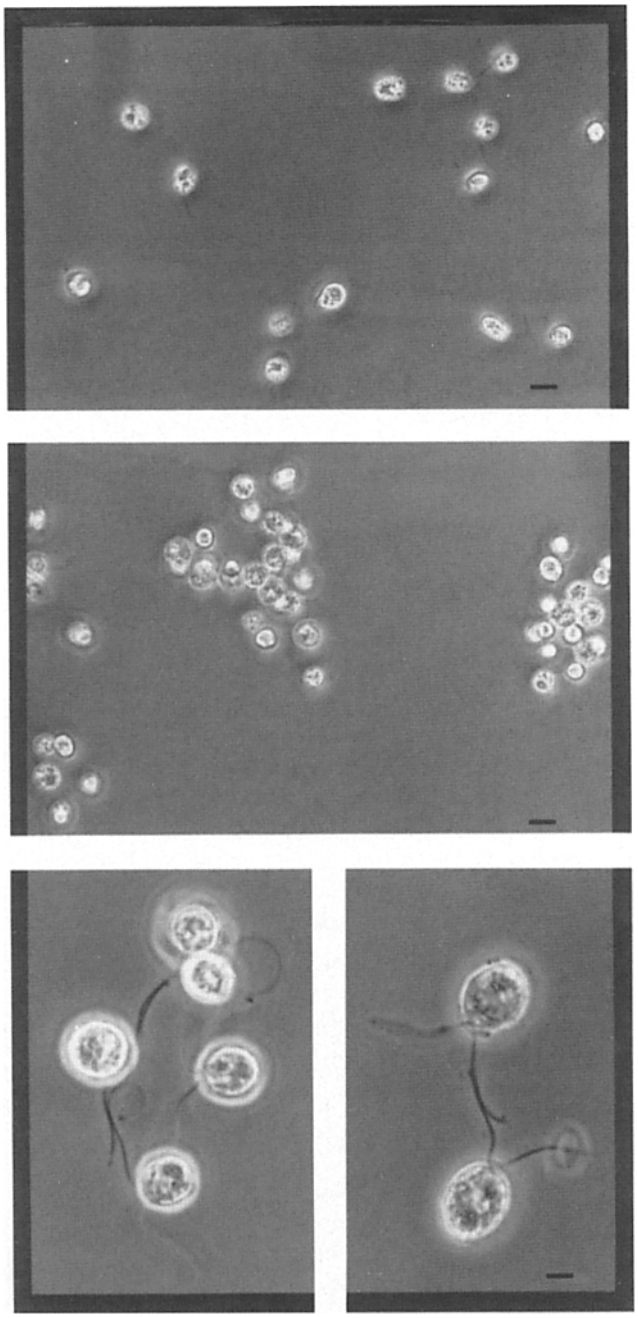
Previous studies have demonstrated that gametes of *C. eugamatos* increase their adhesivity during the mating reaction (5, 28). Fig. 1 documents that this also occurs during a *C. reinhardtii* mating: the agglutinability of the *plus* cells increases fivefold and that the *minus* cells sevenfold, with the expected subsequent decline in agglutinability coincident with the occurrence of cell fusion (18).

Enhanced Agglutinability Induced by cAMP

By using the same corpse-inactivation assay as in Fig. 1, both mt^+ and *imp-1* mt^+ gametes are also found to become more agglutinable when they are not mated but simply presented with 10 mM db-cAMP plus 1 mM IBMX (Table I). The kinetics of this increase parallels other mating responses (21) with the effect first evident after a 10-min exposure and maximal by 20 min, the lag presumably due to slow db-cAMP permeation. The magnitude of the increase ranges from four- to eightfold, with much of the variability contributed by the initial titers. Simultaneous exposure to 10 μg/ml cycloheximide, a potent inhibitor of protein synthesis in *C. reinhardtii* (3, 14), has no effect on this increase (Table I).

Evidence that Enhanced Adhesivity Results from Increased Agglutinins

During the mating reaction, adhesive foci rapidly move to the flagellar tips ("tipping") (9, 15), and it has been proposed that the resultant concentration of agglutinin proteins serves to stabilize the adhesion reaction (9). Several approaches were used to ask whether the increased agglutinability in-



Figures 2–4. Mixtures of mt^+ and mt^- corpses (at 1×10^7 cells/ml in NSHSM) fixed without treatment (Fig. 2) or after 30-min exposure to 10 mM db-cAMP + 1 mM IBMX in NFHSM (Fig. 3). Fig. 4 shows representative cells from the Fig. 3 sample; adhesive contacts are made all along the flagella and not just at the tips. Bars: (Figs. 2 and 3) 9 μm; (Fig. 4) 4 μm.

duced in gametes of a single *mt* by db-cAMP/IBMX was due to a rearrangement, and specifically a tipping, of existing agglutinin proteins.

The first entailed what can be called a "corpse mating." Glutaraldehyde-fixed gametes ("corpses") elicit a sex-specific adhesion from living gametes (10, 30), but when mt^+ and mt^- corpses are mixed together, they fail to adhere to one another (10; Fig. 2). If, however, gametes are separately treated with db-cAMP/IBMX for various periods, glutaraldehyde fixed and washed, and then mixed together, they display an increasingly rapid and extensive adhesion (Fig. 3). When the agglutinated corpses are observed at higher magnification (Fig. 4), the flagella are often adherent at various positions along their lengths and not just at the tips, ruling out full-scale tipping as the cause of the increased adhesivity.

Since a smaller scale "patching" of preexisting agglutinins would not be detected in the corpse-mating experiment, agglutinin titer was directly assessed. Control and db-cAMP/IBMX–stimulated gametes were deflagellated and their flagella were harvested, extracted with the detergent octylglucoside, and the extracts dialyzed against water and bioassayed (1). As shown in Table II, the flagella of treated gametes carried three serial dilutions more agglutinin activity than controls, an eightfold increase. Again, cycloheximide exposure did not affect the magnitude of the increase. Detergent extraction is expected to dissipate any patched configurations, and indeed, if one postulates the existence of detergent-resistant patches, one would predict a decrease in the resultant serial dilution titer compared with unpatched controls, rather than the observed increase. Therefore, this experiment appears to rule out local rearrangements as an explanation for the increased adhesivity.

Two other alternatives remained: preexisting agglutinins might somehow become activated, or additional agglutinins might be added to the flagellar surface. These alternatives could be evaluated by visualizing the density of agglutinin proteins on the flagellar surface before and after cAMP exposure using the quick-freeze deep-etch technique.

Agglutinins can be visualized by this technique only on flagella that have adsorbed to mica and are then largely fractured away, leaving behind the bottom-most (adsorbed) membrane for replication. When such images are obtained using flagella from unmated gametes, the agglutinins are seen disposed in linear rows along the longitudinal flagellar axis (11). Since, at most, only one row of agglutinins is ever encountered, we proposed (11) that the total flagellar surface must carry only a few such rows, the rest of the surface being agglutinin free. Quantitating this pattern for the present study,

Table II. Effect of db-cAMP/IBMX Incubation on Agglutinin Titer

Gamete type	Agglutinin titer		
	Control	db-cAMP/IBMX–treated	Fold increase
mt^+ flagella			
Experiment 1	2^7	2^{10}	8
Experiment 2	2^4	2^7	8
mt^- flagella			
Experiment 1	2^6	2^9	8
Experiment 2	2^4	2^7	8
mt^+ cell bodies			
Experiment 1	2^6	0	
Experiment 2	2^5	0	

Gametes (wt mt^+ or mt^-) at 4×10^7 cells/ml were incubated for 40 min in 10 mM db-cAMP + 1 mM IBMX in NFHSM. Cells were deflagellated, and the flagella and cell bodies were harvested separately and extracted with 30 and 100 mM octylglucoside, respectively. The extracts were dialyzed to remove detergent and bioassayed for agglutinin titer (1). The data are expressed as 2 to the power of the number of serial twofold dilutions required to reach the endpoint of detectable adhesion.

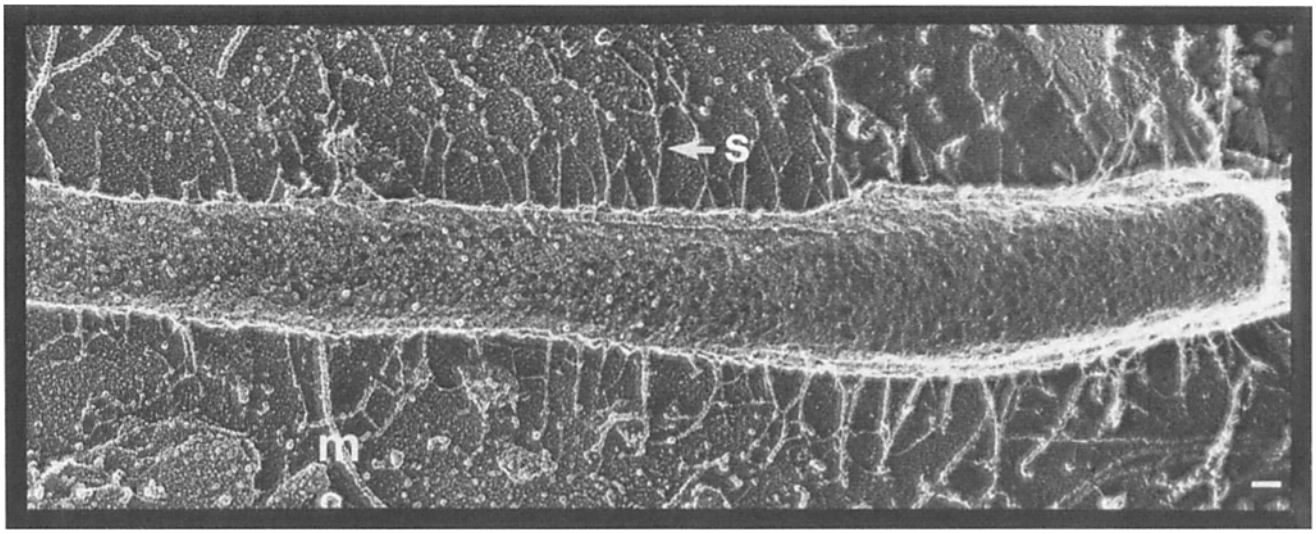


Figure 5. Flagellum isolated by pH shock from a gamete exposed to 10 mM db-cAMP + 1 mM IBMX for 40 min. Flagellum was adsorbed to polylysine-coated mica, in the presence of sucrose, washed in buffer, and quick-frozen, fractured, and deep-etched. *S*, stout fibers of agglutinin proteins; *m*, mastigoneme. Bar, 50 nm.

we found that when 26 control flagella were scored, 69% displayed one agglutinin row and 31% displayed no rows.

When fractured db-cAMP/IBMX-treated flagella are similarly analyzed, the images indicate that many more agglutinin rows populate the flagellar surface: of 20 flagella scored, none was devoid of agglutinin, 25% displayed one agglutinin row, and 75% now displayed two rows, one on each side (Fig. 5). There is no evidence for "patches": the stout fibers (*S*) formed by the agglutinin fibrils (9) are evenly spaced along each row in the same fashion as controls. Moreover, the density of agglutinins along a row is equivalent in control and treated samples. What appears to change, therefore, is the number of such rows per flagellum. While these observations do not rule out the occurrence of some "activation" of preexisting agglutinins, they demonstrate directly that db-cAMP/IBMX-stimulated gametes carry more abundant agglutinin displays, thereby obviating the need to postulate activation.

In studies to be reported elsewhere, we show that during the mating reaction, numerous vesicles bleb from the flagellar tips, cross-bridged by interacting agglutinins. In deep-etch replicas of quick-frozen db-cAMP/IBMX-treated gametes, no such vesicles are observed at their flagellar tips. Therefore, the recruitment of agglutinin is not accompanied by any such obvious form of membrane flow, and its mechanism is unknown.

Evidence that Additional Agglutinins Are Recruited from a Cytoplasmic Pool

Since cycloheximide does not interfere with the db-cAMP/IBMX-induced increase in adhesivity, the additional agglutinins presumably derive from a presynthesized pool. Saito et al. (24) have shown that when deflagellated gametes are broken in a French press, "cell body-agglutinins" are released and can be subsequently bioassayed. In the present study, deflagellated gametes were lysed with octylglucoside and their cell body-agglutinins were bioassayed. As documented in Table II, control extracts displayed good cell body-agglutinin activity whereas no activity could be detected in the lysates from db-cAMP/IBMX gametes, even after they were concentrated by lyophilization. Apparently, therefore, cAMP stimulates a recruitment to the flagellar surface of the cell body pool, the location of which is unknown (except that it is presumably intracellular since no agglutinin proteins are detected by quick-freeze deep-etch transmission electron microscopy on the surface of the *C. reinhardtii* plasma membrane).

Inhibitor Studies

The drug trifluoperizine (TFP), a calmodulin antagonist, blocks several mating responses (6, 21), but the blocked cells can be rescued by db-cAMP/IBMX (21). This pattern was observed as well for agglutinin recruitment: TFP blocks the rise in agglutinability in mating mixtures (data not shown) but has no effect on the increase induced by db-cAMP/IBMX (Table I).

The drug H-8 inhibits the activity of protein kinases, with a particular affinity for cAMP-dependent protein kinases (14), and it blocks wall loss and mating structure activation during *C. reinhardtii* matings (21). In the present study, H-8 was found to inhibit agglutinin recruitment during mating (Fig. 1). (We also assessed the effect of H-8 on flagellar tip activation since this was not analyzed in our previous report. In a mating wherein 50% of flagellar tips were activated when gametes were fixed after 3 min [$n = 67$], no tips were activated [$n = 59$] in a parallel H-8-treated sample).

Agglutinin Recruitment in "Reluctant Maters"

Our earlier study documented that db-cAMP/IBMX fails to restore agglutinability to mutants directly blocked in agglutinin biosynthesis (21). There exist, in addition, a number of *Chlamydomonas* strains whose adhesion is poor but not absent, strains we can designate reluctant maters. In the extreme, agglutination can be so poor that mating type, a useful genetic marker, is difficult to score. Application of db-cAMP/IBMX to such strains has proved to greatly improve their adhesivity.

One example is the *C. smithii* strain, a natural isolate dis-

tinct from, but interfertile with, *C. reinhardtii* (2), which has been extensively used in RFLP map construction (7, 23a). When *C. smithii* mt^+ is mixed with *C. reinhardtii* mt^-, agglutination ranges from poor to nondetectable, and the yield of zygotes is very low. If *C. smithii* is first incubated in db-cAMP/IBMX and then mated, however, widespread agglutination is observed, and when the yield of chloroform-resistant zygotes (17) is analyzed, the treated gametes generate 100 times more zygotes than the controls.

A second example is the mutant strain *gam*-1 mt^- which, when allowed to differentiate into gametes in liquid medium at 35°C, usually agglutinates poorly and hence fails to either transmit or receive sexual signals (8). If such gametes are incubated in db-cAMP/IBMX at 35°C for 20 min, their adhesivity improves markedly and they agglutinate with plus gametes as avidly as wild-type *minus* controls. There persists, nonetheless, a defect in cell fusion: in an experiment where cAMP/IBMX-treated wild-type gametes at 35°C displayed 70% fusion after 20 min of mating, the comparably treated *gam*-1 × mt^+ cells displayed only 4% fusion. Thus db-cAMP/IBMX by-passes the "upstream" defects in the *gam*-1 mutant and directly exposes the fusion block.

Zygotic Disadhesion

When two gametes fuse to form a QFC, the flagella become nonadhesive within seconds and the cell breaks away from the remaining clump of agglutinating gametes and swims freely (18). If, on the other hand, gametes are first incubated in db-cAMP/IBMX and then mated in the presence of the reagents, the QFCs remain in the clumps for many minutes, still agglutinating. Since their sexual adhesiveness is eventually lost, the drop in cAMP that normally occurs with QFC formation (21, 23) is apparently not required for disadhesion. A possible interpretation of this delay, therefore, is that the disadhesion mechanism takes longer to operate on flagella bearing the increased number of agglutinins present on db-cAMP/IBMX-treated cells.

Discussion

Snell and co-workers first showed (26, 27) that *Chlamydomonas* gametes carry only a portion of their agglutinin protein on the flagellar surface and mobilize presynthesized and then newly synthesized pools during the mating reaction (see also 4 and 24). Since mating entails both flagellar membrane vesiculation and agglutinin inactivation, it has been generally assumed that these events stimulate the mobilization of the pool, that is, that gametes somehow recognize that their flagellar surface display is depleted and respond by replenishing the supply.

The data presented here indicate that elevated levels of intracellular cAMP, the normal consequence of sexual adhesion (21, 23), are a sufficient stimulus to recruit the cytoplasmic pools: when gametes of a single mating type are stimulated with db-cAMP and IBMX, their pools are exhausted and their flagella acquire up to an eightfold increase in agglutinin titer, an increase that cannot be explained as a spatial rearrangement of existing proteins. It is further shown that certain *Chlamydomonas* strains that display flagellar agglutinin poorly can be stimulated to increase their adhesiveness by treatment with the db-cAMP/IBMX cocktail.

It is important to stress that whereas cAMP exposure does not induce any detectable rearrangements in the agglutinin display, such rearrangements clearly occur during the native mating reaction (11, 15): adhesive foci move to the flagellar tips, and agglutinin is concentrated there. Therefore, during the mating reaction, adhesivity is clearly increasing by both rearrangement and de novo recruitment, and is continuously being lost by inactivation and vesiculation; additional experimentation will be required to sort out the relative contributions of each. The important point being made here is that it is possible to elicit recruitment alone, without the occurrence of rearrangement, inactivation, or vesiculation, simply by elevating cAMP levels in gametes of a single *mt*, and that this recruitment is capable of rendering the gametes eightfold more adhesive.

When recruitment alone is operative, then the additional agglutinin is apparently added to the flagellar surface in an interesting fashion: the unmated flagellum appears to carry only one to two longitudinal rows of agglutinin, whereas the stimulated flagellum carries many more rows. If, as we have postulated (11), each row overlies a microtubule doublet, then if an unstimulated gamete were to carry one row, a stimulated gamete could carry up to nine rows, and eightfold increase in titer. Perhaps as a consequence of this added baggage, stimulated gametes swim in a slow, awkward fashion (although cAMP effects on flagellar motility may contribute here as well), and QFCs take much longer to disagglutinate.

The following picture therefore emerges. Adhesion initially involves the interaction of *plus* and *minus* agglutinins displayed constitutively by unmated gametes. Their interaction stimulates a rise in cAMP levels, and mating responses are induced that may entail protein phosphorylation (21). One of these responses is a mobilization of additional agglutinin; this would both contribute to overall adhesivity and replace an anticipated loss of agglutinin by inactivation/vesiculation. Note, however, that mobilization occurs even if replacement is not elicited by inactivation/vesiculation; that is, gametes are not "sensing" a loss of agglutinins per se, but rather are preprogrammed to anticipate such a loss, and compensate with a recruitment of the pool. Thus adhesion operates in a positive feedback fashion until a steady-state loss/replacement level is achieved, at which point intracellular cAMP levels are maximal and remain so until fusion occurs (21).

An interesting contrast emerges between hormone-receptor systems and adhesive systems. Hormone receptors, in both lower and higher eukaryotes, are typically "down-regulated" by the presentation of ligand, a process shown in several cases to be mediated by receptor phosphorylation (25). In contrast, several studies of adhesive interactions have detected the same sort of up-regulation encountered here, with initial contacts being "loose" and "reversible" and subsequent contacts being "stronger" and "more stable" (29, 31). These two phases may prove in some cases to entail two different kinds of adhesive interactions rather than an increased titer of a single adhesin, but the biological consequences are comparable: the first contacts are discriminatory and signal generating, the later contacts assure stable interactions and, at least in *Chlamydomonas*, the continued transmission of the signal.

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References

1. Adair, W. S., B. C. Monk, R. Cohen, C. Hwang, and U. W. Goodenough. 1982. Sexual agglutinins from the *Chlamydomonas* flagellar membrane. Partial purification and characterization. *J. Biol. Chem.* 257:4593–4602.
2. Bell, R. A., and J. R. Cain. 1983. Sexual reproduction and hybridization in *Chlamydomonas smithii* and *C. reinhardtii*. (Chlorophyceae, Volvocales). *Phycologia.* 22:243–247.
3. Bloodgood, R. A., E. M. Leffler, and A. T. Bojczuk. 1979. Reversible inhibition of *Chlamydomonas* flagellar surface motility. *J. Cell Biol.* 82:664–674.
4. Cooper, J. B., W. S. Adair, R. P. Mecham, J. E. Heuser, and U. W. Goodenough. 1983. *Chlamydomonas* agglutinin is a hydroxyproline-rich glycoprotein. *Proc. Natl. Acad. Sci. USA.* 80:5898–5901.
5. Demets, R., A. M. Tomson, W. L. Homan, D. Stegwee, and H. van den Ende. 1988. Cell-cell adhesion in conjugating *Chlamydomonas* gametes: a self-enhancing process. *Protoplasma.* 145:27–36.
6. Detmers, P. A., and J. Condeelis. 1986. Trifluoperazine and W-7 inhibit mating in *Chlamydomonas* at an early stage of gametic interaction. *Exp. Cell Res.* 163:317–326.
7. Ferris, P. J., and U. W. Goodenough. 1987. Transcription of novel genes, including a gene linked to the mating type-locus, induced by *Chlamydomonas* fertilization. *Mol. Cell. Biol.* 7:2360–2366.
8. Forest, C. L., D. A. Goodenough, and U. W. Goodenough. 1978. Flagellar membrane agglutination and sexual signalling in the conditional *gam-1* mutant of *Chlamydomonas*. *J. Cell Biol.* 79:74–84.
9. Goodenough, U. W., and D. Jurivich. 1978. Tipping and mating-structure activation induced in *Chlamydomonas* gametes by flagellar membrane antisera. *J. Cell Biol.* 79:680–693.
10. Goodenough, U. W., and R. L. Weiss. 1975. Gametic differentiation in *Chlamydomonas reinhardtii*. III. Cell-wall lysis and microfilament-associated mating structure activation in wild-type and mutant strains. *J. Cell Biol.* 67:623–637.
11. Goodenough, U. W., W. S. Adair, P. Collin-Osdoby, and J. E. Heuser. 1985. Structure of the *Chlamydomonas* agglutinin and related flagellar surface proteins in vitro and in situ. *J. Cell Biol.* 101:924–941.
12. Goodenough, U. W., P. A. Detmers, and C. Hwang. 1982. Activation for cell fusion in *Chlamydomonas*: analysis of wild-type gametes and nonfusing mutant strains. *J. Cell Biol.* 92:378–386.
13. Heuser, J. E. 1983. Procedure for freeze-drying molecules adsorbed to mica flakes. *J. Mol. Biol.* 169:155–195.
14. Hidaka, H., M. Inagaki, S. Kawamoto, and Y. Sasaki. 1984. Isoquinolinesulfanimides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase. *Biochemistry.* 23:5036–5041.
15. Homan, W. L., C. Sigon, W. van den Briel, R. Wagter, H. de Nobel, D. Mesland, A. Musgrave, and H. van den Ende. 1987. Transport of membrane receptors and the mechanics of sexual fusion in *Chlamydomonas eugamatos*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 215:323–326.
16. Deleted in proof.
17. Levine, R. P., and W. T. Ebersold. 1960. The genetics and cytology of *Chlamydomonas*. *Annu. Rev. Microbiol.* 14:197–216.
18. Lewin, R. A. 1952. Studies on the flagella of algae. I. General observations on *Chlamydomonas moewusii* Gerloff. *Biol. Bull. (Woods Hole).* 103: 74–79.
19. Martin, N. C., and U. W. Goodenough. 1975. Gametic differentiation in *Chlamydomonas reinhardtii*. I. Production of gametes and their fine structure. *J. Cell Biol.* 67:587–605.
20. Matsuda, Y., T. Saito, T. Yamaguchi, and H. Kawase. 1985. Cell wall lytic enzyme released by mating gametes of *Chlamydomonas reinhardtii* is a metalloprotease and digests the sodium perchlorate-insoluble component of cell wall. *J. Biol. Chem.* 260:6373–6377.
21. Pasquale, S. M., and U. W. Goodenough. 1987. Cyclic AMP functions as a primary sexual signal in gametes of *Chlamydomonas reinhardtii*. *J. Cell Biol.* 105:2279–2292.
22. Pijst, H. L. A., F. A. Ossendorp, P. van Egmond, A. M. I. E. Kamps, A. Musgrave, and H. van den Ende. 1984. Sex-specific binding and inactivation of agglutination factor in *Chlamydomonas eugamatos*. *Planta (Berl.).* 160:529–535.
23. Pijst, H. L. A., R. van Driel, P. M. W. Janssens, A. Musgrave, and H. van den Ende. 1984. Cyclic AMP is involved in sexual reproduction of *Chlamydomonas eugamatos*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 174: 132–136.

23a. Ranum, L. P. W., M. D. Thompson, J. A. Schloss, P. A. Lefebvre, and C. D. Silflow. 1988. Mapping flagellar genes in Chlamydomonas using restriction fragment length polymorphisms. *Genetics.* 120:109–122.

24. Saito, T., Y. Tsubo, and Y. Matsuda. 1985. Synthesis and turnover of cell body-agglutinin as a pool of flagellar surface-agglutinin in *Chlamydomonas reinhardtii* gamete. *Arch. Microbiol.* 142:207–210.
25. Sibley, D. R., J. L. Benovic, M. G. Caron, and R. J. Lefkowitz. 1987. Regulation of transmembrane signaling by receptor phosphorylation. *Cell.* 48:913–922.
26. Snell, W. J., and W. S. Moore. 1980. Aggregation-dependent turnover of flagellar adhesion molecules in *Chlamydomonas* gametes. *J. Cell Biol.* 84:203–210.
27. Snell, W. J., and S. Roseman. 1979. Kinetics of adhesion and deadhesion of *Chlamydomonas* gametes. *J. Biol. Chem.* 254:10820–10829.
28. Tomson, A. M., R. Demets, C. A. M. Sigon, D. Stegwee, and H. van den Ende. 1986. Cellular interactions during the mating process in *Chlamydomonas eugametos*. *Plant Physiol. (Bethesda).* 81:522–526.
29. Umbrit, J., and S. Roseman. 1975. A requirement for reversible binding between aggregating embyronic cells before stable adhesion. *J. Biol. Chem.* 250:9360–9368.
30. Wiese, L., and R. F. Jones. 1963. Studies on gamete copulation in heterothallic Chlamydomonads. *J. Cell. Comp. Physiol.* 61:265–274.
31. Wilcox, D. K., and M. Sussman. 1981. Serologically distinguishable alterations in the molecular specificity of cell cohesion during morphogenesis in *Dictyostelium discoideum*. *Proc. Natl. Acad. Sci. USA.* 78:358–362.
32. Witman, G. B., K. Carlson, J. Berliner, and J. L. Rosenbaum. 1972. *Chlamydomonas* flagella. I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. *J. Cell Biol.* 54: 507–539.