FLAGELLAR MEMBRANE AGGLUTINATION AND SEXUAL SIGNALING IN THE CONDITIONAL *GAM-1* MUTANT OF *CHLAMYDOMONAS*

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ABSTRACT

The temperature-sensitive gametogenesis-defective mutant, *gam-1* is sex-limited, expressed only in mating type minus (mt^-), and can sexually agglutinate but not fuse at the restrictive temperature (35°C) with gametes of wild type (*wt*) mt^+. Thin-section, freeze-cleave, and scanning electron microscopy reveal that the *gam*-1 phenotype is dependent on both the temperature at which the cells undergo nitrogen starvation (and therefore gamete formation) and the temperature at which the cells are maintained during the 12 h before mating. Under all conditions of gametogenesis at 35°C, each *gam-1* cell produces a normal-appearing membrane-associated mating structure that fails to activate in response to flagellar agglutination. Varying with the conditions of gametogenesis, on the other hand, are the agglutination and signaling properties of the *gam-1* flagella. The two mutant phenotypes displayed by *gam-1* have been denoted *gam-1*-I and *gam-1*-II. An agglutination reaction involving *gam-1*-I cells does not result in activation of the *wt* mt^+ mating structure. A more stable agglutination reaction, which can result in activation of the *wt* mt^+ mating structure, is characteristic of *gam-1*-II cells, but because the *gam-1* mt^- mating structure still fails to activate, cell fusion is precluded. We conclude that the *gam-1* mutation affects flagellar component(s) involved in establishing an effective, signal-generating agglutination reaction.

KEY WORDS mutant · temperature sensitivity · flagellar membrane · sexual agglutination · signaling · mating structure

The sexual cycle of the single-celled eukaryote *Chlamydomonas reinhardi* represents an easily controlled developmental process. Gametogenesis is initiated when the haploid cells are starved for nitrogen (11). Both mating types then develop the ability to agglutinate by their flagellar tips (6, 11), a reaction that occurs immediately when gametes of opposite mating types are mixed. During gamete formation, mating-type-specific mating structures also develop in association with the plasma membrane (2, 6, 9, 14, 17). These mating structures remain unactivated unless stimulated by some sort of sexual agglutination. The activated structure of mating-type plus (mt^+) is a long,

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microfilament-filled tube that is readily detected by many forms of microscopy (2, 6, 9, 12, 14). The activated mating-type minus (mt^-) mating structure is difficult to distinguish from its unactivated counterpart except by freeze-fracture electron microscopy, where it appears as a particle-rich area of the cell membrane (17). Fusion is accomplished when these two structures, brought into proximity by the agglutination reaction, initiate the rapid process of zygote formation (under appropriate conditions an entire population can form quadriflagellated zygotes in 5–10 min).

A particularly effective method for studying gametogenesis appears to be the analysis of mutants defective in the various gamete-specific properties required for zygote formation. One such mutant, *gam-1*, carries a temperature-sensitive defect: it can agglutinate but not fuse at the restrictive temperature (35°C), while behaving normally at the permissive temperature (25°C) (5). When *gam*-1 cells that have undergone gametogenesis at 35°C are shifted down to 25°C, zygotes are first detected after ~45 min, although at least 1½ h is needed before a large number of zygotes are seen. This recovery is cycloheximide insensitive (5). Quantitative loss of zygote-forming ability requires at least 12 h at 35°C if gametogenesis takes place at 25°C (3). A particularly interesting property of the *gam-1* mutation is that its expression is sex-limited, affecting zygote formation in mt^- but not mt^+ gametes (5).

The present paper describes experiments that probe the cellular nature of the *gam-1* defect. We conclude from these experiments that the *gam-1* mutation affects the ability of mt^- cells to participate effectively in two steps of the signal-generating agglutination reaction at the restrictive temperature. As a result, agglutination is not followed by mating-structure activation in *gam-1*, and the ability to cause wild-type (*wt*) mt^+ activation proves to depend upon the conditions under which the *gam-1* cells are shifted to 35°C. In all cases, no gamete fusion occurs. Preliminary reports of some of these observations have been previously published (4, 7).

MATERIALS AND METHODS

Wt mt^+ and mt^- of strain 137c, and the mutants *gam-1* mt^- (5) and *imp-5* mt^+ (9), both derived from 137c, were used for these experiments.

Cell cultures were routinely maintained on yeast acetate (YA) medium (5) or Tris acetate phosphate (TAP) (10). Continuous illumination was maintained for all growth and gametogenic regimes. For liquid induction of gametogenesis, cells were suspended in autoclaved tap water, in liquid nitrogen-free, high salt medium (HSM-N) (10), or in nitrogen-free TAP medium (TAP-N). For plate induction of gametogenesis (10), cells were streaked or poured onto one of the N-free media solidified with 1.5 or 2% agar. Plate gametes can also be obtained when cells have exhausted the nitrogen from complete medium. Zygotes were matured on minimal acetate (MA) medium (5), after a standard light-dark-light cycle (5).

Procedures for thin-section, freeze-fracture, and negative staining were as described previously (1, 9). Specimens were examined with a Philips 300, Hitachi HU-llc, or a Zeiss ME10 electron microscope. Samples for scanning were fixed by a modification of the procedure previously described (1). Cells were first fixed in cold 3% glutaraldehyde in 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethane sulfonic acid (HEPES), pH 7, for at least 12 h. The glutaraldehyde was then replaced with cold 2% OsO_4 in 4 mM potassium phosphate buffer, pH 6. After 2–4 h in OsO_4, washed cells were layered onto a nucleopore filter held in a Millipore filter holder (Millipore Corp., Bedford, Mass.), and the preparation was exposed to increasing concentrations of acetone, or increasing concentrations of ethanol followed by 100% acetone. After critical point drying and coating with carbon and Au/Pd, the cells were observed with an AMR model 1000 scanning electron microscope.

Cells were scored for mating-structure activation as follows: The unactivated mt^+ mating structure can be recognized by thin-section or freeze-fracture electron microscopy (9, 10), while the fertilization tubule, the activated mt^+ structure, is detectable by the above techniques as well as by negative staining and SEM. The mt^- mating structure can be seen by thin-section and freeze-cleave analysis (9, 10), but activation of this structure can only be unambiguously determined by freeze-fracture electron microscopy. Cells were deflagellated by the pH shock method described by Witman et al. (18).

RESULTS

Growth Conditions Affecting the Gam-1 Phenotype

Conflicting reports on the agglutination properties of *gam-1* (4, 5) have proved to be the consequence of variations in the conditions used to induce gametogenesis.

Cultures of both *wt* and mutant *Chlamydomonas* strains are routinely grown on a solid complete medium at 25°C. After a 4–5-day growth period, the cells begin to exhaust the nitrogen in the medium and, as a result, they differentiate into gametes (10). Before mating, such cells are usually suspended in a liquid N-free medium for 1–2 h. Because of the temperature-

sensitivity of the *gam-1* mutation, a minimum of 12 h in liquid medium at the restrictive temperature (35°C) had been previously found to be necessary to insure against leakiness (i.e., to ensure that no zygotes will be formed when *gam-1* is mated with wt^+) (3). In the present study, the following growth and gametogenesis regimes were employed to analyze the expression of the *gam-1* mutation.

GAMETIC DIFFERENTIATION AT 25°C, SHIFT-UP IN LIQUID: Under this regime, *gam-1* cells are allowed to undergo differentiation on plates containing complete medium, for 1 wk at 25°C, as a consequence of exhausting available nitrogen (as described above). To induce the temperature-sensitive mutant phenotype, the cells are suspended in liquid N-free medium and the suspension is shifted to 35°C overnight (12 h being the minimum time necessary to lose all normal zygote-forming ability, as documented in reference 3).

DIFFERENTIATION AT 25°C, SHIFT-UP ON SOLID MEDIA: Under this regime, *gam-1* cells also differentiate into gametes for 1 wk on 25°C plates containing complete medium. To induce the temperature-sensitive mutant phenotype, however, the cells are either left on their nitrogen-depleted plates or transferred to fresh plates containing solidified N-free media, and are maintained at 35°C overnight. 1–2 h before mating, they are suspended in a liquid N-free medium at 35°C.

VEGETATIVE GROWTH FOLLOWED BY INDUCTION OF GAMETOGENESIS AT 35°C: Vegatative growth is initiated by inoculating TAP plates with ~2 ml of a cell suspension, and the plates are maintained at either 25° or 35°C for 2 days, during which period the nitrogen is not exhausted and differentiation does not occur (11). The cells are then either suspended in N-free liquid or placed on N-free plates, and gametogenesis is allowed to occur overnight at 35°C.

As noted in the Introduction, both the agglutinating and signaling abilities of *gam-1* cells depend on the mode of gametogenic induction. The first and third methods for gametic induction, namely, 25°C differentiation followed by a shift-up in 35°C liquid, or else a 35°C differentiation, prove to generate gametes with an apparently identical phenotype. Observations made on cells of these two classes have therefore been pooled, and the gametes prepared in either fashion are designated at Type I gametes (*gam-1*-I). These are contrasted with Type II gametes (*gam-1*-II) that are prepared by the second method, namely a shift-up on 35°C solid medium.

In the sections that follow, we describe and compare the agglutination and signaling properties of *gam-1*-I and *gam-1*-II gametes. In the Discussion section, we consider how the various growth conditions employed may generate gametes having these two different phenotypes.

Sexual Agglutination by Gam-1-I Gametes

Gam-1-I gametes retain at least part of the normal mt^- flagellar agglutinability, as evinced by their ability to recognize specifically, and adhere to, the flagella of *wt* mt^+ gametes. The agglutination reaction that develops is, however, different from a *wt* (*wt* mt^- × *wt* mt^+) reaction in a number of respects.

First, whereas the *wt* cells can be seen by phase microscopy to adhere by their flagellar tips (1, 11, 12), the flagella in a *gam-1*-I × *wt* mating interact quite differently: many tips are obviously free, and the agglutination occurs either somewhat down from the tip or very near the cell body. The overall effect is that the cell bodies in the agglutinating clumps appear much closer together than is characteristic of *wt*. This effect is not seen after cells have been shifted to 25°C for several hours.

A second abnormal feature of the *gam-1*-I agglutination reaction was discovered when we attempted to fix agglutinating cells for examination with the scanning electron microscope. Whereas *wt* cells are readily fixed in their agglutinating configurations at 25°C (9) and at 35°C by the addition of low concentrations of glutaraldehyde, repeated attempts to cross-link agglutinating *wt* mt^+ × *gam-1*-I mt^- cells with a range of glutaraldehyde concentrations were uniformly unsuccessful: the cells immediately came apart. Again, glutaraldehyde cross-linking can be readily achieved when these cells have been shifted to 25°C for several hours.

A third unusual feature of the agglutination between *gam-1*-I and *wt* gametes is that it is more sensitive to the ionic strength of the medium. Specifically, the cells appear to form larger clumps in distilled or tap water than they do in 2.5 mM sodium acetate. This sensitivity explains, at least in part, the weak and sporadic agglutination previously reported (4), when matings were carried out in nitrogen-free, high salt medium. Further

analysis revealed that liquid made 50–100 mM with a variety of salts could disrupt the agglutination reaction of both *gam-1*-I $mt^- \times wt\ mt^+$ and of $wt\ mt^- \times wt\ mt^+$, an effect that occurred whether or not the solution was buffered. If, however, the ions were not added immediately upon mating of *wt* × *wt*, some of the cells formed clumps that could not be disrupted by the ions, and the percentage of cells in these clumps increased very rapidly with time until the majority of cells in the population were quadriflagellate (that is, had mated). When *gam-1*-I × *wt* matings were analyzed in the same fashion, all of the cell clumps fell apart regardless of the length of time the agglutination reaction had proceeded.

Fourth, the *gam-1*-I × *wt* agglutinating clumps are very susceptible to mechanical disruption from as simple an operation as pipetting agglutinating cells from a test tube to a slide.

Finally, if *gam-1*-I cells are deflagellated, the isolated flagella themselves will effect an isoagglutination of $wt\ mt^+$ gametes, which has the same abnormal properties as the agglutination of intact *gam-1*-I cells with *wt*.

Taken together, these results suggest that a mt^--specific flagellar recognition can take place between *wt* and *gam-1*-I gametes at the restrictive temperature but that this interaction is distinctly different from the *wt* interaction that generates a signal-to-fuse.

Sexual Agglutination by Gam-1-II

The agglutination reaction between *gam-1*-II gametes and $wt\ mt^+$ is very different from that described above and appears, instead, to be similar to a *wt* agglutination reaction. The cells adhere by their flagellar tips. The clumps of mating cells can be fixed in their agglutinating configurations with glutaraldehyde and are not unusually susceptible to mechanical disruption. Furthermore, flagella obtained from 35°C plate-induced *gam-1* cause an apparently normal isoagglutination reaction.

Analysis of Mating-structure Activation in a Gam-1-I *Mating*

For thin-section and freeze-cleave transmission electron microscope analysis, and for scanning electron microscopy, an aliquot of *gam-1*-I gametes was mated with $wt\ mt^+$ gametes or with flagella from these gametes. Additional aliquots of these *gam-1* cells were shifted down to 25°C and mated with the mt^+ gametes or flagella after varying lengths of time.

The unactivated *gam-1*-I mt^- mating structure at 35°C proves to be indistinguishable from its *wt* mt^- counterpart (17) by both thin-section and freeze-fracture analysis. Therefore, the nonfusing phenotype of *gam-1* is clearly not caused by a failure to produce an mt^- mating structure at 35°C. When *gam-1*-I cells are mixed with $wt\ mt^+$ at 35°C, however, no evidence of *gam-1* mt^- mating-structure activation is encountered in either thin-section (Fig. 1) or freeze-cleave (Fig. 2) specimens (Table I). Activation does not occur until the mating mixtures are shifted down to 25°C. During recovery, which begins after 45 min and has been followed for 3 h, activated images of the *gam-1*-I mt^- mating structures are encountered (Table I), and the cells go on to fuse.

These observations might be taken to suggest that the *gam-1*-I mating structure, although normal in appearance at 35°C, is incapable of activating at the restrictive temperature. Examination of the $wt\ mt^+$ cells in the 35°C mating mixtures, however, suggests that the situation is more complex. Whereas $wt\ mt^+$ cells activate and fuse normally when mixed with $wt\ mt^-$ cells of flagella at 35°C, no fusion or activation of mt^+ mating structures is observed when $wt\ mt^+$ cells are mixed with *gam-1*-I cells (Table I), even though an agglutination reaction is occurring. Scanning electron microscopy also reveals no mt^+ activation. It is only after temperature shift-down that the $wt\ mt^+$ cells begin to activate (Table I). In other

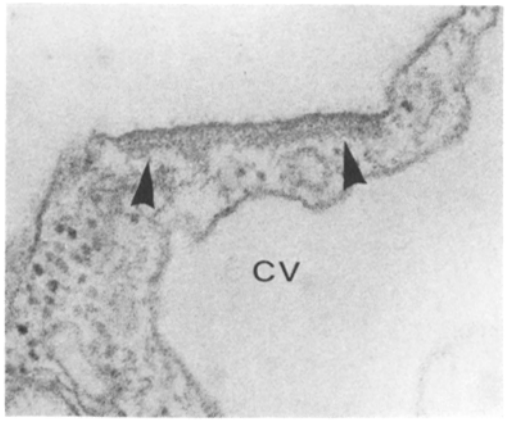

FIGURE 1 An unactivated *gam-1*-I mt^- mating structure (thin-section) is indicated by arrowheads. From a 60-min incubation of 35°C (H_2O) liquid-induced *gam-1* × $wt\ mt^+$ (*cv*, contractile vacuole). × 101,000.

words, it appears that the 35°C *gam-1*-I agglutination fails to send a signal to fuse, not only to the mutant cells but to the *wt* cells as well. The data in Table I represent *gam-1*-I cells that were allowed to become gametic at 25°C before shift-up. Identical results have been obtained with *gam-1*-I cells obtained by all methods of gametogenesis described previously (see also Table III).

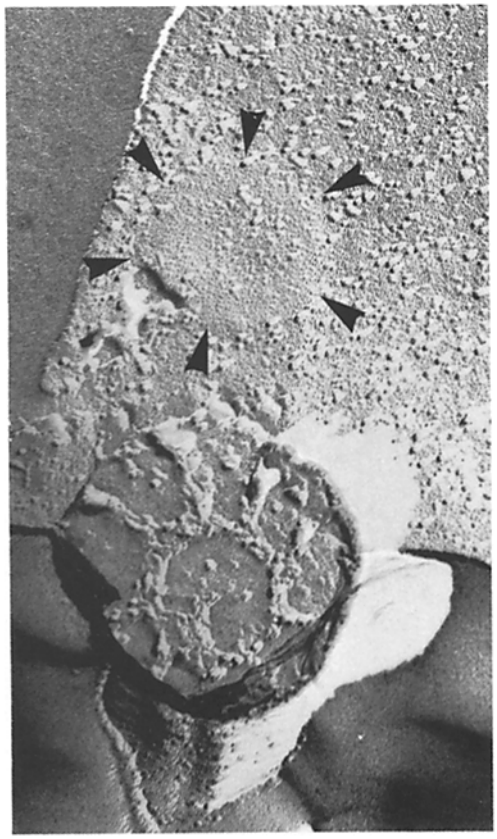

FIGURE 2 An unactivated *gam-1*-I mating structure (freeze-fracture, E face), the circular area on the membrane that is essentially particle-free, is delimited by arrowheads. In the foreground, a basal body and its associated struts (16) are seen in cross-fracture. × 101,000.

Analysis of Mating-Structure Activation in a Gam-1-II Mating

In a mating between *gam-1*-II cells and *wt* mt^+, the *gam-1* mating structures are again found to be unactivated (Table II and Fig. 3 and 4). That *gam-1* mating structures can take on an activated morphology at all was shown by shifting such a mixture to 25°C: activated images, such as those shown in Fig. 5–7, were obtained after the cells had been at 25°C for at least 45 min (see also Table II). We should note in passing that the doming of the central area of the activated mt^- mating structure, described in reference 17, was not observed in the present study, nor has it been observed in *wt* mt^- controls. Possibly the amphotericin used to obtain isolated flagella caused the doming effect. The arrangements of particles in the unactivated and activated mt^- mating structures were otherwise comparable to those reported in the earlier study (17).

In striking contrast to the "nonstimulating" agglutination of *gam-1*-I, the *gam-1*-II cells can elicit normal mating-structure activation in their *wt* mt^+-partners (Fig. 8 and 9 and Table II), with the percent of activated cells varying somewhat from one cross to the next. Thus, the *wt* cells are now able to detect that sexual agglutination has occurred, even though the *gam-1*-II cells still do not respond and cell fusion still fails to occur.

Analysis of Antiserum Effects on Cell Fusion

The experiments presented above, and analyzed in the Discussion section are consistent with the notion that *gam-1* cells are unable to fuse with *wt* cells at 35°C because their flagella are abnormal and incapable of establishing a signal-generating agglutination reaction. An independent test of this hypothesis was therefore performed. It has been shown that for various *imp* mutant strains that have a defective sexual agglutinin but a nor-

TABLE I

Effect of Temperature on Mating-Structure Activation in gam-1-I mt^- × *wt* mt^+ *Crosses*

	No. *gam-1* mating structures unactivated	No. *gam-1* mating structures activated	No. *wt* mating structures unactivated	No. *wt* structures activated
Mixed at 35°C for 15–60 min	10	0	40	0
Mixed at 35°C for 15 min, shifted to 25°C for a minimum of 45 min	3	2	8	7

25°C gametogenesis, 35°C overnight in liquid. Data from thin-section and freeze-fracture observations.

TABLE II

Effect of Temperature on Mating-Structure Activation in Gam-1-II × wt mt⁺ Crosses

	No. *gam-1* mating structures unactivated	No. *gam-1* mating structures activated	No. *wt* mating structures unactivated	No. *wt* mating structures activated	No. mating structures unactivated
35°C × opposite *mt* flagella	15	0	12	44	–
35°C × opposite *mt* cells	–*	0	–*	35	92
25°C × opposite *mt* flagella	1	9	26	19	–

Data from scanning and freeze-fracture observations.

* Because the *mt* of unactivated cells can be difficult to determine accurately, all unactivated cells are grouped into one column for cell × cell matings.

FIGURE 3 An unactivated *gam-1*-II *mt*⁻ mating structure (freeze-fracture, E face). From a 10-min incubation of 35°C (TAP-N) plate-induced *gam-1* × *wt mt*⁺ gametic flagella. Bracelet grooves (arrowheads) (16) surround the flagellum. The circular mating structure at the top right is essentially particle free. × 104,500.

FIGURE 4 An unactivated *gam-1*-II *mt*⁻ mating structure (freeze-fracture, P face) is seen in the bottom left. From the experiment described in Fig. 3. The flagellar bracelet is seen above the mating structure. × 104,500.

mal signaling capacity, administration of antiserum directed against the flagellar surface bypasses the agglutination defect and allows the mutant cells to fuse normally with *wt* cells of the opposite

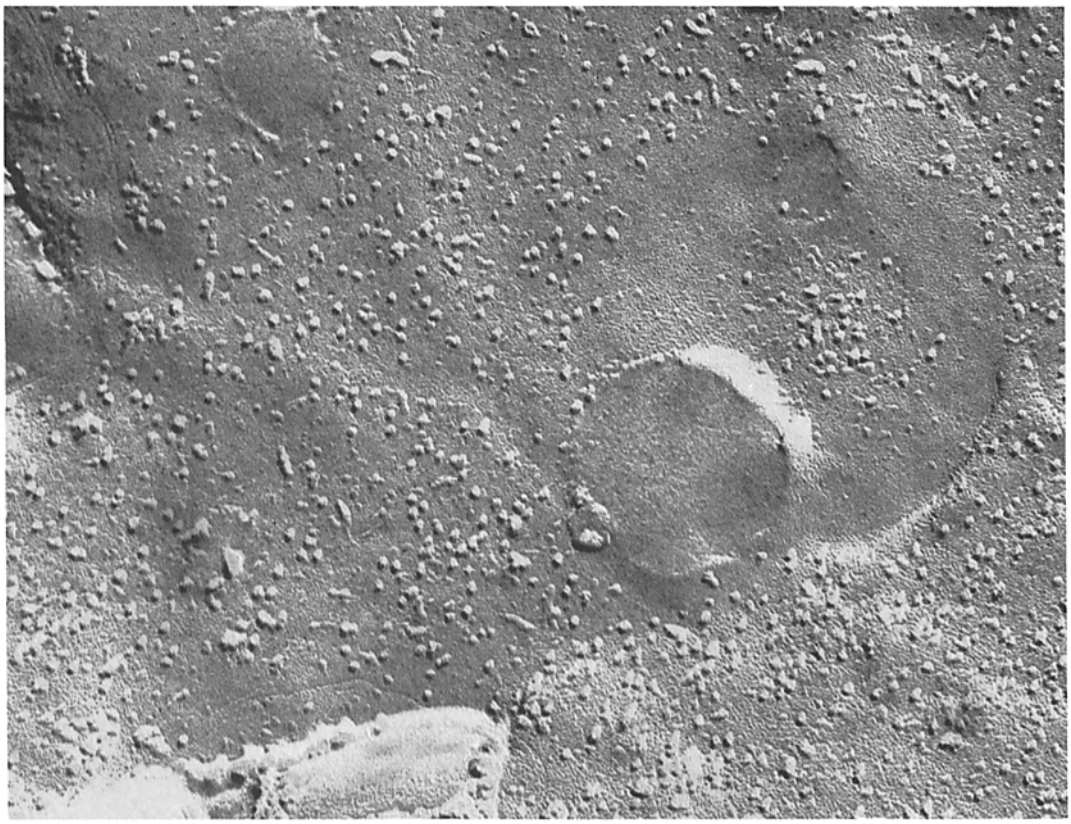

FIGURE 5 An activated *gam-1*-II mt^- mating structure (freeze-fracture E face). From a 10-min incubation at 25°C (TAP-N) plate-induced *gam-1* × *wt* mt^+ gametic flagella. The mating structure, containing particles in the center, is seen at the right. Flagellar bracelet grooves from both flagella can be seen to the left of the mating structure. The round structure in the lower left of the mating structure is probably an artifact. × 104,500.

mt.[1] We therefore asked whether *wt* mt^+ × *gam-1* mt^- mating mixtures could be induced to fuse if antibody were provided.

A wide range of antiserum concentrations, with antiserum: cell suspension ratios ranging from 2:1 to 1:500, fails to elicit any zygote formation between *gam-1* and *wt* gametes as long as the temperature remains at 35°C. In contrast, good zygote formation occurs when the nonagglutinating *imp-5* mutant strain is mated at 35°C in the presence of any of these concentrations of antiserum. Furthermore, when the *gam-1*-I cells are shifted down to 25°C in the presence of antiserum, fusion can occur.

[1] Goodenough, U. W., D. Jurivich, and S. Brauser. Antibody-induced mating-structure activation and cell fusion in *Chlamydomonas* gametes. Manuscript submitted for publication.

DISCUSSION

Although the gene product (or products) specified by the normal *gam-1* allele have not yet been identified, our results, summarized in Table III, suggest that this product is a flagellum component and not, for example, a component of the cell fusion apparatus. First, the flagella of *gam-1*-I cells are clearly abnormal in their agglutination pattern, (Table III, line 1) an abnormality that persists even when the flagella are isolated from the cells, and second, the ability of *gam-1* flagella to signal activation to *wt* cells is dependent on the same set of induction conditions as is the ability of these flagella to agglutinate normally (Table III, line 2). Clearly this point cannot be proven until thermolabile molecular species are demonstrated in *gam-1* flagella; experiments designed to detect such flagellar components are now in progress.

Assuming that the *gam-1* defect indeed affects

FIGURE 6 An activated *gam-1 mt*$^-$ mating structure (freeze-fracture, E face), is seen in the top right. From the experiment described in Fig. 5. Bracelet grooves from both flagella can be seen below the mating structure. × 95,000.

the flagellum, we can explain the various *gam-1* phenotypes in the following way.

If *gam-1 mt*$^-$ cells undergo gametogenesis at 35°C, they assemble defective gametic flagella which can recognize *wt mt*$^+$ flagella but lack the ability to translate this initial recognition event into what we can denote as an "effective" signal-generating agglutination (see also reference 13). As a result, neither the *wt* nor the normal gametes respond to the agglutination by activating their mating structures, and the mutant strain is said to exhibit a *gam-1*-I phenotype (Table III, columns 1–4).

If *gam-1 mt*$^-$ cells instead undergo gametogenesis at 25°C and are then shifted to 35°C for ~16 h, the resultant phenotype depends critically on whether the cells are transferred to liquid or solid medium. In liquid medium at 35°C throughout differentiation; neither they nor *wt mt*$^+$ respond to agglutination (Table III, column 5). Shift-up on solid medium, on the other hand, produces *gam-1* cells that can now signal to their *wt mt*$^+$ partners, even though they cannot respond to agglutination themselves, a state that we denote as *gam-1*-II (Table III, column 6).

The most likely explanation for the difference between shift-up in liquid or solid medium is provided by the studies of Bergman et al. (1) and Snell (12), where it was demonstrated that *C. reinhardi* gametes continuously shed flagellar membrane vesicles into liquid media. It seems reasonable to postulate that a normal *gam-1* gametic flagellum, assembled at 25°C, could be converted into a mutant *gam-1*-I flagellum by a continuous sloughing-off and replacement process

FIGURE 7 An activated *gam-1 mt*$^-$ mating structure (freeze-fracture, P face) is seen in the lower right. From the experiment described in Fig. 5. A flagellar bracelet is seen above the mating structure. The round structure seen in the upper left of the mating structure is probably an artifact. × 104,000.

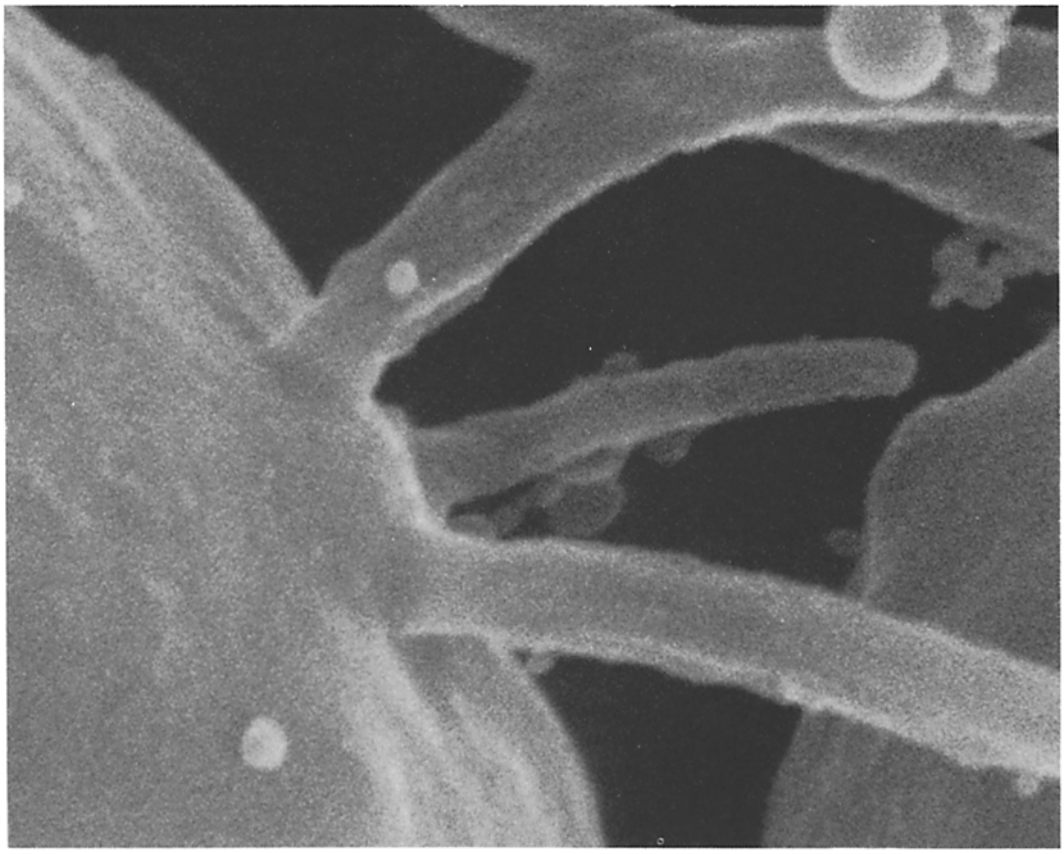

FIGURE 8 An activated *wt* mt^+ mating structure as seen by scanning electron microscopy, the easiest way we have found to definitively show mt^+ activation. From a 10-min mating of 35°C (TAP-N) plate induced *gam-1* × *wt* mt^+ gametes. The mating structure can be seen to attain a normal length (here *1.25* μm). (Forest and Goodenough, in preparation). × 91,200.

occurring at 35°C in liquid medium. Normal *gam-1* gametes shifted to 35°C on solid media would not, on the other hand, be expected to carry out such a vesiculation process since they are immobile. Indeed, we find that, like *wt* cells (11), many *gam-1* gametes fail to grow any flagella on plates (the percent of unflagellated cells increasing with increasing culture age), and the flagella that are present are only half the length of those found on gametes suspended in liquid for 1 h. Therefore, the full-length flagella that assemble when *gam-1*-II cells are suspended in liquid might well be expected to display both normal features (derived from their 25°C differentiation period) and mutant features that developed as a consequence of the 12-h temperature shift-up.

The distinct properties of the *gam-1*-I and *gam-1*-II cells provide, we believe, clues as to the nature of the flagellar interactions that accompany mating in *Chlamydomonas*. If we regard *gam-1*-I cells as manifesting a "pure" *gam-1* phenoype, then this sex-limited mutation clearly prevents any signaling interactions. The inability to generate a signal appears correlated with the lack of preference for the flagella tips during the agglutination reaction; it is also correlated with a sexual agglutination that is unusually sensitive to glutaraldehyde, ionic strength, and mechanical disruption. Finally, *gam-1* flagella cannot be stimulated to signal by the application of antiserum. Because the antiserum is presently postulated to function by bringing signaling components together in the membrane,[1] it would clearly be ineffective when applied to a membrane where signaling components were absent.

One way to explain such properties is to postulate that mt^- gametic flagella possess a vectorial concentration mechanism which, when activated

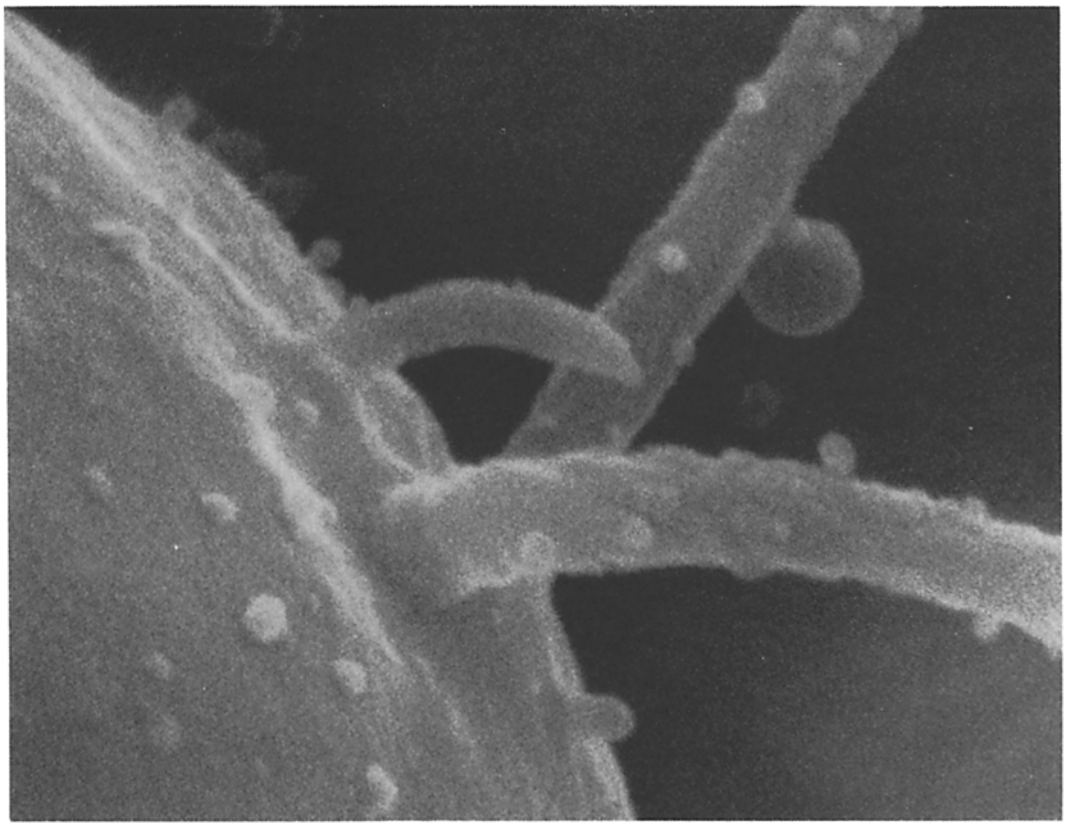

FIGURE 9 An activated *wt* mt^+ mating structure (scanning electron microscopy). From the experiment described in Fig. 8. This structure shows the typical bend seen in scanning views of the mt^+ structure (Forest and Goodenough, in preparation) and inferred from thin-section studies (9). × 95,000.

TABLE III

Phenotype of gam-1 mt^- after 35°C Overnight

Prior Growth Conditions	35°C Vegetative*		25°C Vegetative*		25°C Gametic‡	
	Liquid	Plate	Liquid	Plate	Liquid	Plate
Final 16 h in N-free at 35°C in/on						
1. Agglutination	mutant	mutant	mutant	mutant	mutant	*wt*
2. Ability to activate mt^+	–	–	–	–	–	+
3. Ability to fuse with mt^+ (under normal mating conditions)	–	–	–	–	–	–
4. Phenotype	*gam-1*-I	*gam-1*-I	*gam-1*-I	*gam-1*-I	*gam-1*-I	*gam-1*-II

* 2 days growth on solid medium containing nitrogen.

‡ 5 or more days growth on solid medium containing nitrogen.

by sexual or antiserum agglutination, brings dispersed signaling components in the flagellar membrane towards the flagellar tip, the signaling reaction taking place when a sufficient density of components builds up at the tip. If we postulate that the *gam-1* locus specifies one or more components of this concentration system and that a comparable mt^+-specific system exists in the mt^+ gametic flagellum, we can then imagine that during a normal mating, signaling components are moved to the tips of one or both agglutinating flagella. In a *gam-1*-I × *wt* mt^+ mating, no such interactions are possible and the adhesion remains "loose" and readily disrupted. Finally, in a *gam-*

1-II × *wt* mt^+ mating, sufficient normal *gam-1* product (produced during the period of 25°C growth) is present in the *gam-1*-II flagellum to elicit signal generation in mt^+ and a "tight" agglutination between the two mating types, but not enough is present to achieve the requisite, signal-generating concentration at the *gam-1* flagella tips.

Although alternate pictures of the *gam-1* phenotype can, of course, be contrived, our working hypothesis at present is to propose that the same feature(s) of flagellar membrane organization, and perhaps even the same molecular species, are involved in both tight adhesion and signal generation, and that these feature(s) are rendered temperature-sensitive by the sex-limited *gam-1* mutation.

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