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Flagellar Adhesion and Deadhesion in Chlamydomonas Gametes: Effects of Tunicamycin and Observations on Flagellar Tip Morphology

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The aggregation-dependent loss of flagellar adhesiveness in Chlamydomonas reinhardi has been correlated with changes in flagellar tip morphology during adhesion and deadhesion. As aggregating mt^- and impotent (able to adhere, but not fuse) mt^+ gametes begin to disaggregate in the presence of the protein synthesis inhibitor cycloheximide, there is a concomitant change in flagellar tip morphology from the activated bulbous form to the nonactivated tapered shape. The requirement of protein-synthetic activity for the maintenance of flagellar adhesiveness during aggregation may be due in part to turnover of proteins involved in formation or stabilization of activated flagellar tips.

Incubation of aggregating gametes with tunicamycin indicates that, like protein synthesis inhibitors, this inhibitor of glycosylation also causes adhering gametes to deadhere. The results suggest that protein glycosylation may be essential for maintenance of adhesiveness during aggregation.

Key words: Chlamydomonas, flagellar adhesion and deadhesion, tunicamycin

During the mating reaction in the biflagellate alga, Chlamydomonas reinhardi gametes of opposite mating types adhere to each other via their flagella, form pairs composed of cells of opposite mating types, and fuse to become zygotes. (See [1] for a review.) Our laboratory has been interested in several aspects of this mating reaction including the initial adhesive interaction between flagella as well as the signaling for cell wall release and mating structure activation that occurs during the adhesive interaction. Recent results from Mesland et al [2] have shown that this signaling event is closely correlated with a change in flagellar tip morphology, which also occurs immediately upon adhesion. Our own laboratory has recently established that the initial flagellar adhesion reaction in this organism is a highly dynamic reaction in and of itself in which adhesion sites are rapidly and irreversibly modified as part of the adhesive interaction [3, 4]. This was determined by experiments showing that when gametes were mixed with flagella isolated from gametes of the opposite mating type, there was an isoagglutination (rapid aggregation) of the cells followed quickly, however, by disaggregation. Although the cells in such gamete-flagella mixtures were still adhesive to freshly added flagella, recovery and retesting of the deadhered flagella revealed that these organelles were no longer adhesive [3].

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Further investigation showed that if these gamete-flagella aggregation experiments were done in the presence of inhibitors of protein synthesis, the gametes also lost their adhesiveness owing to their interaction with the flagella of the opposite mating type. Other experiments with mixtures of mating-type-minus (mt^-) gametes with impotent (mt^+) gametes demonstrated that turnover of adhesive sites is indeed an integral part of the adhesion between live gametes of opposite mating types [4]. This loss of adhesion sites in the presence of protein synthesis inhibitors occurred as a result of the adhesive interaction, because gametes that were incubated separately in inhibitor for several hours did not lose their adhesiveness until they were mixed in the continued presence of the inhibitor with gametes of the opposite mating type (Fig. 1).

The experiments reported here were carried out to investigate 1) the relationship between aggregation-induced loss of adhesiveness in protein synthesis inhibitors and changes in flagellar tip morphology implicated in signaling, and 2) the effects of incubation of aggregating gametes with tunicamycin, an inhibitor of protein glycosylation.

MATERIALS AND METHODS

Cultures

Cultures of 21 gr mt^+, 6,145c mt^-, and impotent mt^+ cells of Chlamydomonas reinhardi were grown axenically on a light-dark cycle in defined medium as

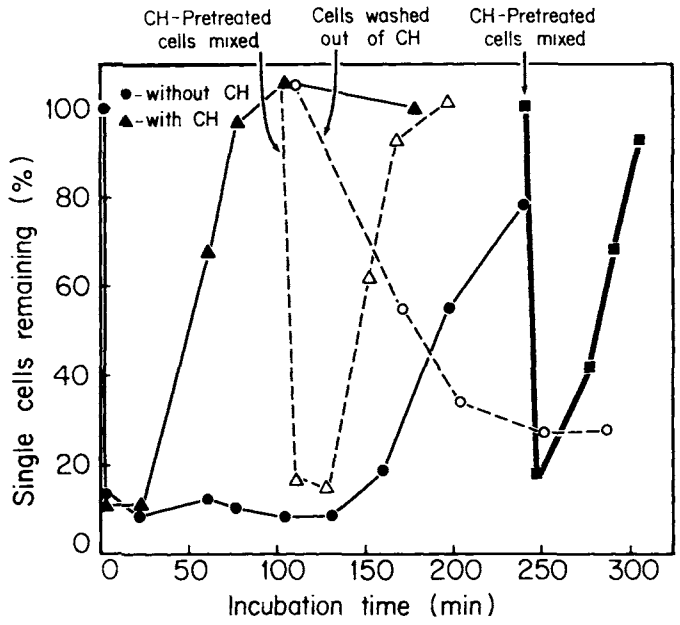

Fig. 1. Effect of CH on aggregation of mt^- and imp mt^+ gametes. Mt^- and imp mt^+ gametes were mixed together at 22°C with (▲) or without (●) cycloheximide (CH)(10 μg/ml), and aggregation was measured with the Coulter counter as previously described [4]. To determine if the effect of CH was reversible, the disaggregated gametes were washed out of inhibitor at 111 min after mixing (○). For the pretreatment experiment gametes were incubated separately in CH for 104 min (△) or 239 min (■) before mixing (arrows). Data from [4].

previously described [5]. Gametes were induced by resuspending vegetatively growing cells in nitrogen-free medium.

Materials

Tunicamycin was a gift from Dr. Hamill of Lilly, and octyl glucoside was obtained from Sigma. All other reagents were obtained as previously described [3–5].

Methods

Aggregation assay. A Coulter model ZBI electronic particle counter was used to measure cell aggregation as previously described [4]. Briefly, the counter is set to detect only single cells, and, as cells aggregate in suspension, the number of single cells is reduced and the loss of single cells is used as a measure of aggregation.

Electron microscopy. To observe alterations in flagellar tip morphology, aggregating cells were fixed in 1% glutaraldehyde in nitrogen-free medium for 15 minutes on ice, extracted with 30 mM octyl glucoside in nitrogen-free medium containing 0.1 mM dithiothreitol as described by Mesland et al [2], negatively stained with 1% uranyl acetate, and examined in the Philips 301 electron microscope. Over 100 flagella per time point were scored as having bulbous tips (activated) or nonbulbous tips (nonactivated) as described by Mesland et al [2].

RESULTS

When mt^- and impotent mt^+ gametes are mixed together in the presence of cycloheximide (CH), they begin to disaggregate within 35–40 minutes after mixing and, by 90–100 minutes, 90% of the cells are singles again. In the absence of cycloheximide, the cells stay aggregated for several hours (Fig. 1). We decided to investigate how adhesion or deadhesion between live cells of opposite mating types was correlated with changes in flagellar tip morphology as reported by Mesland et al [2]. These workers reported that gametes mixed with gametes or with isolated flagella of the opposite mating type show a new tip morphology different from that observed in nonaggregating gametes. In Mesland's experiments with gamete-flagella mixtures, the activated flagellar tip morphology was maintained as long as the cells aggregated. Figure 2A shows an unactivated flagellum that has a smooth tapered tip, and Figure 2B and C shows activated flagella with tips that have a much more bulbous morphology. Flagella with bulbous tips were not seen in samples taken from nonaggregating cell suspensions. It can also be seen in Figure 2C that there is an accumulation of dense material surrounding the microbules near the tip of the activated flagellum.

To determine if there were changes in flagella tip activation during adhesion or deadhesion in cycloheximide, mt^- and impotent mt^+ gametes were mixed together in the presence of cycloheximide, and, at various times after mixing, the amount of aggregation was measured with the Coulter counter, and the extent of flagellar tip activation (FTA) was determined by electron microscopy. The results shown in Table I indicate that at 35 minutes, when 88% of the cells were aggregating, FTA was 74%. However, as the cells begin to disaggregate in cycloheximide, the extent of FTA decreased, and by 79 minutes, when only 5% of the cells were

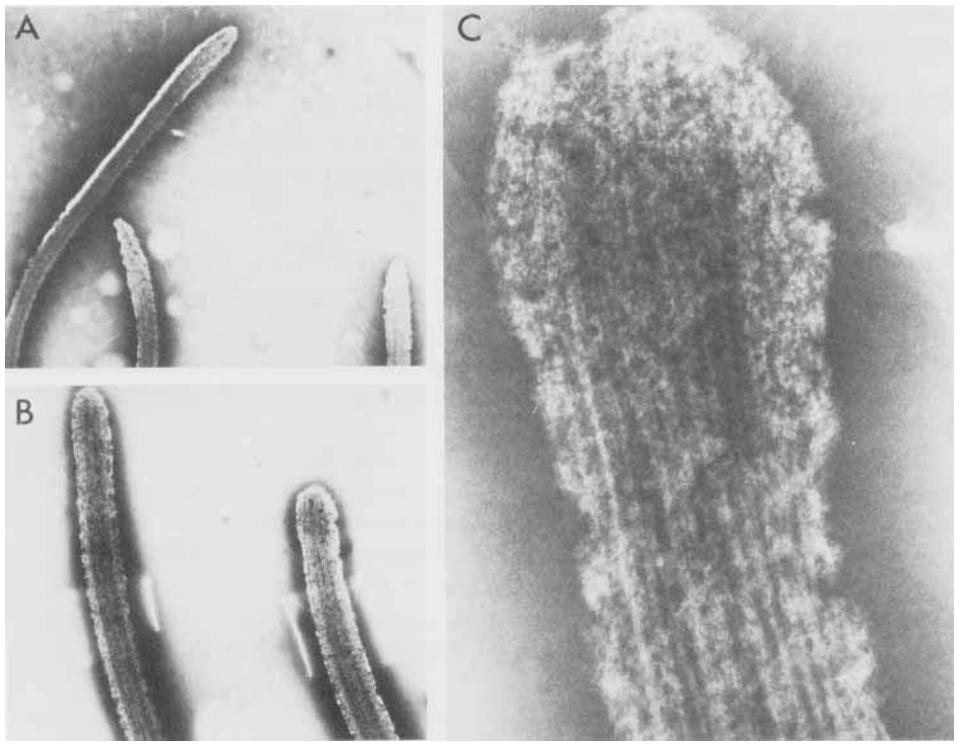

Fig. 2. Changes in flagellar tip morphology during adhesion. Nonaggregating (A) and aggregating (B, C) gametes were prepared for electron microscopy as described in Materials and Methods. Magnification A ~ 7,500 ×;B ~ 10,000; C ~ 60,000.

TABLE I. Change in Flagellar Tip Activation During Adhesion in CH

Incubation time (min)	Cells aggregating (%)	Activated Tips (%)
35	88	74
51	38	32
79	5	12

aggregated, FTA was reduced to 12%. These results indicate that activated flagella tip morphology is closely correlated with adhesion between live gametes of opposite mating types.

To determine if protein glycosylation might also be necessary for the maintenance of flagellar adhesiveness during aggregation, mt^- and impotent mt^+ gametes were mixed together in the presence of 5μg per ml of tunicamycin. At this concentration of tunicamycin, the rate of protein synthesis is 90–100% that of untreated cells (data not shown) [6]. The results indicate that this inhibitor also causes loss of adhesiveness of aggregating cells. As can be seen in Figure 3, the cells aggregate in the presence of tunicamycin for at least 10–15 minutes, but by 65 minutes 40% of the cells have disaggregated, and by 120 minutes over 80% of

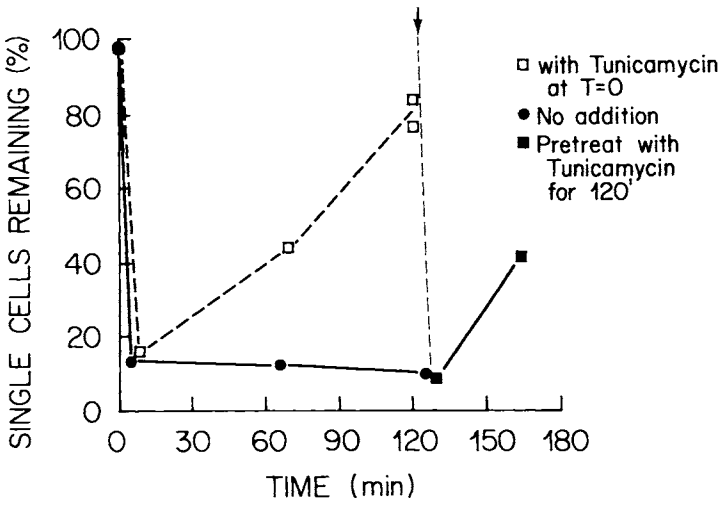

Fig. 3. Effect of tunicamycin on aggregation of mt⁻ and impotent mt⁺ gametes. Mt⁻ and impotent mt⁺ gametes in nitrogen-free medium (3 ml each, 8 × 10^6 cells per ml) were mixed together at 22°C without (closed circles) or with (open squares) 5μg/ml of tunicamycin, and aggregation was measured with the Coulter counter as described [4]. Gametes of both mating types were also pretreated separately for 120 min and mixed together in the continued presence of the inhibitor, and aggregation was measured with the Coulter counter (closed squares).

the cells were single again. Examination of these deadhered cells by phase-contrast microscopy revealed that the gametes were fully flagellated and motile. The effects of tunicamycin on adhesiveness were thus not due to a general toxic effect on the cells. As with cycloheximide, the loss of adhesiveness in tunicamycin occurred only during aggregation, because gametes kept separately in tunicamycin for 2 hours before mixing in the continued presence of the inhibitor were still able to aggregate as well as nonpretreated cells. It thus appears that protein glycosolation may play a role in maintenance of flagella adhesiveness during aggregation.

DISCUSSION

Our previous work on gamete adhesion and deadhesion was consistent with the hypothesis that flagellar adhesion in Chlamydomonas involves the continuous loss and replacement of flagella surface adhesion molecules [3], and inhibitor studies suggested that these molecules might be proteins [4]. The present results with tunicamycin indicate that glycosylation of flagellar surface molecules may also be required for maintenance of adhesiveness during adhesion. As with protein synthesis inhibitors, tunicamycin treatment of nonaggregating gametes did not affect their adhesiveness, but adhesion sites were lost only during adhesion. We recognize that tunicamycin may be interfering with processes only indirectly related to adhesion, but the results are not inconsistent with the idea that glycosylated molecules are directly responsible for adhesiveness. According to this idea, during aggregation these molecules are lost and are normally replaced. If ag-

gregation occurs in the presence of tunicamycin, however, these molecules do not reappear in a functional form.

Bloodgood [6] has previously reported that tunicamycin affects other functions associated with the surface of the Chlamydomonas flagellum. In studies on the active movement of later microspheres on the surface of the flagella of vegetative cells (flagellar surface motility), Bloodgood found that tunicamycin treatment of cells caused a gradual loss in the ability of vegetative cells both to bind and to move the microspheres. Although we have investigated different processes, the results suggest that glycosylated molecules may serve several important functional roles on the flagellar surface.

The results presented above also indicate that activated flagellar tip morphology is closely correlated with cell adhesion. As cells aggregate, flagellar tips become activated, and as disaggregation proceeds the activated tip morphology is progressively lost. These results are similar to those found by Mesland et al when they studied the extent of flagella tip activation during adhesion or deadhesion in gamete-flagella mixtures. These workers found that as the cells aggregated with isolated flagella, the percentage of activated tips increased, and during disaggregation the percentage of activated tips decreased. Our present results confirm the observation that flagellar tip morphology is modified during adhesion, and furthermore we show here that, as disaggregation occurs in gamete-gamete mixtures, the activated tip morphology is lost.

Since the flagella of aggregating cells initially adhere over most of their length but rapidly become adhesive primarily at the tip, the altered tip morphology has been suggested to be induced as a consequence of the accumulation of adhesion molecules at the flagellar tip and to serve as the signal for events at the cell body. However, the data are also consistent with the notion that the activated tip morphology may be more than just a result of adhesion, but might indeed be necessary for continued adhesion. Thus the requirement of protein synthesis and/or glycosylation for maintenance of adhesiveness during aggregation may in part be a result of the turnover of flagellar molecules involved in the formation and stabilization of the activated tip structure. Since the tip morphology does not appear until cells are aggregating, this hypothesis would explain why the adhesiveness of nonaggregating cells is insensitive to the inhibitors. The hypothesis can be tested by determining if these inhibitors also prevent the continued expression of activated flagellar tips induced by stimuli other than adhesion.

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