Light Affects Flagellar Agglutinability in *Chlamydomonas eugametos* by Modification of the Agglutinin Molecules

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**ABSTRACT**

The effect of light on the sexual competence of a light-sensitive mating type minus strain (mt-) of *Chlamydomonas eugametos* obtained by crossing a light-sensitive mating type plus strain (mt+) with a light-in sensitive mt- strain is described. As previously demonstrated for the mt+ parent, this study of one of the mt- offspring shows that (a) a light-sensitive mechanism affects flagellar agglutinability in a rapid process that does not require protein synthesis; (b) only the activity of the flagellar agglutinins (glycoproteins responsible for agglutination) is susceptible to light while agglutinins on the cell body surface are not affected by light. We further demonstrate that (a) membrane vesicles naturally released from nonagglutinable dark gametes remain inactive. Extracts of these vesicles also remain inactive even though they contain agglutinin-like components; (b) inactive mt- agglutinin is present in extracts of flagella from nonagglutinable dark gametes by comparison of its chromatographic, electrophoretic, and immunogenic properties with those of active agglutinin. When purified of all other flagellar proteins, it remains inactive; (c) a monoclonal antibody directed against the sexual agglutination site of the mt- agglutinin discriminates between active and inactive agglutinins when present in a native state on the flagellar surface, but is unable to discriminate between them when they are denatured in sodium dodecyl sulfate-electrophoresis gels and blotted onto nitrocellulose. Taken collectively these observations suggest that light activation involves the chemical modification of the agglutinins in situ on the flagellar surface.

The first step in the sexual reproduction of the unicellular green alga *Chlamydomonas eugametos* is the highly specific mutual adhesion of gametes of opposite mating types (mt- and mt+). This agglutination process takes place between the flagellar surfaces, and initiates other mating reactions such as flagellar tip activation (2) and the transport of membrane components to the flagellar tip (tipping) (6, 10). Subsequently, the gametes activate their mating structures by which they eventually fuse to give rise to vis-à-vis pairs.

Förster and Wiese (5) showed that the mating competence of mt+ cells of *C. eugametos* depends on light. No vis-à-vis pairs were formed when nonilluminated mt+ gametes were mixed with competent mt- cells. Earlier, we reported that it is the agglutinability of the mt+ cells that depends on light (12). When dark-treated, nonagglutinable gametes were illuminated, maximal agglutinability was achieved within 15 min. These cells lost their agglutination activity within 30 min of returning to the dark. Other mating reactions such as flagellar tip activation and tipping (both induced by wheat germ agglutinin) were light-independent.

Agglutination activity is the consequence of two different agglutinins present on the flagella of mt+ and mt- gametes, respectively. They have been identified as large glycoproteins, bound extrinsically to the membranes of flagella and cell bodies of all gametes (11, 12, 18, 21). However, only the agglutinin bound to the flagella seems to be influenced by the light, for the same level of agglutination activity can be extracted from the cell bodies of dark gametes as from light gametes (12). Nonetheless, it should be appreciated that as a consequence of the continuous presence of the gametic cell wall during the mating reaction, these agglutinins are not involved in agglutination. It seems that a membrane barrier, present in the transition zone between the flagellum and cell body, separates the plasma membrane into two independent domains (20) and that the light-controlled mechanism only operates in the flagella. What is the nature of this mechanism? Since protein synthesis is not necessary for activation or deactivation, it was postulated that the mechanism might involve the *in situ* modification of agglutinin rather than the rapid turnover of these molecules. To confirm this hypothesis, it was necessary to isolate inactive agglutinin from dark gametes. However, the mt+ gametes used to study this phenomenon are not suitable, for its agglutinin is relatively labile and present in only limited quantities on the flagella. Since light sensitivity is not sex-linked (12), we constructed a light-sensitive mt- strain that does not possess these limitations. As an extra advantage, we possess a monoclonal antibody that is specific for the mt- agglutinin (WL Homan, unpublished data) by which we can monitor its presence independent of its biological activity. In this report we illustrate that just as much inactive agglutinin is present on the flagella of dark gametes as there is active agglutinin on the flagella of light gametes. One may therefore assume that light stimulates a mechanism in the flagella, whereby the agglutinin molecules are modified and activated *in situ*.

**MATERIALS AND METHODS**

Cell Cultures. Gametes of the *Chlamydomonas eugametos* strains UTEX 9 (mt+) and UTEX 10 (mt-) from the Culture Collection of Algae, University of Texas at Austin, were crossed. The zygotes were germinated and resulting tetrad cells were isolated and cloned as described by SCHURING *et al.* (22). UTEX 9, UTEX 10, and the daughter cells were cultivated in Petri dishes on agar-containing medium in a 12 h light/12 h dark cycle.
regimen as described by Mesland (15). Cell suspensions were
obtained by flooding 2- to 4-week-old cultures with a 10 mm
Hepes buffer (pH 7.6) just before the start of the dark period.
Cells referred to as dark gametes were put into the dark directly
after flooding and harvested at the same time as comparable
light-treated cells. Illumination of dark-treated cell suspensions
occurred at 20°C, using white fluorescent light with an intensity
of 6.8 W · m⁻².

Isolation of Flagella and Isoagglutinin. Flagella were ampu-
tated by the pH-shock technique described by Witman et al.
(23). Flagella and cell bodies were separated by centrifugation on
a cushion of 25% sucrose (1000g, 15 min). The flagella, present
on the top of the cushion, were purified by repeating the sepa-
ration procedure once. Subsequently, the flagella were pelleted by
centrifugation at 12,000g for 20 min. Isoagglutinin was iso-
lated as described by Homan et al. (9) except for the use of a
Tris buffer that was replaced by a 10 mm Hepes buffer, pH 7.6.
For the isolation of flagella from nonilluminated cells, the pH-
 shock procedure was performed in the dark. The isolation of
membrane vesicles naturally released by gametes into the me-
dium (isoagglutinin) (9) from dark-treated cell cultures was
performed in darkness until the cells were removed from suspension
by centrifugation (1000g, 15 min). The agglutinability of ampu-
tated flagella and isoagglutinin was not affected by light.

Extraction of Biologically Active Material. Mt⁻ cell bodies,
flagella and isoagglutinin were extracted in 3 ml GTC for 30 min
at room temperature and centrifuged at 50,000g for 30 min. The
glycoproteins in the supernatant were separated from GTC by
gel filtration in water using a G-25 sephadex column (Pharma-
cia). Any insoluble material was removed by centrifugation at
50,000g for 15 min.

Biological Assays. The agglutination activity of mt⁻ cells was
quantitated by determining the highest dilutions of a standard
suspension of mt⁺ flagella that still evoked isoagglutination of
mt⁻ gametes (12). The agglutinin activity of flagella and isoagglutinin
was assayed by determining the highest dilution of these particles
that still caused isoagglutination of gametes of the opposite
mating type. Biological activity in GTC-extracts was detected
after dialysis adding mt⁺ gametes (9). In the presence of mt⁺
agglutinin, mt⁻ gametes accumulate at the water/air interface
and without aggregating, lie there twitching in a manner resem-
bling the movement of sexually agglutinating gametes.

Monoclonal Antibody. The monoclonal antibody Mab 66.3
has been used in this study as a specific label for the mt⁻
agglutinin. Although its characteristics will be presented in detail
by W. L. Homan (unpublished data), a summary is given here
to justify its acclaimed specificity. The hybridoma clone was first
selected because the supernant completely blocked the bio-
logical activity of isolated mt⁺ agglutinin bound to Sepharose beads.
The purified antibody is even able to block the agglutinability of
intact mt⁻ gametes. mt⁻ agglutinability is not affected. The
specificity of Mab 66.3 for extracted mt⁻ protein was tested by
immunoblotting after separation in SDS-electrophoresis gels, by
affinity chromatography using Mab 66.3 bound to protein A-
Sepharose, and by immunoprecipitation. Using all those tech-
niques, the affinity for the mt⁻ agglutinin was obvious. Only
when using the affinity chromatography technique did it appear
to bind another component. However, since this component was
not detected in the original cell extract, we conclude that it is
either a degradation product from the mt⁺ agglutinin or a com-
ponent that is present in such low concentrations that it could
only be detected after concentration on the column. It was never
detected in any of the other techniques used. When Mab 66.3
was tested for binding to intact cells in the immunofluorescence
test, it only labeled the flagella of gametes and not those of
vegetative cells which do not synthesise the agglutinin. In this
article, we shall demonstrate that Mab 66.3 binds to the flagella
of some strains in the light, when they are agglutinable, but not
to the same cells in the dark, when they are nonagglutinable.

Immuno-fluorescence. After fixation in 1.25% glutaraldehyde,
cells were washed 3 times in phosphate-buffered saline (pH 7.2)
and subsequently incubated for 15 min with a 20 times
diluted solution of the Mab 66.3 in PBS. Excess of antibody was
removed by washing 3 times with PBS. Flagella-bound Mab 66.3
was labeled with 1:100 diluted goat-anti-mouse IgG conjugated
with fluorescein isothiocyanate (H and L chain, Tago Inc., Bir-
lingame, CA). The cells were examined under a Zeiss (Ober-
kochen, FRG) fluorescence microscope. When binding of Mab 66.3
on the flagella of living cells was examined, cells were fixed
in glutaraldehyde after a 15-min incubation with Mab 66.3.

Anion Exchange Chromatography. GTC extracted material
from flagella and isoagglutinin was chromatographed over a QAE
Sephadex A-25 column (φ 16 mm, length 55 mm, Pharmacia)
in 10 mm histidine buffer pH 6.0, containing 50 mm NaCl. After
a washing step with 15 ml of the same buffer, the components
that bound to the resin were stepwise eluted with 200 mm and
400 mm NaCl in the same buffer.

Gel Electrophoresis and Immunoblotting. Lyophilized GTC-
extracted material was dissolved in sample buffer and the com-
ponents separated by SDS-PAGE according to Laemmli (13) in
1 mm thick 8 × 8 cm slab gels containing a 2.2 to 20% acrylamide
gradient. Approximately the first 5 mm of the gel consisted of
2.2% acrylamide. Immunoblotting was performed as described by
Homan et al. (8).

RESULTS

Light Activation of Agglutinability in an mt⁻ Strain of C.
eugametos. The light sensitivity of sexual competence in C.
eugametos gametes has been described for the mt⁺ strain UTEX
9, but this strain is unsuitable for isolating the agglutinins from
the flagella because of the limited amounts present. We have
therefore constructed a light-sensitive mt⁻ strain (henceforth
designed as 5.39.4) obtained by crossing UTEX 9 with the light
insensitive mt⁻ strain UTEX 10 (22). The kinetics of activation
and deactivation of flagellar agglutinility in this strain is illus-
trated in Figure 1. Compared with the original mt⁺ strain, the
attainment of maximum agglutinability usually took twice as
long, whereas the deactivation in the dark usually went faster.
However, these are not mating type-linked differences but simply
variations between strains, for we have isolated mt⁻ strains which
exhibit similar characteristics and also mt⁻ strains that more
closely resemble the UTEX 9 parent. A more pronounced dif-
ference is seen in their sensitivity to CHI. In UTEX 9, both light-
activation and dark-inactivation took place independent of CHI-
treatment (12), but treatment of 5.39.4 with CHI prevented the
activation of agglutinability in the light, whereas deactivation
was unaffected (Fig. 2). This difference in CHI-sensitivity is again
not sex-linked but a common variation between strains (Table
1). We propose that this effect is not due to an inhibition of the
synthesis of the mt⁺ agglutinin. This stems from the fact that the
iron chelator α,α-bipyridyl, which, like CHI, completely inhibits
the synthesis of active agglutinin, during gelatinogenese or in
mt⁻ strains (20), had no effect on the light-activation process (Fig. 2).
A possible explanation is that in some strains the proteins in-
olved in activation are subject to a rapid turnover. When
gametes were treated for decreasing lengths of time with CHI,
before illumination, it became obvious that also a short pretreat-
ment had a profound effect, even though the inhibition was not
absolutely (Fig. 3). This could indicate an extremely rapid turnover
of components involved in activation, or alternatively a more direct effect of CHI on the activation process. Since the protein synthesis inhibitor Emetine also resulted in a comparable effect (data not presented), we prefer the former explanation. Cells that were activated in the light and treated with CHI maintained their agglutinability for several hours (Fig. 2). Nonetheless, their light activation mechanism was inoperative, for when they were inactivated in the dark, they could not be reactivated while maintained in the presence of CHI. Thus once light activation has occurred, the mechanism need not be retained in an operative state; inactivation does not immediately follow exposure of light-activated gametes to CHI.

Table 1. Characterization of Some Strains Isolated from Zygotes 5.39 and 17.17, Obtained by Crossing of C. eugametos UTEX 9 and 10

<table>
<thead>
<tr>
<th>Tetrad Products</th>
<th>Mating Type</th>
<th>Light Sensitivity</th>
<th>CHI Sensitivity</th>
<th>Isoagglutinin Production</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.39.1</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>27</td>
</tr>
<tr>
<td>5.39.2</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>26</td>
</tr>
<tr>
<td>5.39.3</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>27</td>
</tr>
<tr>
<td>5.39.4</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>27</td>
</tr>
<tr>
<td>17.17.1</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>210</td>
</tr>
<tr>
<td>17.17.2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>26</td>
</tr>
<tr>
<td>17.17.3</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>26</td>
</tr>
<tr>
<td>17.17.4</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>26</td>
</tr>
</tbody>
</table>

* Not determined.

Although it is the flagella that are involved in sexual agglutination, most of the agglutinin in a cell has been shown to be present on the outside of the cell body (21). We recently demonstrated that a membrane barrier separates the flagellar membrane from that of the cell body (20) and that glycoproteins are...
therefore not able to diffuse from one membrane domain to the other. An illustration of this is that only those agglutinins confined to the flagellar surface of UTEX 9 are subject to a light-controlled activation system. Having produced a new light-sensitive mt- strain, we had to test whether in this strain the light sensitivity was also restricted to the flagella. Thus GTC extracts of both membranes from light- as well as from dark-treated gametes were obtained and tested for biological activity. The results are summarized in Table II. The vast majority of mt- agglutinin from this strain is present on the cell body surface and its activity is not influenced by light. In contrast, the activity of the fraction at the flagellar surface is strictly controlled by a light-sensitive mechanism as we have shown before (12).

**Isoagglutinin Production in Light and Dark.** Most *Chlamydomonas* gametes naturally release membrane vesicles into the culture medium which, when added to gametes of the opposite mating type, evoke isoagglutination. These vesicles, referred to as isoagglutinin, are thought to originate from the flagellar membrane by budding (1, 14, 16). The question was raised whether isoagglutinins produced in the dark, show isoagglutinative activity, or in other words, whether the flagellar membrane becomes agglutinable when naturally released from any restraints imposed by the living cell in the dark. In order to answer this question, we first had to be sure that isoagglutinins were indeed released from the flagella and not from the cell body where, as already explained, the light-sensitive mechanism does not operate. In Figure 4, the glycoprotein composition of isoagglutinins is compared with that of flagella and the cell body membrane. Isoagglutinins contain nearly all of the typical flagellar components but none of the major cell body glycoproteins. This is considered convincing evidence that isoagglutinins do not originate from the cell body surface. We therefore studied the release of isoagglutinin into the medium from 5.39.4, exposed to light or kept in the dark. The results are presented in Figure 5. Active isoagglutinins were only found in cultures in the light. Production of isoagglutinin in general is not a light-dependent process for all light-insensitive strains yield as much in the dark as in the light (Table I). It is only the light-sensitive strains that do not release active isoagglutinin in the dark. The conclusion is that they are only produced from agglutinable flagella. This was also illustrated when gametes of 5.39.4 were transferred from the dark to the light. They became agglutinable within 10 min, and within 2 h, they had produced a considerable quantity of active isoagglutinin (Fig. 5), but when the light activation was blocked by CHI treatment, the production of active isoagglutinin was similarly prevented.

Next, we investigated whether in the dark inactive cells produce inactive isoagglutinin. To this end, particulate material was isolated from both dark- and light-grown cultures containing approximately $2 \times 10^9$ cells. While the optical density of both preparations was the same, only that from the illuminated culture was biologically active (titer $2^4$ compared with inactive). When GTC extracts were made of the two different samples, that from the dark culture was inactive, that from the light culture had a titer of $2^5$ in the twitch assay. The two extracts were subjected to SDS-PAGE. Just as much glycoprotein-staining material was present in the extract of particles obtained from dark cells as in that of the particles obtained from light cells. The pattern of glycoprotein bands was similar to the high mol wt region of that shown in Figure 4. Thus it is clear that dark-grown gametes release inactive membrane vesicles from their flagella and that the state of activity or inactivity is maintained after release. It was not possible to activate these vesicles by illumination, yet as far as one can judge from the stained gels, the agglutinin band seemed to be equally present in both types of particles (Fig. 6A). Thus the inactivity of flagellar membranes in the dark seems not due to the complete absence of agglutinins or a temporary restraint imposed by the intact membrane, but rather to a chemical modification, and when the agglutinin is isolated from the living cell, the state of activity (inactive or active) is maintained.

**Inactive Agglutinins.** In order to reinforce the conclusion stated above, that the light-activation/dark-inactivation involves the chemical modification of flagellar agglutinins, it was considered important to demonstrate that inactive agglutinins are present on the flagella of dark grown gametes. Accordingly, flagella were isolated from nonagglutinable dark gametes as well as from agglutinated light gametes and GTC-extracts were made from them. The mt- agglutinin was then partially purified from other high mol wt species by anion exchange chromatography using a

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**Table II. Influence of Light on Isoagglutination Activity of Isolated Flagella and GTC Extracts of Strain 5.39.4**

A nonilluminated cell suspension with $2 \times 10^9$ cells was divided into two equal parts of which one part was illuminated for 1 h. Subsequently, the flagella were amputated by a pH shock (23) and separated from the cell bodies on a sucrose cushion. A small portion of the flagella was assayed for isoagglutination activity as described in "Materials and Methods." The rest of the flagella and the cell bodies were extracted with 3 M GTC. After removal of GTC from the supernatant by gel filtration, the extracts were assayed for biological activity by making a series of twofold dilutions of the extracts and determining the highest dilution that still evokes twitch activity in mt- gametes (9).

<table>
<thead>
<tr>
<th>Cell Suspension</th>
<th>Dark</th>
<th>Light</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated flagella</td>
<td>$&lt;2^0$</td>
<td>$2^4$</td>
</tr>
<tr>
<td>Extracts of flagella</td>
<td>$&lt;2^0$</td>
<td>$2^4$</td>
</tr>
<tr>
<td>Extracts of cell bodies</td>
<td>$2^4$</td>
<td>$2^9$</td>
</tr>
</tbody>
</table>
**DISCUSSION**

The kinetics of light-activation/dark-inactivation in the mt− strain 5.39.4 are essentially the same as those described for the original mt+ strain UTEX 9 (12), from which it was derived. The mechanism in both strains is expressed only in the flagella and does not involve the turnover of agglutinins. This may be concluded from the fact that the mechanism in 5.39.4 is insensitive to α,α-dipyridyl-treatment which blocks agglutinin synthesis in this and other mt+ strains (20), and from the fact that the mechanism in several strains is independent of protein synthesis. Instead, activation/deactivation involves the regulation of the activity of agglutinins on the flagellar surface, for they can be extracted as inactive species from the flagella of dark-grown cells. Since the state of activity or inactivity is not affected by purification of the agglutinin in SDS, we may conclude that inactivation is not a restraint imposed on the agglutinin by association with another flagellar protein, rather it is a covalent modification which is irreversible once the cell is killed or the agglutinin is extracted. In some strains, including 5.39.4, light activation is inhibited when protein synthesis is prevented during activation. This implies that one of the components involved in triggering activation or in the mechanism of activation has a very rapid turnover. However, once the agglutinin is activated, protein synthesis is not needed to maintain the activated state, for 5.39.4 can be treated with cycloheximide for several hours in the light, without agglutinability being seriously reduced. Thus inactivation does not automatically follow when the activation system is switched off. It seems likely therefore, that activation and inactivation are two independent processes that are separately triggered, the one in the light and the other in the dark. What do these processes involve? Clearly the mechanism is operative with respect to mt+ and mt− agglutinin molecules which have two different binding sites. The modification is subtle because the electrophoretic and chromatographic properties of the active and inactive agglutinin are alike. Similarly, while the native inactivated mt− agglutinin cannot bind the Mab 66.3 (in contrast to the active agglutinin) it can do so when the molecule is denatured after SDS-electrophoresis and blotting. These results suggest that a minor modification of the molecule affects its activity, e.g. (de)phosphorylation or (de)sulfation. This would imply that particular ectoenzymes such as kinases and phosphatases are operative at the outer surface. Such enzymes have been demonstrated by Ehrlich et al. (4). We are presently trying to detect their presence.

The monoclonal antibody used in this study, Mab 66.3, did
not bind to the flagella of cells in the dark. This is an interesting result because the Mab was selected for its ability to block the activity of isolated mt− agglutinin. In the meantime Fab fragments have been demonstrated to block the agglutinability of living mt− gametes, without affecting that of mt+ gametes (WL Homan, unpublished data). Thus there was already good evidence for believing that Mab 66.3 binds to, or close to, the sexual adhesion site of the mt− agglutinin. The new finding, that Mab-binding to the flagella of 5.39.4 is strictly correlated with flagellar agglutinability in the light, provides particularly convincing evidence for this contention. However, the antigenic epitope does not seem to include the site modified during inactivation, for when the inactive agglutinin is denatured, the epitope becomes available to bind Mab 66.3. Thus agglutinin-inactivation in the dark, while possibly a minor modification of an amino acid or sugar residue, seems to result in a change of conformation whereby the antigenic epitope becomes cryptic and the sexual adhesion site inaccessible. The modification of the agglutination activity may therefore not involve regions directly involved in adhesion, but a site in the vicinity that affects the accessibility of the adhesion site. The idea that the adhesion site is not destroyed in the dark is strongly supported by the fact that low concentrations of glutaraldehyde can partially activate nonagglutinable gametes (data to be presented elsewhere).

The production of isoagglutinin is a well known property of the mt+ strain of C. eugametos that was used to establish the identity of the mt− agglutinin (7, 18). It was assumed that these membrane vesicles originated from the flagellar membrane because their properties reflected those of the flagellar membrane and because Bergman et al. (1) obtained electron micrographs which seemed to demonstrate the loss of isoagglutinin from the flagellar membrane of C. Reinhardtii. From the present results it is confirmed that isoagglutinins originate from the flagellar membrane. Isoagglutinins which are produced in the dark are...
inactive. Since the light-sensitive activation mechanism is restricted to the flagellar membrane and does not affect agglutinins on the cell body surface, there can be little doubt that agglutinins bud from the flagellar membrane.

When a function of a plant cell is influenced by the light/dark cycle, it is natural to consider whether it is via photosynthesis, since the source of energy could be limiting in the dark. However, cells kept in the dark are not depleted of energy, for swimming, flagellar growth and glycoprotein synthesis are not affected by the dark. Furthermore, the generation of ATP by oxidative phosphorylation in darkness is 10 times higher than the amount of ATP that can be generated by photosynthesis under the low light intensities (0.2 W/m$^2$) that are sufficient to induce agglutinability. Thus the generation of ATP is unlikely to play a key role in the light-activation. Other products of photosynthesis are not involved either, since inhibition of photosystem 2 by dichloro-phenyl-diemthylurea does not affect the light-activation (R Kooijman, unpublished observations). In conclusion then, while the light receptor may well lie in the cell body and could then involve a signal diffusing to the flagellar membrane, the photosynthetic machinery is not directly involved.

From the evidence presented, we can safely conclude that the light controlled activation/inactivation in _C. eugametos_ is an example of post-translational modification of sexual activity. Since the flagella can be readily isolated and the plasma membrane is the only membrane present, this system provides a good model for studying the topography of membrane proteins in general, and particularly for studying their interactions to explain this regulation mechanism. The flagella membrane exhibits other characteristics that makes it an exciting study object. For example, we have recently demonstrated that the agglutinins that become involved in sexual agglutination, are transported to the flagellar tips (10). Therefore there is not only an interesting interplay of components external to the membrane, regulating agglutinin activity, but also internally, where the agglutinins can become attached to the cytoskeleton and redistributed over the surface. What is more, the activity of flagellar agglutinins is more complicated than has so far been considered, for the control of agglutinability exists at different levels. First of course, there is the mechanism of gametogenesis in which the synthesis of agglutinins is triggered. Second, the density of agglutinin in the membrane is subjected to a circadian rhythm, being appreciably higher at the beginning of the light period than at the end of the day. This has been explained as a rhythm in agglutinin synthesis and incorporation into the flagella, and the only effect of light is in setting the clock that determines the rhythm (3). Third, as we have seen, the complement of flagellar agglutinins is in some strains subject to a light-controlled modification of activity. Lastly, it has been shown that during sexual agglutination, the level of agglutinability rises dramatically (about 10$^3$ or 2$^{14}$ when expressed as a dilution titer) independent of the original ground state activity (R Demets, A Tomson, unpublished observations). This could be due to different causes, for example the incorporation of new agglutinins into the flagellar membrane, their rearrangement in the membrane, _e.g._, clustering to form more effective adhesions, or a form of agglutinin activation independent of the light activation described here. We are presently trying to sort out these possibilities.

**Acknowledgment**—We thank Jeroen Staal for his help in the light-activation experiments.

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