Flagellar Adhesion between mt+ and mt− *Chlamydomonas* Gametes Regulates Phosphorylation of the mt+-specific Homeodomain Protein GSP1*

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During fertilization in *Chlamydomonas*, flagellar adhesion between mt+ and mt− gametes induces a cAMP-dependent signal transduction pathway that prepares the gametes for cell fusion and zygote formation. Previously, our laboratory identified a homeodomain protein (GSP1) whose expression was restricted to the cell bodies of mt+ gametes and whose transcript level was up-regulated during flagellar adhesion. In this report, we describe a new form of GSP1 that appears early during gamete interactions. Immunoblot analysis showed that in addition to the 120-kDa form of GSP1 normally present in mt+ gametes, a 122-kDa form was detected when the cells were mixed with mt− gametes. The more slowly migrating form of GSP1 was detectable within minutes after gametes were mixed together, and its appearance did not require new protein synthesis. Thus, the 122-kDa form represents a post-translational modification of the pre-existing 120-kDa form of GSP1. Moreover, conversion to the 122-kDa form did not require cell fusion. Although the 120-kDa form was expressed 10 h after vegetative cells were transferred to gametic induction medium, the 122-kDa form was detected only after mt+ gametes were induced to undergo the sexual signaling that accompanies fertilization. Incubation of mt+ gametes with dibutyryl cAMP led to the appearance of the 122-kDa form of GSP1, and the cyclic nucleotide-dependent protein kinase inhibitor H-8 inhibited the adhesion-induced conversion. Incubation of GSP1 immunoprecipitated from signaling mt+ gametes with alkaline phosphatase showed that the conversion was due to phosphorylation. The results indicate that flagellar adhesion induces a rapid, cAMP-dependent phosphorylation of the homeodomain protein GSP1 early during fertilization in *Chlamydomonas*.

During fertilization in both unicellular and multicellular organisms, the interactions between gametes induce signal transduction cascades that prepare the cells for fusion (1). Although studies of fertilization in a variety of species have identified several molecules involved in gamete interactions (2, 3), much remains to be learned about the molecules and the signaling pathways involved in gamete activation and fusion as well as the transcriptional regulation (4) of molecules during and immediately after fertilization.

In the unicellular, biflagellated green alga *Chlamydomonas reinhardtii*, fertilization is initiated when mt+ (mating type plus) and mt− (mating type minus) gametes adhere to each other along the lengths of their flagella via adhesion molecules, the mt+ and mt− agglutinins (5, 6). Agglutinin interactions initiate a protein kinase-dependent signaling pathway that leads to activation of a gamete-specific flagellar adenyl cyclase (6–10). The subsequent increase in intracellular cAMP rapidly induces multiple cellular events required to activate the gametes for cell body adhesion and fusion to form a zygote (7, 10).

Cellular responses associated with activation by cAMP include the following: movement of agglutinin molecules from the plasma membrane of the cell body onto the flagellar membrane to replace agglutinins that are lost as a consequence of adhesion (11–13); up-regulation of synthesis of agglutinin molecules (11); regulated secretion of a serine protease required to convert a periplasmic prometalloprotease to an active matrix-degrading metalloprotease (14, 15); release and degradation of the extracellular matrix (cell wall) (7); activation of apically localized mating structures (7, 16); and finally, adhesion and fusion of the cell bodies via the activated mating structures (7, 16). Gamete fusion itself generates signals for cellular responses that prepare the new quadriflagellated cell for the zygotic phase of the life cycle. Pre-existing flagellar agglutinins are inactivated; synthesis of new agglutinins no longer can be detected; flagella are resorbed; and a new, highly impervious zygote cell wall is assembled (17–19). Although these responses have been well characterized at the cellular level, few of the molecular events that accompany gamete activation and zygote development have been studied (9, 14, 15, 17, 19, 20).

Recently, a screen in our laboratory carried out to identify molecules regulated by flagellar adhesion in *Chlamydomonas* (21) yielded a cDNA that encodes a homeodomain protein, GSP1, which is the first homeodomain protein to be identified in *Chlamydomonas* (22). Analysis by Southern blotting and immunoblottedting showed that the gene for GSP1 is present in cells of both mating types (i.e. it is not linked to the mating type locus), the protein is expressed only in mt+ gametes; it is not expressed in either mt− or mt+ vegetative cells or in mt− gametes. Here we show that GSP1 undergoes a post-translational modification, detected as a shift in SDS-PAGE mobility, within minutes after adhesion between mt+ and mt− gametes. Our results indicate that the GSP1 mobility shift does not require cell fusion and is a consequence of adhesion-induced phosphorylation.

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1 The abbreviations used are: PAGE, polyacrylamide gel electrophoresis; TLCK, N-tosyl-l-lysine chloromethyl ketone HCl; TPCK, L-1-tosylamido-2-phenylethyl chloromethyl ketone; ALLN, N-acetyl-Leu-Leu-norleucinal.
**EXPERIMENTAL PROCEDURES**

Materials—Unstained and Kaleidoscope prestained molecular mass markers were from Bio-Rad. Leupeptin, chymostatin, pepstatin A, 1,10-phenanthroline, E-64 (trans-epoxysuccinyl-l-1-tosylamido-4-guanidino/butane HCl), benzamidine, N-tosyl-l-lysine chloromethyl ketone HCl (TLCK), l-tosyl-amido-2-phenylethyl chloromethyl ketone (TPCK), okadaic acid, and sodium orthovanadate were from Sigma. N-Acetyl-β-glucosaminidase (ALN) and 4-2-aminoethyl)benzenesulfonyl fluoride HCl were from Calbiochem. Calf intestinal alkaline phosphatase was from Roche Molecular Biochemicals. All other chemicals were of reagent grade.

**Cells and Cell Culture—C. reinhardtii strains 21gr (mt +) [cc-1690], 614Sc (mt -) [cc-1689], and imp-1 (mt +) [cc-462] (available from the Chlamydomonas Genetics Center, Duke University, Chapel Hill, NC) were grown at 22 °C with aeration prior to mixing at 29 °C and Maximum I of Sager and Martin Instruments). The supernatant was discarded; and the sedimented cells were flash-frozen in liquid N2. After at least 1 h in liquid N2, sedimented samples were removed to a buffer containing 1% SDS, 25 μg/ml ALLN, 100 μM leupeptin, 100 μM E-64, 50 mM NaF, 100 μM β-glycero phosphate, 100 μM sodium orthovanadate, 1 μM okadaic acid, and 50 μM Tris, pH 8.0, at 37 °C was immediately added to the sample. After incubation at 37 °C for 5 min, 2.25 ml of buffer containing 1% Nonidet P-40 and 150 mM KCl was added to yield a final SDS concentration of 0.1%. This mixture was centrifuged at 50,000 g for 10 min at 4 °C (Model TLA100.3 rotor and Optima TLX ultracentrifuge, Beckman Instruments). The supernatant, which contained GSP1, was used for immunoprecipitation as described below.

Anti-GSP1 antibody-coated beads were prepared by incubation of protein A-Sepharose beads (Amersham Pharmacia Biotech) with 10 μl of the once-purified anti-GSP1 polyclonal antibody. After 1 h at 4 °C, the beads were harvested by centrifugation in a buffer containing 0.5% SDS, 1 μM E-64, 1 mM benzamidine, 20 μg/ml chymostatin, 1 μM ALLN, and 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride HCl in 1 mM Hepes, pH 6.8, and 20 μl of the washed GSP1-bead complex was mixed with 1 unit of calf intestinal alkaline phosphatase and incubated at 30 °C. After 30 min, the beads were washed once with 20 volumes of phosphate-buffered saline by centrifugation, and the samples were prepared for analysis by SDS-PAGE and immunoblotting.

For electrophoretic analysis of samples, immunopurified GSP1 from 5 × 105 mt + gametes was mixed with SDS-PAGE sample buffer (final concentrations: 20% glycerol, 4% SDS, 0.2 μl diethiothreitol, 0.05% bromphenol blue, 0.1 μl diethiothreitol, and 0.0625 μl Tris, pH 6.8) (26); boiled for 4 min; and subjected to electrophoresis on Laemmli 7% polyacrylamide minislab gels (27) at 20 mA for 3.5 h. After electrophoresis, proteins were transferred to Immobilon P membranes as described above. Membranes were blocked and washed as described above and incubated with a 1:5000 dilution of the once-purified anti-GSP1 polyclonal antibody. All subsequent immunoblotting steps were carried out as described above.

**RESULTS**

Gametic Adhesion Induces a Shift in GSP1 Mobility—To study GSP1 during fertilization, mt - and mt + gametes were mixed together for 60 min, and GSP1 was analyzed by SDS-PAGE and immunoblotting. Fig. 1 shows that GSP1 migrated as a single band at ~120 kDa in control, unmixed mt + gametes; but, after mixing for 60 min, a new, more slowly migrating form of GSP1 of ~122 kDa was detected. Surprisingly, the new 122-kDa form of GSP1 appeared rapidly after mixing. Fig. 2A shows that 5 min after mixing gametes of opposite mating types together, when 64% of the cells had fused to form zygotes, almost the entire mobility of GSP1 was in the 122-kDa form. By 15 min, when ~85% of the cells had formed zygotes, roughly equal amounts of the two forms were present and remained detectable through the 60-min time point. A control, unmixed sample of mt + gametes incubated under the same conditions for 60 min contained only the 120-kDa form of GSP1.
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Modification of GSP1 during Flagellar Adhesion—This report describes the adhesion-induced post-translational modification of the mt⁺-specific homeodomain protein GSP1 during fertilization in Chlamydomonas. Using immunoblotting to identify GSP1 in cells, we discovered that mixing mt⁺ gametes with mt⁻ gametes led to the appearance of a 122-kDa form of GSP1 (Fig. 1), which had not been detected in previous experiments that used only unmixed cells (22). The appearance of the more slowly migrating form of GSP1 was surprisingly

with mt⁻ gametes (Fig. 4). Furthermore, only the 120-kDa form of GSP1 was present during gametogenesis. The 122-kDa form of GSP1 was not detectable in these differentiating cells either prior to or after the appearance of agglutinins. A control experiment showed that the 122-kDa form appeared upon mixing mt⁺ gametes with mt⁻ gametes (Fig. 4, lane +/−).

The Appearance of the 122-kDa Form of GSP1 Is a Consequence of cAMP Signaling—To learn more about the pathway responsible for the mobility shift of GSP1, we examined whether cAMP-mediated signal transduction was involved. Incubation of gametes in dibutyryl cAMP and papaverine has been shown to induce the cellular responses normally observed during flagellar adhesion (7). To induce sexual signaling, mt⁺ gametes were incubated with dibutyryl cAMP and papaverine or mixed with mt⁻ gametes, and GSP1 was analyzed by immunoblotting. The results in Fig. 5 show that the cAMP treatment was sufficient to induce the appearance of the 122-kDa form of GSP1 (lane 3). Only the 120-kDa form was present in untreated cells (lane 1); as expected, control cells mixed with mt⁻ gametes contained the 122-kDa form of GSP1 (lane 2).

Since several studies have shown that sexual signaling through cAMP is inhibited by H-8 (an inhibitor of cyclic nucleotide-dependent protein kinases) (7, 9), we examined whether the change in GSP1 mobility showed a similar sensitivity to H-8. When mt⁺ and mt⁻ gametes were preincubated in H-8 and mixed together in the continued presence of the inhibitor, only the 120-kDa form of GSP1 was detected (Fig. 6, lane +H-8/mt⁻). In contrast, control cells contained the 122-kDa form of GSP1 (lane −H8/mt⁺). Consistent with earlier studies (7, 9), H-8 had no effect on flagellar adhesion, but prevented zygote formation (Fig. 6). These results indicated that adhesion-induced signaling through a cAMP-dependent pathway, perhaps through the activity of a cAMP-dependent protein kinase, was necessary and sufficient to induce the appearance of the 122-kDa form of GSP1.

DISCUSSION

Modification of GSP1 during Flagellar Adhesion—This report describes the adhesion-induced post-translational modification of the mt⁺-specific homeodomain protein GSP1 during fertilization in Chlamydomonas. Using immunoblotting to identify GSP1 in cells, we discovered that mixing mt⁺ gametes with mt⁻ gametes led to the appearance of a 122-kDa form of GSP1 (Fig. 1), which had not been detected in previous experiments that used only unmixed cells (22). The appearance of the more slowly migrating form of GSP1 was surprisingly

synthesis) for 10 min, mixed together, and analyzed for GSP1 by SDS-PAGE and immunoblotting. Fig. 2B shows that cycloheximide had no effect on the extent of zygote formation or the appearance of the 122-kDa form of GSP1. Thus, the appearance of the 122-kDa form of GSP1 was due to pre-existing GSP1.

In the experiment shown in Fig. 2A, the appearance of the 122-kDa form of GSP1 was concomitant with formation of zygotes. To ascertain if modification of GSP1 involved components from the mt⁻ cytoplasm, we determined if the appearance of the more slowly migrating form of GSP1 required cell fusion. To do this, the above experiment was carried out with the mt⁺ fusion-defective mutant imp-1. Gametes of imp-1 undergo normal flagellar adhesion and sexual signaling with mt⁻ gametes, but are unable to fuse (16). The 122-kDa form of GSP1 was present after 30 min of mixing wild-type mt⁺ gametes and imp-1 mt⁻ gametes, although, as expected, no zygotes were observed in these samples (Fig. 3). Moreover, the 122-kDa form in signaling imp-1 gametes was first detectable within 5 min of mixing with mt⁻ gametes. These results indicated that adhesion-induced signals and molecules within the mt⁻ gametes were sufficient to lead to conversion of the 120-kDa form of GSP1 to the 122-kDa form.

The 122-kDa Form of GSP1 Does Not Appear during Gametogenesis—Previous examination of the timing of GSP1 expression during sexual differentiation indicated that GSP1 was first detectable at the time that cells expressed other gametic markers such as the flagellar adhesion molecule, agglutinin (22). Using SDS-PAGE and sample loading conditions optimized to detect the two forms of GSP1, we reexamined GSP1 during sexual differentiation to determine whether the 122-kDa form of the molecule appeared at any time during gametogenesis. As shown in Fig. 4, within 10 h of transferring vegetatively growing mt⁺ cells to nitrogen-free medium to induce gametic differentiation, expression of the 120-kDa form of GSP1 was detectable. Consistent with previous observations (22), GSP1 expression was coincident with the appearance of other gamete-specific proteins such as agglutinin, as assessed by the ability of the cells to undergo agglutination when mixed

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mt⁺ gametes (5 × 10⁵) were incubated alone (Unmixed) or with an equal number of mt⁻ gametes for 60 min to form zygotes (Mixed) and analyzed by immunoblotting with the anti-GSP1 antibody. Migration of Kaleidoscope prestained molecular mass markers is shown on the left (in kilodaltons); the arrowhead indicates the higher molecular mass form of GSP1. (The molecular mass of GSP1 was previously reported to be ~140 kDa based on the migration of a different lot of Bio-Rad prestained markers (22). Since then, we have used several different lots of prestained markers as well as unstained markers and found that the protein in non-mating mt⁻ gametes consistently migrates with an apparent molecular mass of ~120 kDa, as indicated under “Results.”)
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Fig. 2. The shift in GSP1 mobility occurs early during zygote formation and does not require new protein synthesis. A, to examine the timing of appearance of the 122-kDa form of GSP1, an equal number of mt+ and mt− gametes (each at 2 × 10^6 cells/ml) in nitrogen-free medium were mixed together. After 0, 1, 5, 15, 30, and 60 min, samples containing 5 × 10^6 mt+ cells were removed and analyzed by immunoblotting with the anti-GSP1 antibody. Time of mixing is shown above the sample lanes, and the arrowhead indicates the 122-kDa form of GSP1. Lane 60C contains 5 × 10^6 control mt+ gametes incubated for 60 min without mixing with mt− gametes. The percent of cells forming zygotes is indicated below the corresponding sample. ND, not determined. B, to examine the role of protein synthesis in the appearance of the 122-kDa form of GSP1, mt+ and mt− gametes (each at 2 × 10^6 cells/ml) were separately pretreated for 10 min with (+) and without (−) 10 μg/ml cycloheximide (CH). Equal numbers of mt+ and mt− gametes were mixed together in the continued absence or presence of the inhibitor for 15 min and then analyzed by immunoblotting with the anti-GSP1 antibody. The higher molecular mass form of GSP1 is indicated by the arrowhead, and the percent of cells forming zygotes is shown below.

Fig. 3. The shift in GSP1 mobility occurs in the absence of cell fusion. Imp-I mt+ gametes (2 × 10^6 cells/ml) were mixed with an equal number of wild-type mt− gametes for 0, 1, 5, 15, and 30 min, and samples containing 5 × 10^6 mt+ cells were analyzed by immunoblotting with the anti-GSP1 antibody. The arrowhead indicates the 122-kDa form of GSP1. The absence of zygotes 30 min after mixing is indicated below the corresponding lane.

Fig. 4. The higher molecular mass form of GSP1 appears only during sexual signaling. Vegetatively growing mt+ cells were transferred to nitrogen-free medium (N-free) (t = 0 h) to induce gametic differentiation. At various times during gametogenesis (indicated above each lane), 5 × 10^6 mt+ cells were removed and prepared for immunoblotting with the anti-GSP1 antibody. mt+ cells in nitrogen-free medium for 16 h were mixed with an equal number of mt+ gametes for 15 min, and samples containing 5 × 10^6 mt+ cells were also analyzed (lane +/−). The presence of flagellar agglutinins was assessed in the phase-contrast microscope by determining the ability of the mt+ cells to undergo flagellar adhesion with mt− tester gametes. The extent of agglutination (formation of large clumps of mt+ and mt− cells adhering to each other via their flagella) is shown below each lane: −, 0% agglutination; +, 25%; ++, 50%; ++++, 90%.

Fig. 5. Increases in cAMP alone induce the shift in mobility of GSP1. mt+ gametes (2 × 10^6 cells/ml) were incubated in culture medium alone (mt+), with an equal number of mt− gametes (+/−), or with 15 μM dibutyryl cAMP and 0.15 mM papaverine (cAMP) for 60 min, and 5 × 10^6 mt+ cells were analyzed by immunoblotting.

rapid, being detectable between 1 and 5 min after gametes were mixed together (Fig. 2A).

These initial experiments did not indicate whether the 122-kDa form of GSP1 represented a newly synthesized molecule or a modified form of pre-existing GSP1. Because flagellar adhesion has been shown to lead to the synthesis of new flagellar agglutinins (11), it was possible that fertilization also was associated with induction of synthesis of a new form of GSP1 in mt− gametes or even in mt+ gametes. The result that the appearance of the more slowly migrating form of GSP1 was not blocked by the protein synthesis inhibitor cycloheximide, however, demonstrated that the new form was derived from pre-existing GSP1. In addition, the results indicated that the molecules responsible for the conversion also pre-existed within the cells and were activated early during fertilization.

We were able to determine if cell fusion was required for the shift in mobility of GSP1 by use of the sterile mt− mutant of *Chlamydomonas*, imp-1. Gametes of *imp-I* express normal levels of flagellar agglutinins, adhere to mt− gametes, and undergo activation of adenyl cyclase and all of the cellular responses normally associated with increases in cAMP. Because of a lesion in the fus1 gene (16, 30), however, they are unable to undergo cell-cell fusion. The result (Fig. 3) that the shift in GSP1 mobility also occurred during adhesion and signaling of *imp-I* gametes indicated that adhesion-associated signals alone were sufficient to induce the change in GSP1; signals generated during cell fusion were not required for the modification. Moreover, all of the molecules required for the modification of GSP1 also were present within mt+ gametes.

The 122-kDa Form of GSP1 Is Expressed Only in Signaling Gametes—Having shown that flagellar adhesion alone brought
about conversion of GSP1, we next examined whether the more slowly migrating form of GSP1 was present at other stages in the life of a gamete. We showed previously (22) that GSP1 appears at the same time during gametogenesis that mt \(^+\) cells acquire flagellar agglutinins. In those experiments, however, we were not aware that GSP1 could be present in different electrophoretic forms, and the SDS-PAGE methods may not have been sufficient to distinguish between a 120- and a 122-kDa band. When we re-examined GSP1 during gametogenesis, we found that only the 120-kDa form of GSP1 was detectable at any time in unmixed mt \(^+\) gametes. Thus, the 122-kDa form of GSP1 appears in mt \(^+\) gametes only under the unique conditions that obtain as a consequence of interactions with mt \(^-\) gametes.

**Conversion of GSP1 Is Induced by cAMP Signaling and Is Due to Phosphorylation of GSP1**—A key consequence of flagellar adhesion is a rapid and dramatic increase in the intracellular level of cAMP (7, 10). Pasquale and Goodenough (7) and others (12, 13) have shown that incubation of gametes of a single mating type in this cyclic nucleotide along with a phosphodiesterase inhibitor induces all of the cellular responses in gametes that are associated with flagellar adhesion, including loss of cell walls, erection of mating structures, and movement of agglutinins from the cell body onto the flagella. Not surprisingly, we found that incubation of mt \(^+\) gametes with dibutyryl cAMP and a phosphodiesterase inhibitor also induced the post-translational modification of GSP1 (Fig. 5).

Moreover, H-8, an inhibitor of cyclic nucleotide-dependent protein kinases, blocked conversion of GSP1 even though the H-8-treated gametes underwent normal flagellar adhesion. Previous studies have shown that in mt \(^+\) and mt \(^-\) gametes mixed together in the presence of H-8, adenyl cyclase is activated, and cAMP levels undergo increases indistinguishable from those of untreated cells (9). In H-8-treated cells, however, the cellular events that normally accompany flagellar adhesion are blocked, including release of cell walls, activation of mating structures, and cell-cell fusion (7). The result that GSP1 conversion also is blocked by this inhibitor adds another event to this group of cellular responses activated by cAMP and blocked by H-8.

Biochemical experiments demonstrated that the adhesion-induced change in migration of GSP1 was due to phosphorylation. GSP1 immunopurified from signaling mt \(^+\) gametes and incubated with alkaline phosphatase was converted from the 122-kDa form to a 119-kDa form (Fig. 7). Consistent with this result, alkaline phosphatase treatment of GSP1 immunopurified from non-mating mt \(^-\) gametes also reduced its apparent molecular mass to 119 kDa. These results indicate that GSP1 undergoes a basal level of phosphorylation in mt \(^+\) gametes after its synthesis during gametogenesis and that flagellar adhesion leads to further phosphorylation of the molecule.

In earlier studies, we have shown that protein kinases are required for regulation of the gamete-specific adenyl cyclase that is activated during flagellar adhesion (8, 9). In related studies, we identified a protein (SksC) that serves as a substrate for a protein kinase whose activity is regulated by flagellar adhesion (31, 32) and is probably upstream of adenyl cyclase. The evidence presented here indicates that protein phosphorylation also occurs as a downstream event in this signaling pathway. To our knowledge, GSP1 is the first molecule to be identified whose phosphorylation state is regulated by the increased levels of cAMP present during fertilization in *Chlamydomonas*.

With the available information, it is not possible to determine if the increased level of phosphorylation is due to activation of a protein kinase(s) or inhibition of a protein phosphatase(s). GSP1 contains several consensus sites for phosphorylation by cAMP-dependent protein kinase (data not shown), and a simple interpretation of our results is that GSP1 is phosphorylated on one of these sites by a cAMP-dependent protein kinase. On the other hand, it is possible that regulation of GSP1 phosphorylation is controlled farther downstream in the pathway initiated by cyclic nucleotide-dependent protein kinases.

**Possible Roles for GSP1**—The role of the adhesion-induced phosphorylation of GSP1 in *Chlamydomonas* gametes and zygotes remains unknown. The presence of a homeodomain in the molecule and the mt \(^+\) gamete-specific expression of GSP1 suggest that it could be a transcription factor that regulates gene expression in gametes or zygotes or both. For example and by analogy to the role of the homeodomain protein Mata2 in *Saccharomyces cerevisiae* (33), after cell fusion, GSP1 might form a complex with molecules from the mt \(^+\) gamete (34, 35). On the other hand, if GSP1 functioned solely after cell fusion to regulate gene expression, then phosphorylation of GSP1 prior to cell fusion would seem to be unnecessary. In other words, since expression of GSP1 is sex-limited (i.e. expressed only in mt \(^+\) gametes), its presence in the mt \(^-\)/mt \(^+\) cytoplasm formed by cell fusion should be sufficient for induction of zygotic genes without prior phosphorylation. The observation that phosphorylation of GSP1 occurs in the absence of cell fusion suggests that phosphorylation of GSP1 could play a role during signaling in mt \(^+\) gametes (Fig. 3), possibly to up-regulate synthesis of a gamete-specific molecule such as agglutinin that undergoes rapid turnover during flagellar adhesion (11, 13).

Now that a protein has been identified whose phosphorylation is regulated (either directly or indirectly) by cAMP, it becomes possible to study the molecules responsible for this post-translational modification. Such studies should offer new molecular insights into the entire adhesion-induced signaling pathway during fertilization.

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