Plant & Cell Physiol. 19(7): 1253–1261 (1978).

Mating type specific induction of cell wall lytic factor by agglutination of gametes in *Chlamydomonas reinhardtii*

Yoshihiro Matsuda, Shin-ichi Tamaki[1] and Yoshihiro Tsubo[2]

Department of Biology, Faculty of Sicence[1] and School of General Education[2], Kobe University, Kobe 657, Japan

(Received February 28, 1978)

When mt^+ and mt^- gametes of *Chlamydomonas reinhardtii* were mixed, shedding of cell walls took place in both mating types during massive agglutination and/or pairing. This was caused by a cell wall lytic factor that had been induced by flagellar agglutination and excreted into the medium by cells concurrently with their cell wall release.

When glutaraldehyde-fixed gametes and isolated flagella of one mating type caused isoagglutination of live gametes of the other mating type, the live mt^+ gametes induced the lytic factor and shed their walls, whereas none of the live mt^- did this. The cell walls of mt^- gametes were lost only when the lytic factor, which had been excreted by mt^+ gametes into the medium, acted from the outside. These data imply that mt^+ gametes are responsible for the induction of the lytic factor by agglutination, which acts on cell walls of both mating types either endogenously or exogenously.

Key words: Cell wall lysis — *Chlamydomonas* — Mating type specificity.

Sexual reproduction in *Chlamydomonas reinhardtii* involves gametic differentiation from vegetative cells and the mating reaction between mating-type plus (mt^+) and minus (mt^-) gametes. Gametogenesis can be induced by the simple removal of nitrogen compounds from the culture medium (*15*). The mixing of gametes of opposite mating types induces the mating reaction, which is known to proceed through at least four steps: a massive flagellar tip agglutination, mating pair formation, cytoplasmic connection by fertilization tubule, and cell fusion to form a zygote (*15*). These sequential events have been studied biochemically, morphologically and genetically (*4–8*, *10*, *19–21*, *24*).

In this alga, lysis of gamete cell walls takes place during the mating process as a necessary prelude to cell fusion (*2*, *6*). It is caused by a cell wall lytic factor that has been induced in gametes by flagellar agglutination (*2*). However, the mechanism of induction of the factor as well as the nature of its lytic action remain obscure. In this paper, we present evidence that the lysis event of the gametic cell wall during mating involves a mating type-specific trait; only mt^+ gametes induce the lytic factor by agglutination and release it into the medium, where it acts on the cell wall of mt^- from the outside.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

Materials and methods

Strains

The mating-type plus (mt^+) and minus (mt^-) strains of *Chlamydomonas reinhardtii* used in the present study were 6301B and 6301D, respectively (*16*). Single cell clones were selected every two months to maintain mating ability. A non-mating mutant, fus^-, was obtained from our 21 gr (mt^+) (*13*) stock culture as a spontaneous mutant. This is capable of sexual agglutination with mt^- gametes, but can form zygotes very rarely (see **Results**). A mutant without a cell wall, CW-15 (*3*), was obtained from the Cambridge Algae Collection through the kindness of Dr. D. R. Davies of the John Inns Institute.

Vegetative and gametic cultures

Cells were grown vegetatively in a minimal salt medium (M) under constant illumination as described elsewhere (*11*). Cultures were illuminated concurrently with two types of fluorescent bulbs (daylight and white types) at 6000 lux. All cultures were maintained at 25°C with aeration. To obtain gametic cells, vegetative cultures in the late logarithmic phase ($2–2.5 \times 10^6$ cells/ml) were aseptically harvested by centrifugation at $1500 \times g$ for 3 min. They were washed once with induction medium and resuspended in this medium at a final density of 5×10^6 cells/ml unless otherwise specified. The induction medium consisted of 1/5 the concentration of the medium deprived of the nitrogen source (NH_4NO_3) and supplemented with 0.2 g/liter of sodium acetate. Each cell type was induced separately at 25°C under a light intensity of 3000 lux with moderate shaking.

Determination of mating efficiency

Mating tests were carried out by mixing equal numbers of the opposite mating-type gametes in 1–2 ml volumes. Mixed cells were placed in a small test tube, left to stand for 30 min at 25°C under the light (3000 lux) and then fixed with a drop of 10% glutaraldehyde. The number of cells possessing two flagella (unmated gametes) and three to four flagella (zygotes) were determined in a hemocytometer by phase contrast microscopy and the mating efficiency was calculated according to the following equation:

$$\text{mating efficiency } (\%) = \frac{(\text{number of zygotes}) \times 2 \times 100}{(\text{number of zygotes}) \times 2 + \text{number of unmated gametes}}$$

Quantitative determination of wall-less cells

To distinguish between the normally walled and wall-less cells in a given cell suspension, a simple heating method was developed. A known number of cells was heated in a small test tube at 60°C for 2 min and shaken vigorously with a Vortex mixer for about 15 sec. A drop of 10% glutaraldehyde was then added and the reduction in cell number was found with the hemocytometer. In a preliminary test using a vegetatively grown wild-type and the CW-15 mutant, the former cells remained intact after the heat treatment, whereas the latter wall-less cells had ruptured into small fragments that could not be counted with the hemocytometer. Thus, the proportion of wall-less cells present in the cell cultures could be quantified by this method.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

Flagellar isolation

Flagella were detached from gametic cells by the "STEEP+calcium" procedure of Witman et al. (*25*). About 1×10^9 cells were pelleted at room temperature by centrifugation at $3000 \times g$ for 10 min, washed once with 10 mM Tris-HCl buffer (pH 7.8), and resuspended in an equal volume of this buffer. The cell suspension was cooled to 10°C and about five times the volume of cold STEEP (0.15 M sucrose, 15 mM Tris-HCl, 2.5 mM EDTA·2 Na, 11% ethanol, and 30 mM KCl) was added. The mixture was stirred vigorously with the Vortex mixer, supplemented with $CaCl_2$ to a final concentration of 20 mM and again vigorously agitated for 2 min. The detached flagella were separated from the cell bodies by loading the mixture (15 ml) on 25 ml of 25% sucrose-10 mM Tris-HCl and centrifuging it at $3000 \times g$ for 10 min in a swinging bucket-type rotor. A white band at the interface was removed and centrifuged at $20,000 \times g$ for 30 min. The flagellar pellet thus obtained was washed once with the induction medium, resuspended in 2–5 ml of this medium, and the concentration was measured with the hemocytometer by phase contrast microscopy.

Gametic cell fixation

Glutaraldehyde was added directly to gametic cultures or condensed cell suspensions to a final concentration of 2%. Cells were fixed on ice for 1 hr and then washed three times with the induction medium by centrifugations of $1000 \times g$ for 5 min. They were then resuspended in the medium at a final density of about 10^8 cells/ml.

Results

Under the induction conditions described in **Materials and methods**, fully differentiated gametes were obtained after about 9 hr of induction following nitrogen removal. Mating efficiency, when the wild-type mt^+ and mt^- gametes were mixed, was about 90%. When fus^- mutant cells were induced, they normally agglutinated with the wild-type mt^-, but could very rarely fuse to form zygotes (less than 1% mating efficiency). During the induction, the increase in cell number for mt^+ cells (including fus^- mutant) was not significant and was usually 10–30% for mt^- cells. Almost all unmated gametes of both mating types had cell walls, as monitored by the heating method described in **Materials and methods**. Note here that occasional cultures of the mt^- exhibited an increase of 100% or more in the cell number during the induction, and these cell populations contained at most 35% of heat-sensitive, presumably wall-less cells. However, this phenomenon as well as the extensive cell multiplication were not necessarily related to the mating competency of the mt^- gametes. Thus, we used only walled gametes showing minimal cell multiplication during a 9-hr induction period in the following experiments to investigate the lytic mechanisms of cell walls during mating.

Induction of wall lysis during mating

Shedding of cell walls during mating was preliminarily observed by phase contrast microscopy. Soon after the complementary gametes were mixed, many cells began to shed their walls in large and small agglutinating clusters or in mating pairs. A bud-like extrusion of protoplasm through the break of cell walls at the anterior end was temporarily seen. The cell walls were then shed into the medium.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

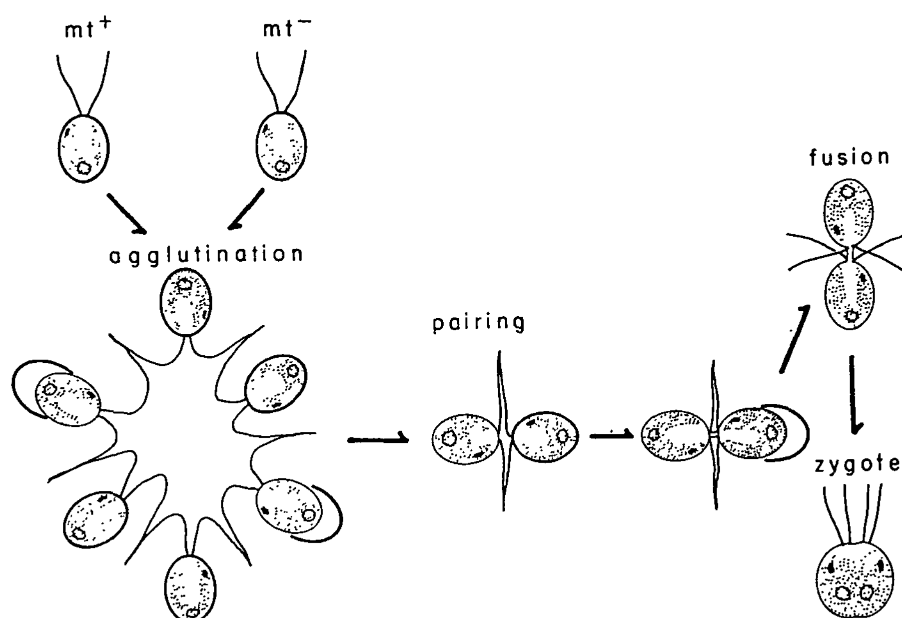

Fig. 1. *Schematic drawing of the induction of cell wall release during the mating process between wild-type mt+ and mt− gametes.* This was based on phase contrast observations of early mating mixtures. Cells surrounded with cell walls were represented by thick lines, and naked protoplasts by thin lines. For explanation, see text.

When we followed the time of cell wall release for opposite mating-type gametes during the mating process, a significant difference was noticed in early mating mixtures; one cell in each pair shed its cell wall during the previous massive agglutination or soon after pairing, while the other shed it only after a fertilization tubule (*4*) connected the two cells (Fig. 1). However, this difference became indistinguishable in mating pairs formed during the later period after mixing. It appeared that both gametes had already lost their walls before pairing.

Table 1 *Effects of the culture medium separated from mating suspensions on vegetative and gametic cell walls*

Test cells		Cell number per ml ($\times 10^4$)		% wall-less cells
Strain	Culture condition	Before heating	After heating	
Wild-type, mt+	vegetative	146	54	63
, mt−		118	73	38
, mt+	Gametic	178	2	99
, mt−		225	0	100
fus− , mt+		177	1	99

A mixed suspension of wild-type mt+ and mt− gametes that had been incubated for 30 min at 9-hr induction period (mating efficiency 88%) was centrifuged at $15,000\times g$ for 30 min. The medium (0.5 ml) was added to vegetative or gametic cells (0.5 ml). They were incubated for 30 min then the percent of wall-less cells was measured.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

When fus$^-$ mutant (mt$^+$) gametes agglutinated with wild-type mt$^-$ gametes, the release of cell walls also occurred. In this mixture, agglutination continued for at least 1 day without zygote formation and many cells were ruptured, probably due to vigorous collision between naked protoplasts.

As pointed out by Claes (*2*), a cell wall lytic factor was excreted into the medium of the mating mixture by cells concurrently with cell wall release. This is shown quantitatively in Table 1. A mixture of wild-type gametes that had been preincubated for 30 min at the 9-hr induction period was centrifuged at $15{,}000 \times g$ for 30 min to separate the medium from the cells. The medium was then added to suspensions of vegetative or gametic cells in equal volumes, which were incubated for 30 min. During the incubation, no agglutination of the test gametes was observed. If an agglutination-causing factor such as detached flagella (see below) or membrane vesicles (*1*, *21*, *24*) had been present, such a phenomenon would be expected. About half of the vegetative and almost all of the gametic cells were converted to naked protoplasts by the lytic factor acting from the outside. Similar cell wall lysis took place with the medium obtained from the mixture of fus$^-$ (mt$^+$) and mt$^-$ gametes.

Induction of wall lysis during isoagglutination

Since both mating-type gametes in the mixture are made naked by the lytic factor acting endogenously or exogenously, at least two possible explanations exist for the lytic mechanisms: (a) both mating-type cells can induce the lytic factor endogenously by agglutination, or (b) only one mating-type cell loses its cell wall due to an endogenous factor, while the other type loses it by the action of an exogenous factor. To distinguish between these mechanisms, we conducted isoagglutination experiments as follows.

When fully competent gametes of either mating type were fixed with glutaraldehyde and mixed with live gametes of the opposite mating type in a 1 : 1 proportion, apparently normal agglutination took place. The agglutination did not occur when the glutaraldehyde-fixed gametes were mixed with live gamees of the same mating type or with vegetative cells of the opposite mating type. As shown in Table 2, when live mt$^+$ gametes (including the mutant) agglutinated with fixed mt$^-$ gametes, 25–50% of the cells lost their walls during 30-min incubation. In contrast, when live mt$^-$ gametes agglutinated with fixed mt$^+$, virtually no cell wall lysis occurred.

Table 2 *Induction of cell wall lytic factor by isoagglutination of gametes caused by glutaraldehyde-fixed cells*

Combination		Agglutination	Cell number per ml ($\times 10^4$)		% wall-less cells
Live gametes	Fixed gametes		Before heating	After heating	
mt$^+$	mt$^+$	−	507	490	3
mt$^+$	mt$^-$	+	502	368	27
fus$^-$ mt$^+$	mt$^-$	+	474	245	48
mt$^-$	mt$^-$	−	483	485	0
mt$^-$	mt$^+$	+	487	482	1
mt$^-$	fus$^-$ mt$^+$	+	507	505	0

Equal numbers of live gametes and glutaraldehyde-fixed gametes were mixed and incubated for 30 min.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

Table 3 *Induction of cell wall lytic factor by isoagglutination of gametes caused by isolated gametic flagella*

Combination		Agglutination	Cell number per ml ($\times 10^4$)		% wall-less cells
Gametes	Flagella		Before heating	After heating	
mt^+	mt^+	−	304	310	−2
mt^+	mt^-	+	305	3	99
fus^- mt^+	mt^-	+	320	0	100
mt^-	mt^-	−	374	366	2
mt^-	mt^+	+	374	367	2

Isolated gametic flagella were given at about twice the number of gametes. Incubation time was 30 min.

Since glutaraldehyde-fixed cells were completely resistant to the heat treatment even after they had been incubated with the medium containing the lytic factor, the above results indicate that only live mt^+ gametes shed their walls by agglutination.

Table 3 further clarifies this point with isolated gametic flagella. Isolated flagella of either mating type were given to gametes of the same or opposite mating type at about twice the number of gametes. Widespread agglutination occurred with the opposite mating type, and the agglutination clumps grew into large clusters until the cells visibly settled at the bottom of the test tube. After a 30-min incubation, the mt^+ gametes were mostly converted into naked protoplasts (Table 3) and also had excreted the lytic factor into the medium, which acted exogenously on the test gametes of both mating types (Table 4). On the other hand, the isoagglutination reaction of mt^- gametes was not accompanied by cell wall lysis nor by excretion of the factor into the medium (Tables 3 and 4). Similar results were obtained when the number of gametic flagella was greater than a twofold excess.

The above experiments showed that the agglutination-causing activity of glutaraldehyde-fixed gametes and isolated gametic flagella disappears once they have interacted with flagella of opposite mating-type gametes. For example, when isolated flagella and live gametes were mixed at a 2 : 1 ratio as above, agglutination took place very intensively for the initial 20–30 min, then decreased gradually and disappeared completely after about 1 hr. When fresh flagella were added, the gametes immediately isoagglutinated, suggesting that the cells maintain their agglutinability on their flagellar tips. Comparable results were obtained by Wiese (*23*), who observed that the aggluntinability of flagella membrane vesicles, called gamone, disappears once they are adsorbed on gametes of the opposite sex.

Table 4 *Effects of the culture medium separated from isoagglutinating gametic suspensions on gametic cell walls*

Medium source	% lysis of test gametes	
	mt^+	mt^-
mt^+ cells × mt^- flagella	39	98
mt^- cells × mt^+ flagella	0	0

The experimental conditions for isoagglutination were the same as those in Table 3. The culture media separated by centrifugation (0.5 ml) were mixed with mt^+ or mt^- gametic cultures (0.5 ml), which were incubated for 30 min.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

Discussion

In experiments of agglutination caused by glutaraldehyde-fixed gametes and isolated gametic flagella of the opposite mating type, the present results indicated that the mt^+ gametes induced the lytic factor and released their cell walls, whereas the mt^- gametes only agglutinated without cell wall lysis. Cell walls of the mt^- were lost when the lytic factor, which had been excreted by mt^+ gametes into the medium concurrent with the cell wall release, acted exogenously. These data imply that in the normal mating reaction between mt^+ and mt^- gametes, only the former mating type is responsible for the induction of the lytic factor by agglutination, and this factor acts on the cell walls of both gametes either exogenously or endogenously. Phase contrast observations of cell wall release in mating pairs support this idea. Although restricted to pairs that had been formed relatively early after mixing, they showed that each gamete forming a pair shed its wall at different steps of the mating process; one cell shed its wall during massive agglutination or soon after pairing, while the other only after a fertilization tubule connected two cells (Fig. 1). This time lag between the two cells depends on the mating type and whether the lysis of the cell wall is caused an endogenous or exogenous factor. However, in mating pairs formed rather late after mixing, both gametes almost always lost their walls before pairing. This can be explained by the action of a lytic factor, accumulated in the medium during prolonged incubation, on gametes of both mating types which are not ready for pairing.

The present results are in contrast with those obtained by Claes (*2*) and Goodenough and Weiss (*6*), who reported for synchronously induced gametes that both mating-type gametes were converted to naked protoplasts by agglutination caused by gametic flagella (*2*) or glutaraldehyde-fixed gametes (*6*)[1]. The contradiction may be due to the induction history of gametes used by these workers, in addition to differences in methods of quantification for wall-less cells. We preferred using non-synchronized vegetative cultures over synchronized ones because gametogenesis is simpler in the former than the latter. Asynchronous gametogenesis requires less time after nitrogen removal to achieve mating competency. Cell division is not essential to this gametogenesis, and the gametes obtained morphologically resemble the vegetative cells (*19*). In contrast, synchronous gametogenesis is necessarily accompanied by a round of cell division and the total cell number usually increases about fourfold during the induction (*9*). Extreme degradations of cellular components and structures take place during the induction period (*7, 8, 10, 20*). Some of them are thought to be brought about merely by prolonged incubation in the nitrogen-starved medium rather than by a step required in gametogenesis (*10, 19*). Therefore, one must be cautious in interpreting results obtained with synchronous gametes (*10, 19*).

In this alga, another type of lytic factor, which is related to daughter cell liberation from the mother cell wall after cell division, is known (*12, 17, 18*). The factor

[1] During the preparation of this manuscript, Solter and Gibor published a different result using non-synchronized gametes (*22*). They reported that both glutaraldehyde-fixed gametes and isolated gametic flagella, though they caused the agglutination reaction, did not induce the lytic factor. We do not know, however, their precise experimental conditions, such as the ratio of flagella or fixed gametes given to the opposite mating-type cells.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

is excreted into the medium by cells concurrently with daughter cell liberation, where it acts on other cells from the outside (*12*, *18*). Therefore, a suspension of synchronous gametes may contain this type of factor, which in turn may affect gametic cell walls. This may be supported by our finding that some portions of cells were converted to naked protoplasts while in their unmated state during the cell multiplication that occurred in the induction period of asynchronous cultures. Similar wall-less gametes have been observed in the synchronous culture (*6*).

We believe that the induction of cell wall lytic factor by aggluntination may be a mating type-specific trait of the mt^+ gamete. It is well known that in *Chlamydomonas reinhardtii*, the mt^+ gamete synthesizes agglutination factors that are distinct from those of mt^- flagella (*1*, *21*), produces a fertilization tubule to establish cytoplasmic continuity with the mt^- gamete (*4*, *6*), and uniquely transmits to the zygote a group of cytoplasmic genes (*14*). Thus, the mt^+ gamete may play more specific and active roles than the mt^- one during the mating process. Further study of the induction mechanism of the lytic factor in mt^+ gametes by agglutination, as well as the chemical nature of the factor, is necessary.

Gametes carrying fus^- mutation normally agglutinated with the wild-type mt^- gametes and induced the lytic factor during the agglutination, but could form zygotes only very rarely. These phenotypic behaviors resemble those of the imp-1 mt^+ mutant strain of Goodenough et al. (*5*). The fus^- gametes may not be able to elaborate the fertilization tubule, as observed with imp-1 (*6*).

We are grateful to Drs. R. K. Togasaki and R. Schneeman of Indiana University for reading the manuscript. This work was supported in part by a grant from the Ministry of Education, Science and Culture.

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

References

(*1*) Bergman, K., U. W. Goodenough, D. A. Goodenough, J. Jawitz and H. Martin: Gametic differentiation in *Chlamydomonas reinhardtii* II. Flagellar membranes and the agglutination reaction. *J. Cell Biol.* 67: 606–622 (1975).

(*2*) Claes, H.: Autolyse der Zellwand bei den Gameten von *Chlamydomonas reinhardii*. *Arch. Mikrobiol.* 78: 180–188 (1971).

(*3*) Davies, D. R. and A. Plaskitt: Genetical and structural analyses of cell-wall formation in *Chlamydomonas reinhardii*. *Genet. Res. Camb.* 17: 33–43 (1971).

(*4*) Friedmann, I., A. L. Colwin and L. H. Colwin: Fine structural aspects of fertilization in *Chlamydomonas reinhardi*. *J. Cell Sci.* 3: 115–128 (1968).

(*5*) Goodenough, U. W., C. Hwang and H. Martin: Isolation and genetic analysis of mutant strains of *Chlamydomonas reinhardi* defective in gametic differentiation. *Genetics* 82: 169–186 (1976).

(*6*) Goodenough, U. W. and R. L. Weiss: Gametic differentiation in *Chlamydomonas reinhardtii* III. Cell wall lysis and microfilament-associated mating structure activation in wild-type and mutant strains. *J. Cell Biol.* 67: 623–637 (1975).

(*7*) Jones, R. F.: Physiological and biochemical aspects of growth and gametogenesis in *Chlamydomonas reinhardtii*. *Ann. N. Y. Acad. Sci.* 175: 648–659 (1970).

(*8*) Jones, R. F., J. R. Kates and S. J. Keller: Protein turnover and macromolecular synthesis during growth and gametic differentiation in *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* 157: 589–598 (1968).

(*9*) Kates, J. R. and R. F. Jones: The control of gametic differentiation in liquid cultures of *Chlamydomonas*. *J. Cell Comp. Physiol.* 63: 157–164 (1964).

(*10*) Martin, N. C. and U. W. Goodenough: Gametic differentiation in *Chlamydomonas reinhardtii* I. Production of gametes and their fine structure. *J. Cell Biol.* 67: 587–605 (1975).

(*11*) Matsuda, Y., T. Kikuchi and M. R. Ishida: Studies on chloroplast development in *Chlamydomonas reinhardtii* I. Effect of brief illumination on chlorophyll synthesis. *Plant & Cell Physiol.* 12: 127–135 (1971).

(*12*) Mihara, S. and E. Hase: Studies on the vegetative life cycle of *Chlamydomonas reinhardi* Dangeard in synchronous culture III. Some notes on the process of zoospore liberation. ibid. 16: 371–375 (1975).

(*13*) Sager, R.: Inheritance in the green alga *Chlamydomonas reinhardi*. *Genetics* 40: 476–489 (1955).

(*14*) Sager, R.: *Cytoplasmic Genes and Organelles*. Academic Press Inc., New York, N. Y., 1972.

(*15*) Sager, R. and S. Granick: Nutritional control of sexuality in *Chlamydomonas reinhardi*. *J. Gen. Physiol.* 37: 729–742 (1954).

(*16*) Sager, R. and Y. Tsubo: Genetic analysis of streptomycin-resistance and -dependence in *Chlamydomonas*. *Z. Vererbungsl.* 92: 430–438 (1961).

(*17*) Schlösser, U.: Enzymatisch gesteuerte Freisetzung von Zoosporen bei *Chlamydomonas reinhardii* Dangeard in Synchronkultur. *Arch. Mikrobiol.* 54: 129–159 (1966).

(*18*) Schlösser, U. G.: Entwicklungsstadien- und sippenspezifische Zellwand-Autolysine bei der Freisetzung von Fortpflanzungszellen in der Gattung *Chlamydomonas*. *Ber. Deutsch. Bot. Ges.* 89: 1–56 (1976).

(*19*) Schmeisser, E. T., D. M. Baumgartel and S. H. Howell: Gametic differentiation in *Chlamydomonas reinhardi*: Cell cycle dependency and rates in attainment of mating competency. *Dev. Biol.* 31: 31–37 (1973).

(*20*) Siersma, P. W. and K.-S. Chiang: Conservation and degradation of cytoplasmic and chloroplast ribosomes in *Chlamydomonas reinhardtii*. *J. Mol. Biol.* 58: 167–185 (1971).

(*21*) Snell, W. J.: Mating in *Chlamydomonas*: A system for the study of specific cell adhesion I. Ultrastructural and electrophoretic analyses of flagella surface components involved in adhesion. *J. Cell Biol.* 68: 49–69 (1976).

(*22*) Solter, K. M. and A. Giborr: The release of carbohydrates during mating in *Chlamydomonas reinhardi*. *Plant Sci. Lett.* 8: 227–231 (1977).

(*23*) Wiese, L.: On sexual agglutination and mating-type substances (gamones) in isogamous heterothalic *Chlamydomonas* I. Evidence of the identity of the gamones with the surface components responsible for sexual flagellar contact. *J. Phycol.* 1: 46–54 (1965).

(*24*) Wiese, L.: Nature of sex specific glycoprotein agglutinins in *Chlamydomonas*. *Ann. N. Y. Acad. Sci.* 234: 383–395 (1974).

(*25*) Witman, G. B., K. Carlson, J. Berliner and J. L. Rosenbaum: *Chlamydomonas* flagella I. Isolation and electrophoretic analysis of microtubules, matrix, membranes, and mastigonemes. *J. Cell Biol.* 54: 507–539 (1972).

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016

Downloaded from http://pcp.oxfordjournals.org/ at Penn State University (Paterno Lib) on September 16, 2016