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Transcription of Novel Genes, Including a Gene Linked to the Mating-Type Locus, Induced by *Chlamydomonas* Fertilization

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Six cDNA clones have been identified that are complementary to transcripts present in young zygotes of *Chlamydomonas reinhardtii* but absent from vegetative and gametic cells. Five early transcripts are synthesized within 5 to 10 min of fertilization; the sixth, late, transcript is not synthesized until 90 min following fertilization. Synthesis of both classes requires cell fusion between gametes. Cycloheximide fails to inhibit early mRNA synthesis, indicating that transcription factors must preexist in the gametes and be activated by cytoplasmic confluence. By contrast, cycloheximide blocks synthesis of the late transcript, suggesting that an early protein product(s) is required for expression of the late gene. Restriction fragment length polymorphism analysis of inter- and intraspecific genetic crosses demonstrates that one of the early genes is very tightly linked to the mating-type locus.

The single-celled alga *Chlamydomonas reinhardtii* undergoes an apparently simple type of fertilization and development (23, 24); gametes of opposite mating type (*mt*), called plus (mt^+) and minus (mt^-), fuse to form a diploid zygote, which quickly begins to synthesize a novel group of proteins, some of which are glycoproteins that go on to assemble into an insoluble wall around the cell (2, 18). In this article we report the cloning of six zygote-specific cDNAs, documenting that at least some of the observed fertilization-induced shifts in protein synthesis (18, 31) are due to novel gene expression in zygotes and not simply to the activation of maternal-mRNA equivalents. We found that five of the six genes are transcribed within 5 to 10 min after fertilization, whereas the sixth is not expressed until 90 min after fertilization; since the expression of the sixth is cycloheximide sensitive, we propose that a protein product of one or more of the early genes is required for the expression of such a late gene. The expression of the early genes precedes nuclear fusion and is cycloheximide insensitive, indicating that factors synthesized by the gametes must interact in the zygote to initiate the early program. Finally, we found that one of the five early genes was linked to the mating-type locus, which will make it possible to probe the genomic organization of this key regulatory element.

MATERIALS AND METHODS

Cells. Except when designated, experiments were performed with strains CC620 (mt^+) and CC621 (mt^-). Vegetative cells were grown in liquid TAP medium (8a) and harvested during logarithmic growth; gametes were produced on TAP agar plates and suspended in N-free HSM (16). Equal numbers of mt^+ and mt^- gametes were mated at 2×10^7 to 5×10^7 cells per ml to produce zygotes. The efficiency of mating, determined as described previously (17), was generally 85 to 95%. Obvious cell wall secretion (as judged by adhesion of zygotes to one another and resistance to lysis with 1% Nonidet P-40) usually begins about 3 h after mating; however, this time has varied from 2.5 to 5 h in different experiments.

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DNA and RNA isolation and characterization. *Chlamydononas* DNA was prepared essentially by the method of Weeks et al. (30), except that the DNA was phenol extracted several times after recovery from CsCl. *Chlamydomonas* RNA used for Northern blot analysis was prepared by the method of Kirk and Kirk (12); RNA used for cDNA cloning was prepared by lysing zygotes in guanidinium isothiocyanate-sodium dodecyl sulfate and pelleting the RNA through CsCl as described by Maniatis et al. (15).

Genomic DNA was fractionated on agarose gels (from 0.8 to 1.1%) containing Tris-borate-EDTA buffer (15) or, in some cases, Tris-acetate buffer (4). The DNA was blotted to nitrocellulose (29) and then prehybridized, hybridized with ^{32}P-labeled probe, and rinsed as described by Church and Gilbert (3). RNA was fractionated on formaldehyde–1.2% agarose gels and blotted to nitrocellulose, without a partial alkaline hydrolysis step (15). Filters were prehybridized and then hybridized to plasmids ^{32}P-labeled by nick translation (21) in a solution containing 50% formamide, 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 1× Denhardt solution, 100 μg of sonicated, denatured salmon sperm DNA per ml, 0.2% sodium dodecyl sulfate, and 10 μg of poly(A) per ml at 55°C for 36 h. The filters were rinsed in 2× SSC–0.2% *N*-lauroyl sarcosine (three changes) and then in 0.5× SSC–0.2% *N*-lauroyl sarcosine (three changes) at 65°C.

cDNA cloning. Poly(A)$^+$ RNA was prepared from total RNA by two passages of the RNA over an oligo(dT)-cellulose column (15). Poly(A)$^+$ RNA prepared from zygotes 3 h after mating was used to produce double-stranded cDNA. First-strand synthesis, second-strand synthesis, and S1 nuclease treatment were performed as described by Huynh et al. (10). Oligo(dC) tails were added to the cDNA with terminal transferase (15), and the cDNA was then annealed with *Pst*I-digested, oligo(dG)-tailed pBR322 (Bethesda Research Laboratories). This preparation was then used to transform *Escherichia coli* RR1. Individual Tet^r Amp^s transformants were transferred to duplicate nitrocellulose filters, amplified on chloramphenicol, and lysed in situ essentially as described by Maniatis et al. (15). The filters were hybridized (3) with [^{32}P]cDNA prepared from poly(A)$^+$ RNA by using the first-strand cDNA synthesis protocol but substituting [α-^{32}P]dCTP for unlabeled dCTP.

Genetic analysis. The inheritance of restriction fragment length polymorphisms (RFLPs) was determined by performing an interspecific cross between *C. smithii* mt^+ (CC1373) and *C. reinhardtii* $imp\text{-}5^-$ mt^- (CC470) by using standard protocols (14). Because of the presence of the *imp-5* marker (a mating type-specific mutation that renders mt^+, but not mt^-, cells nonagglutinating [7]) in the cross, all mt^+ $imp\text{-}5^-$ recombinants will be nonagglutinating. Unfortunately, the *C. smithii* parent also proved to give a poor agglutination reaction, and so progeny that were nonagglutinating could not be assumed to be mt^+ *imp-5*, as in the original scheme. Since three of the four progeny in the two tetrads analyzed gave a scorable mating type, the fourth progeny could be assumed to be mt^+. In the random progeny, however, we chose to use only progeny for which we could score the mating type, meaning that all mt^+ progeny must be $imp\text{-}5^+$. The interpretation of the data does not seem to be affected by these biases. Strains CC124 and CC125 were used in the intraspecific cross of *C. reinhardtii* wild-type mt^+ and mt^- (see Fig. 6).

RESULTS

Relevant features of fertilization and zygote development. Features of *Chlamydomonas* fertilization and zygote development that are pertinent to our study are summarized below; detailed accounts are published elsewhere (6a, 8, 25). Haploid biflagellate vegetative cells, growing mitotically, differentiate into haploid biflagellate gametes when deprived of a nitrogen source. When plus (mt^+) and minus (mt^-) gametes are mixed, they undergo an instantaneous agglutination via their flagella. Adhesion elicits several prefertilization changes, including cell wall loss and the activation of organelles known as mating structures, and pairs of cells then undergo fusion via their mating structures, followed by full cytoplasmic confluence. The time between agglutination and fusion can be as short as 15 s, comparable to fertilization rates in the metazoa. Cell fusion causes the four flagella to disadhere, and the resultant quadriflagellated cell is motile for ca. 3 h. The two nuclei in the zygote begin to fuse after ca. 1 h, and zygote-specific wall proteins begin to be secreted at ca. 3 h, at which time the four flagella are resorbed. In liquid culture, the zygotes secreting wall protein come to stick together, eventually forming large sheets of cells; if plated on 4% agar at low cell density, each cell forms a discrete cell wall and individual zygotes can be recovered for genetic analysis (14). Germination is induced in the laboratory by transferring mature, dormant zygotes to fresh, moist plates in the light; meiosis occurs during the ensuing 12 to 18 h, and the zygote wall is ruptured by secreted factors. It is not known whether the mRNAs controlling meiosis are transcribed before the dormant period and stored or whether they are transcribed only when germination is initiated.

Cloning of zygote-specific cDNA. Poly(A)$^+$ RNA was isolated from zygotes 3 h after gametes were mixed, at which time the cells had just begun to adhere in small clumps and become resistant to lysis with 1% Nonidet P-40, indicating that they had initiated the secretion of zygote wall proteins (18). The poly(A)$^+$ RNA was used to generate cDNA, which was then inserted into the *Pst*I site of pBR322 by using dG-dC homopolymer tails. This preparation was used to transform *E. coli*, and approximately 1,200 Amp^s Tet^r colonies were grown individually on duplicate nitrocellulose filters, lysed in situ, and hybridized with [^{32}P]cDNA from zygotes or from gametes (Fig. 1). Approximately 120 clones that gave preferential hybridization with the zygote probe were rescreened by preparing plasmid mini-preparations from the bacteria, binding the DNA to nitrocellulose with a dot-blot manifold, and hybridizing as before. Thirteen clones that were clearly zygote specific were chosen for further analysis. It should be noted that this screen would identify only abundant mRNAs, and would not identify mRNAs that were present transiently shortly after fertilization.

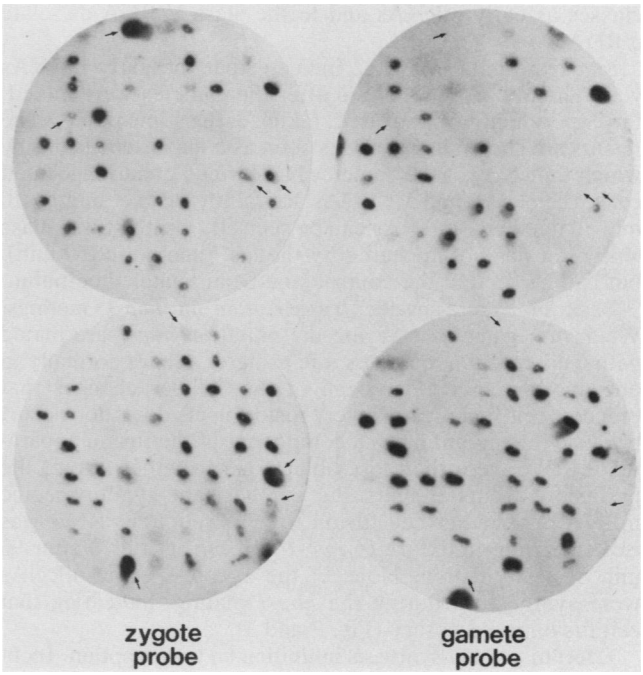

FIG. 1. cDNA screening. *E. coli* transformants were grown on duplicate nitrocellulose filters, lysed in situ, and hybridized with [^{32}P]cDNA made from either zygote poly(A)$^+$ RNA or gamete poly(A)$^+$ RNA. Autoradiograms of two such pairs of filters are shown; arrows point to colonies that hybridized to the zygote but not to the gamete probe.

To determine whether any of the clones had related inserts, the insert DNA was purified from some of the plasmids on low-melting-point agarose gels after digestion with *Pst*I, ^{32}P labeled by nick translation, and then hybridized to the other clones. From the results of such cross-hybridization experiments, the clones were divided into six classes (Table 1). For each class, the plasmid with the largest insert was used for further characterization of the cDNA.

Demonstration of zygote specificity. To confirm that the cDNA clones indeed represented zygote-specific mRNA, they were used to probe Northern blots of total RNA. RNA was isolated from vegetative cells, mt^+ gametes, mt^- gametes, and zygotes at various times after mating, electrophoresed on a formaldehyde–1.2% agarose gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled plasmid DNA from each cDNA class (Fig. 2). Each class hybridized only to a single RNA species, which was present only in zygote RNA (sizes are indicated in Table 1). Control hybridizations with a β-tubulin cDNA clone or with non-zygote-specific clones from our collection (202 and 204) were included to demonstrate that RNA is indeed present in the vegetative and gametic lanes.

The time course studies (Fig. 2) indicate that five of the RNAs (classes I to IV and VI) were already present by 30 min after mating. By contrast, class V RNA did not appear until at least 90 min after mating. We refer to the five RNA

classes as early mRNAs and to the class V RNA as a late mRNA.

Since the data in Fig. 2 indicate that the early mRNAs were already present 30 min after the gametes were mixed, the experiment was repeated, taking earlier time points. The results are shown in Fig. 3. As soon as 5 min after fusion, by which time 87% of the gametes had formed quadriflagellated cells, the class I and VI RNAs are faintly visible, and by 10 min all five early mRNAs can be seen. By contrast, the class V mRNA has not appeared by the last time point (60 min), but can be seen in the control lane from a later time point.

Lack of zygote-specific transcription in *imp-1* matings. When mt^+ gametes carrying the mutation *imp-1* are mated with wild-type mt^- gametes, the gametes adhere normally to one another and undergo all of the cellular changes that precede cell fusion; however, fusion itself does not occur, because the mutant lacks a component of the fusion apparatus (6). We were therefore able to ask whether any of the prefusion events triggers the synthesis of zygote-specific mRNA or whether cell fusion itself is required. RNA was isolated from a mixture of *imp-1* and mt^- gametes after 90 min of agglutination. None of the zygote-specific mRNAs were synthesized during the *imp-1* mating, indicating that cell fusion is necessary (Fig. 2 and 3).

Effect of protein-synthesis inhibition on transcription. Incubation of mature gametes with cycloheximide for as long as 4 h at 10 μg/ml, which has been shown to inhibit protein synthesis in *C. reinhardtii* (22), does not inhibit their ability to agglutinate and fuse; however, if continuously incubated in cycloheximide, the resulting quadriflagellated cells do not fuse their two nuclei (unpublished electron microscope studies), remain flagellated, and fail to form a zygote wall. When zygotes treated with cycloheximide for 4 to 6 h are washed free of the drug, they resume maturation in an apparently normal fashion, resorbing their flagella and secreting a zygote wall.

To assess the effect of cycloheximide on transcription, gametes and quadriflagellated cells were incubated continuously in cycloheximide starting 45 min before mating. RNA was prepared from the resulting zygotes, and Northern blots hybridized with the six cDNA probes. The five early transcripts were synthesized in the presence of cycloheximide, indicating that new protein synthesis is not required for their transcription (Fig. 4). By contrast, the late mRNA, class V, is not produced in the presence of cycloheximide. If the zygotes are washed free of cycloheximide after 3.5 h, they subsequently synthesize the class V mRNA (Fig. 4). These results indicate that factors necessary for the transcription of the early genes preexist in the unmated gametes and require cell fusion to become active; they also suggest that translation of some early zygote mRNA is required for the transcription of later zygote genes.

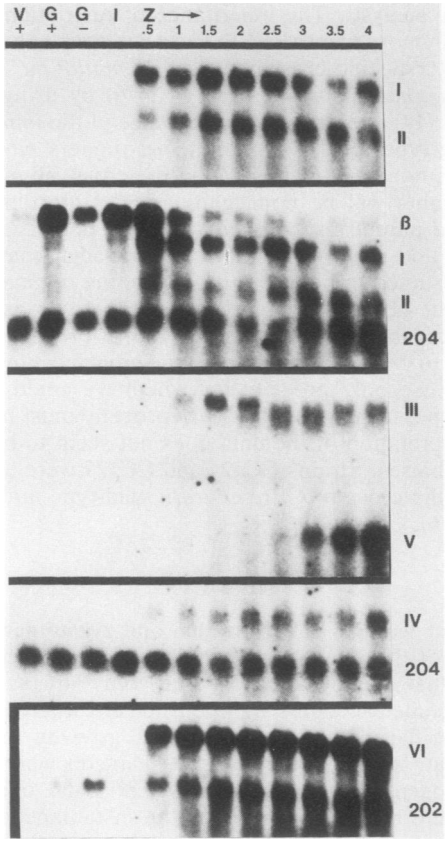

FIG. 2. cDNA hybridization to different life cycle stages. Total RNA (7.5 μg in the zygote lanes, 10 μg in the others) from vegetative cells (V+), mt^+ gametes (G+), mt^- gametes (G−), an *imp-1* × mt^- mating (I), and zygotes (Z) 0.5 to 4 h after mating was electrophoresed on formaldehyde-agarose gels, blotted to nitrocellulose, and hybridized with the six cDNA classes, a β-tubulin cDNA control (β), or two non-zygote-specific controls (202, 204), as indicated. Note that the second panel is the same filter as the first panel, rehybridized with the control probes. Since efforts were made to load equivalent amounts of RNA per lane, the variations in hybridization intensity (e.g., the attenuation of the β-tubulin and class III signal) are possibly significant; however, additional experiments are necessary to assess this question.

TABLE 1. Zygote-specific cDNA clones[a]

cDNA no.	Insert size (bp)	mRNA size[b] (bases)	Class
4	650	1,700	I
5	630	1,700	I
31	550	1,700	I
8	800	1,300	II
19	750	2,200	III
28	1,600	2,200	III
25	450	1,400	IV
1	450	1,100	V
14	300	1,100	V
33	650	1,100	V
7	400	2,400	VI
20	250	2,400	VI
146	1,400	2,400	VI

[a] The 13 zygote-specific cDNA plasmids are listed, along with the size of their cDNA insert, and grouped into six classes on the basis of cross-hybridization. The approximate size of the corresponding RNA is shown for each class.

[b] Sizes were obtained from Northern blot analyses.

Genetic analysis. Genetic analysis of the genes corresponding to the six cDNA classes was done by using RFLPs. Southern blots of *C. reinhardtii* genomic DNA were hybridized with the zygote-specific cDNAs. Each class except III hybridized to a simple pattern of fragments, suggesting that each of these classes is encoded by a single gene. By contrast, the class III probe hybridized to a complex pattern of fragments, which prove to derive from a gene present in 8

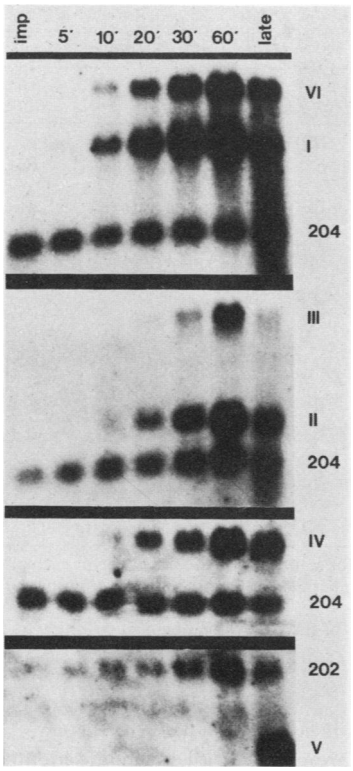

FIG. 3. Time course of zygote RNA expression. RNA was extracted from zygotes 5, 10, 20, 30, and 60 min after mating, electrophoresed on formaldehyde-agarose gels, blotted to nitrocellulose, and hybridized with the indicated cDNA probes. Two probes (202 and 204) that hybridize RNA from all stages of the life cycle were used as controls. RNAs from an *imp-1* × *mt*$^-$ mating and from the last time point in Fig. 2 were also included.

to 10 copies (demonstrated by isolating the genomic equivalents [unpublished observations]). The hybridization pattern of *C. reinhardtii* was then compared with that of *C. smithii*, an independently isolated strain that is interfertile with *C. reinhardtii* (1). Comparisons of genomic Southern blots of the two species identified restriction enzymes that yielded RFLPs for each cDNA probe.

C. smithii mt$^+$ was then crossed with *C. reinhardtii mt*$^-$, and a number of tetrads were isolated. DNA was prepared from each product of two complete tetrads and from eight

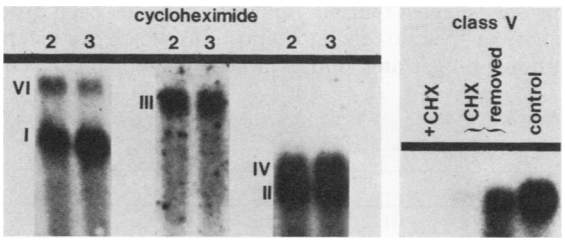

FIG. 4. Effect of cycloheximide on transcription. Gametes were preincubated in cycloheximide for 45 min, mated in cycloheximide for 2 or 3 h, and RNA extracted. On the left, Northern blots of this RNA were probed with the five early cDNAs, showing that they are all being expressed. On the right, the late cDNA, class V, was used to probe RNA from zygotes after 7 h in cycloheximide (+CHX) and RNA from zygotes that were washed out of cycloheximide after 3.5 h and incubated for a further 1.5 h (left) or 3.5 h (right). Zygotes mated for 3 h without cycloheximide are shown in the control lane.

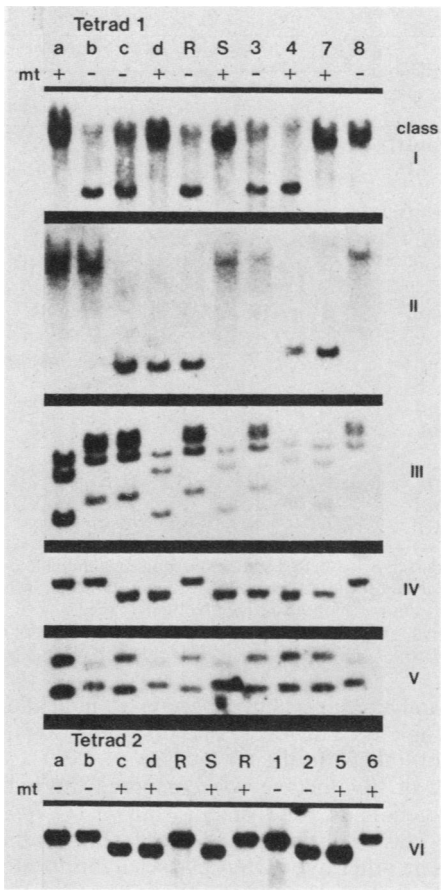

FIG. 5. RFLP mapping. Shown are some of the genomic Southern blots used to map the zygote-specific cDNAs. DNA isolated from the products of tetrad 1 and from four individual progeny are shown in the upper five panels, probed with cDNA classes I to V. In the bottom panel, the class VI probe is used to hybridize DNA from the products of tetrad 2 and the other four individual progeny. The lanes labeled R and S contain DNA from *C. reinhardtii* and *C. smithii*, respectively. The class I probe hybridizes a 9.3-kilobase (kb) *Bam*HI fragment present in *C. reinhardtii* and a high-molecular-weight fragment in *C. smithii*. The class II probe hybridizes a 10-kb *Eco*RV fragment in *C. reinhardtii* and a high-molecular-weight fragment in *C. smithii*. The class III probe hybridizes five *Xho*I fragments in *C. reinhardtii* and three in *C. smithii*, all migrating between 5.8 and 9.1 kb. One fragment in each species is not polymorphic. The multiple fragments are always inherited together. The class IV probe distinguishes a 2.5-kb *Rsa*I fragment in *C. reinhardtii* and a 2.3-kb fragment in *C. smithii*. The class V probe (whose insert contains a *Pst*I site) hybridizes two *Pst*I fragments, which are always inherited together: 0.96 and 0.8 kb in *C. reinhardtii*, and 0.93 and 0.84 kb in *C. smithii*. The class VI probe hybridizes a 4.3-kb *Pst*I fragment in *C. reinhardtii* and a 4.0-kb fragment in *C. smithii*.

additional progeny, each from a different tetrad. These DNA samples were digested with appropriate restriction enzymes, blotted to nitrocellulose from agarose gels, and hybridized with the cDNA probes (Fig. 5). For some cDNA classes, RFLPs were scored by using more than one restriction enzyme; in such cases, all enzymes gave the same result.

The data for all the markers are shown in Table 2. All six markers were inherited 2:2 in the two tetrads as expected. Although the number of progeny analyzed was not large enough for accurate mapping, all six of the markers segre-

TABLE 2. Genetic data[a]

Class	Restriction enzyme	Tetrad 1				Tetrad 2				Single progeny							
		a (mt^+)	b (mt^-)	c (mt^-)	d (mt^+)[b]	a (mt^-)	b (mt^-)	c (mt^+)[b]	d (mt^+)	1 (mt^-)	2 (mt^-)	3 (mt^-)	4 (mt^+)	5 (mt^+)	6 (mt^+)	7 (mt^+)	8 (mt^-)
I	*Bam*HI	S	R	R	S	S	R	S	R	R	S	R	R	R	R	S	S
	*Eco*RV	S	R	R	S	S	R	S	R	R	S	R	R	R	R	S	S
II	*Eco*RV	S	S	R	R	R	R	S	S	S	S	S	R	S	R	R	S
	*Rsa*I	S	S	R	R	R	R	S	S	S	S	S	R	S	R	R	S
	*Xho*I	S	S	R	R	ND	ND	ND	ND	ND	ND	S	R	ND	ND	R	S
III	*Xho*I	S	R	R	S	R	R	S	S	R	R	R	S	S	S	S	R
IV	*Rsa*I	R	R	S	S	R	R	S	S	S	S	S	S	S	S	S	R
V	*Rsa*I	R	S	R	S	R	S	R	S	S	R	R	R	R	S	R	S
	*Pst*I	R	S	R	S	R	S	R	S	S	R	R	R	R	S	R	S
VI	*Pst*I	R	R	S	S	R	S	S	R	S	S	R	S	R	R	S	R
	*Rsa*I	R	R	S	S	ND	ND	ND	ND	ND	ND	R	S	ND	ND	S	R
	*Xho*I	R	R	S	S	R	S	S	R	ND	ND	R	S	ND	ND	S	R

[a] Two tetrads (labeled 1a to d and 2a to d) and eight individual progeny (labeled 1 to 8) were scored for mating type and for RFLPs for each cDNA class (I to VI). The *C. smithii* parent was mt^+, and the *C. reinhardtii* parent was mt^-. Progeny are marked S if they inherited the *C. smithii* polymorphism and R if they inherited the *C. reinhardtii* polymorphism. ND, Not determined.

[b] The mating type could not be scored, but is inferred to be mt^+ on the basis of the mating types of the other three members of the tetrad.

gate as if unlinked; certainly there is no indication of close linkage (Table 3). However, one of the markers, class III, shows tight linkage to the *mt* locus.

Because of this linkage, we compared the hybridization profiles of genomic DNA from mt^+ and mt^- *C. reinhardtii* by using the class III probe and were able to find several RFLPs. (The other five cDNA classes hybridize to the same fragments in both mt^+ and mt^-; this is shown for class VI in Fig. 5, lanes R+ and R−.) These intraspecific polymorphisms were then used to confirm the tight linkage to *mt*. Wild-type mt^+ and mt^- strains of *C. reinhardtii* were crossed, and 54 tetrads were isolated. For each tetrad, the two segregants of like mating type were grown separately and equivalent numbers of cells from each progeny were pooled. Three such sets of pooled progeny, representing six mt^+ (or mt^-) progeny from three tetrads, were then combined, and DNA from the mixture was prepared and analyzed by Southern blotting. If any of the three tetrads had undergone a crossover between *mt* and the RFLP to produce a tetratype, then one-sixth of the cells, and hence one-sixth of the DNA, should show a nonparental hybridization pattern, which we would be able to detect. In fact, only parental hybridization patterns were encountered in the 54 tetrads (Fig. 6), indicating that there is no recombination between *mt* and the RFLP seen by the class III probe.

DISCUSSION

We screened a cDNA library prepared from zygote mRNA by differential hybridization and identified six classes of zygote-specific clones. Using each class as a probe to Northern blots of RNA from various stages of the life cycle, we demonstrated that each class hybridizes to a single transcript present only in zygotes. Five of these genes began to be transcribed within 10 min of zygote formation; the sixth mRNA did not appear until at least 90 min after fusion. Because the mRNAs fail to be expressed in an *imp-1* × mt^- "mating," cell fusion, and not just mating interactions, is required to activate the genes. The two nuclei do not begin to fuse until about 1 h after mating, meaning that nuclear fusion cannot trigger the early transcriptional activity. Moreover, synthesis of the early zygote RNAs occurs in the presence of cycloheximide, suggesting that the initial zygotic transcription does not require new protein products. Cycloheximide does, however, block the synthesis of the one late transcript, consistent with the notion that a product of an early gene is necessary to activate late transcription. The late transcript is produced at about the time of nuclear fusion, but it is not known whether the two events are related.

Our data suggest that mt^+ and mt^- gametes each produce mating type-specific regulatory proteins which are incapable of turning on the transcription of zygote-specific genes in the

TABLE 3. Segregation of the genetic markers[a]

Genetic marker	Genetic marker[b]					
	mt	I	II	III	IV	V
I	PD, T, 3/8					
II	T, PD, 1/8	T, T, 4/8				
III	PD, PD, 8/8	PD, T, 3/8	T, PD, 1/8			
IV	T, PD, 5/8	T, T, 2/8	NPD, PD, 4/8	T, PD, 5/8		
V	T, T, 3/8	T, NPD, 4/8	T, T, 4/8	T, T, 3/8	T, T, 2/8	
VI	T, T, 4/8	T, T, 5/8	NPD, T, 3/8	T, T, 4/8	PD, T, 5/8	T, T, 3/8

[a] Segregation data for all pairwise combinations between the RFLP markers for the six cDNAs and *mt* are shown.

[b] In each three-part listing, the first entry shows segregation for tetrad 1 (T, tetratype; PD, parental ditype; NPD, nonparental ditype); the second shows segregation for tetrad 2; and the third shows what fraction of the eight individual progeny had the parental combination for that pair of markers.

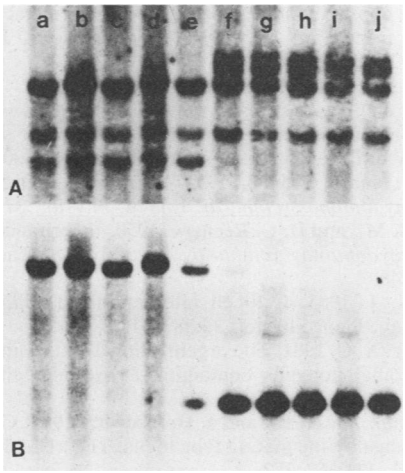

FIG. 6. Genetic linkage of class III to the *mt* locus. (A) In each of lanes a to d, the pooled DNA from the six mt^+ progeny of three tetrads was digested with *Xho*I, electrophoresed on a 0.7% agarose gel in Tris-acetate buffer, transferred to nitrocellulose, and hybridized with the class III probe. Lanes g to j contain similar groups of mt^- progeny. Lanes e and f contain DNA from the mt^+ and mt^- parents, respectively. The absence of the cluster of approximately 10-kb bands from the mt^- parent in the mt^+ progeny, or the 5.8-kb band from the mt^+ parent in the mt^- progeny, indicates the lack of recombination between *mt* and the class III RFLPs. (B) Lanes a to d and g to j are as in panel A, except that the DNA was digested with *Sac*I and electrophoresed on a 0.7% agarose gel in Tris-borate buffer. The probe was a single-copy region from a genomic clone of the class III gene (data not shown). Here, the absence of the 4.3-kb band in the mt^+ progeny and of the 11-kb band in the mt^- progeny confirms the close linkage of *mt* and the class III gene. Lane e contains a 9:1 mixture of DNA from the mt^+ and mt^- parents, respectively; lane f contains a 9:1 mixture of mt^- and mt^+ DNA. The fact that the minority band can be seen in such mixtures indicates that if any of the six progeny in the pooled DNA preparations in the remaining lanes had been recombinant, the blots would be sensitive enough to detect a recombinant band. Faint additional bands in lanes g to j are products of partial digestion.

gamete, but which immediately interact to turn on transcription of zygote-specific genes following cell fusion. In other words, the zygote program is activated by the mixing of the preprogrammed cytoplasms of the mt^+ and mt^- gametes. If this interpretation of the data is correct, then such a mechanism is analogous to the proposed function of the a1 and α2 mating-locus gene products in *Saccharomyces cerevisiae* (27), which interact in diploids to form an a1-α2 product which represses the expression of haploid-specific genes, including the expression of a gene (*RME1*) which inhibits sporulation (19). An important difference between the two systems, however, is that sporulation in *S. cerevisiae* requires not only a1-α2 activity but also nutritional starvation, whereas spore formation in *C. reinhardtii* is apparently independent of nutritional or other external signals. In this sense it is more analogous to egg-sperm fertilization systems, wherein the course of development is set by preprogrammed cytoplasmic factors and by novel gene transcription but not by environmental cues. Additional speculation about the program of transcriptional activation in *Chlamydomonas* zygotes has been presented elsewhere (6a).

The fact that nutritional starvation is not required for observation of the onset of the sporulation program is a major experimental asset of the *Chlamydomonas* system: several investigators have shown, for example, that many of the new gene products that appear during yeast sporulation are in fact related to the starvation response, being present as well in starved nonsporulating homozygous diploids (13). Moreover, the rapid activation (5 to 10 min) of zygote-specificgene transcription in *C. reinhardtii* should be simpler to analyze than the turn-on of yeast sporulation genes, which occurs some 7 h after starvation is imposed (20).

In some systems, such as insect chorion genes (11, 26) or certain *Aspergillus* conidiation genes (9), developmentally regulated genes are physically linked in the genome. However, genetic analysis of the six zygote-specific transcripts by using RFLPs indicates that they are all unlinked. This agrees with the data on zygote maturation mutants in *C. monoica*, which are also unlinked (28). One of the *C. reinhardtii* genes, however, is tightly linked to the *mt* locus and can detect RFLPs between mt^+ and mt^- strains. Although the wild-type strains used in this study were not selected as being isogenic, the five probes not linked to *mt* were not polymorphic, suggesting that the *mt* polymorphism is significant. Whether it results from the fact that recombination is suppressed in the *mt* region (5), promoting the opportunity for genetic drift, or from a functionally significant heterozygosity between the two mating types awaits further analysis. We are currently using this cDNA to isolate homologous genomic DNA clones, since it should allow us to isolate the *mt* locus itself.

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