

Genes Involved in Light Control of Sexual Differentiation in *Chlamydomonas reinhardtii*

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ABSTRACT

Gamete formation requires the sequential action of two extrinsic cues, nitrogen deprivation and blue light. The mutants described here are specifically altered in the light-dependent step. Mutations *lrg1*, *lrg3*, and *lrg4* overcome this light dependence while mutation *lrg2* results in a delayed execution of the light-mediated step. The four mutations are linked. The recessive nature of the *lrg1*, *lrg3*, and *lrg4* mutations implies that they encode elements of negative control in this light response pathway. Analyses of diploids suggest an interaction between the gene products of the mutated loci with a central role for *lrg4*. The *lrg4* mutation is unique also because it overcomes the light dependence of *Chlamydomonas* zygote germination when present in homozygous form. These data indicate that there are common components in the signal chains that control gametogenesis and zygote germination.

OF the numerous environmental factors that influence plant growth and development, light has an especially important role. It not only is a substrate for photosynthesis but also a stimulus for many developmental processes. In higher plants light controls these processes by the combined action of several photoreceptor systems. Several classes of photoreceptors including protochlorophyllide, UV and blue light receptors, and the red/far-red light receptors, the phytochromes, mediate these responses to light. The phytochrome-based photoreceptor system has been characterized in some detail at the molecular level (FURUYA 1993). Molecular analyses of components of the blue light receptor system have implicated a protein with homology to photolyases (AHMAD and CASHMORE 1993) as well as a membrane-associated protein kinase (SHORT *et al.* 1994).

The dissection of the transduction chains from the photoreceptor that receives the light signal to the resulting physiological response has recently progressed significantly. Using putative signalling intermediates and pharmacological agents with known biochemical effects, evidence for an involvement of G proteins, cyclic GMP, Ca²⁺, Calmodulin, and protein kinases in phytochrome-activated signal transduction chains has been obtained (NEUHAUS *et al.* 1993; BOWLER *et al.* 1994a,b). In genetic approaches, mutants with defects in light-controlled development and behavior have been isolated and characterized in several plant species (CHORY 1993a). In *Arabidopsis*, this approach has resulted in the isolation of mutants (*cop* and *det*) that do not require light for greening (CHORY *et al.* 1989, 1991, 1993a; DENG *et al.* 1991). The corresponding genes, marked by point mutations or DNA insertions, should now be clonable

and should provide a basis for the molecular characterization of individual proteins that mediate signal transduction (DENG *et al.* 1992).

Mutants with defects in photoreceptors or a connected chain for signal transduction have also been identified in fungal systems such as *Aspergillus* (MOONEY and YAGER 1990), *phycomyces* (CORRACHANO and CERDA-OLMEDO 1992), *Neurospora* (DEGLI-INNOCENTI and RUSSO, 1984), and *Trichoderma* (HORWITZ *et al.* 1985). Thus, light dependence of conidiation in *Aspergillus nidulans* is abolished by a mutation in gene *veA1* (MOONEY and YAGER 1990). Genetic analysis of *veA1* suppressor mutations suggests a physical interaction of several gene products in this light response pathway (MOONEY *et al.* 1990).

The unicellular and haploid green alga *Chlamydomonas reinhardtii* represents a simple model plant to study the routes by which light controls differentiation. Light is required for the progression through the sexual life cycle in at least two steps: gametogenesis and zygote germination. Gametogenesis is initiated by the removal of a utilizable nitrogen source (SAGER and GRANICK 1954). This induces a program of differentiation accompanied by extensive changes in gene expression patterns (TREIER and BECK 1991). If cells are cultured in the absence of nitrogen in the dark, mating incompetent pregametes accumulate. Irradiation of these pregametes induces further differentiation into sexually mature gametes (TREIER *et al.* 1989; BECK and ACKER 1992). The action spectrum for pregamete to gamete conversion suggests the participation of a UV A/blue light photoreceptor (WEISSIG and BECK 1991). This process requires RNA and cytoplasmic protein synthesis, suggesting that the signal chain activated by light includes a step at the level of gene expression (TREIER *et al.* 1989).

The realization that gamete formation is a two step

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process, in which light plays a pivotal role in the second step, provided a basis for the further characterization of this second step. The characterization of *gas28*, a gene specifically expressed upon irradiation of pregametes (VON GROMOFF and BECK 1993), lends support to the model of a two-step process. Furthermore we isolated mutants affected in the light-mediated step (BUERKLE *et al.* 1993). These mutants (designated *lrg*, for light regulation of gametogenesis) provide access to genes that encode individual components of a signal pathway for a blue light-mediated response in a plant organism. Analysis of this signal pathway in *C. reinhardtii* offers additional advantages: The absence of phytohormone-regulated responses and the lack of phytochrome (BONENBERGER *et al.* 1994) in *Chlamydomonas* provide a less complicated background. Therefore, an interaction or even convergence of signal pathways connected to different photoreceptor systems, as can be expected in higher plants (CHORY 1993a,b), are unlikely to occur.

This report describes the isolation and further characterization of mutants altered in light regulation of gametogenesis. Three mutants exhibit light-independent pregamete to gamete conversion, one mutant is partially defective in this step. Our results suggest that the four loci play a role in light perception or signal transduction. All four markers map to the same single linkage group. A homozygous combination of one of the mutations, *lrg4*, rendered zygote germination light independent. Together, these mutants define a network of genetic loci whose products may interact in the perception/transduction of a blue light signal.

MATERIALS AND METHODS

Strains, culture conditions, and media: Strains 137c *nit1 nit2* of mating types *mt+* and *mt-*, and strain 105 *mt+ arg7-3* were kindly provided by R. MATAGNE (University of Liège, Belgium). Strains CC-1009 *mt-* (wild type) and CC-1705 (*mt-*, *pab1*) were from the *Chlamydomonas* Genetics Center (Duke University, Durham, NC). Mutants *lrg3* and *lrg4* were single cell clones of strains 137c *mt-* and CC-1009, respectively. They represent spontaneous mutations that apparently occurred during maintenance in the culture collection. Progeny from crosses of these mutants with strains CC-1705 and 105 were used for the generation of diploids. Strains harbouring mutations *lrg1* and *lrg2* have been described (BUERKLE *et al.* 1993). Cells were grown photomixotrophically with aeration in a Tris acetate phosphate (TAP) medium (GORMAN and LEVINE 1965). When necessary, the medium was supplemented with L-arginine (100 mg/l) or p-aminobenzoic acid (10 mg/l). White light at $30 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ was provided by fluorescent tubes (Osram L36W/35, Germany).

Gametogenesis and assays for mating ability: Our standard protocol for gametogenesis in light or in darkness was as described previously (TREIER *et al.* 1989). The use of cells grown in liquid medium for gamete formation is of importance to achieve constant test conditions. The percentage of gametes in a test culture was determined as described (BECK and ACKER 1992). To test for the phenotypes of the *lrg1*, *lrg3*, and *lrg4* mutations of progeny from tetrads, gametogenesis was performed in 96-well microtiter plates. Before gametogenesis, cells from tetrads were grown for 5–7 days on plates

(1.5% agar) containing TAP medium and necessary supplements. These cells were suspended in nitrogen-free TAP medium in two microtiter plates to a density of $\sim 5 \times 10^6$ cells per ml. One microtiter plate was incubated with continuous illumination ($30 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$); the other plate was kept in the dark. After 20 hr, an approximately equal number of competent gametes of the opposite mating type was added. To cells incubated in the dark, gametes were added in a dark room illuminated very weakly by a red light. After an additional incubation for 12–24 hr in the dark, the presence or absence of a zygote pellicle in individual wells was scored under a dissecting microscope (HARRIS 1989). The presence of a zygote pellicle was used as an indicator for the formation of gametes by the clone tested. Presence of zygote pellicles only in wells exposed to light indicated a wild-type phenotype. Presence of zygote pellicles in wells of both dark- and light-incubated cells indicated a *lrg1*, *lrg3*, or *lrg4* mutant phenotype. A similar protocol was used to test for the *lrg2* phenotype (BUERKLE *et al.* 1993). In a first step, pregametes were generated by suspending cells of individual clones in nitrogen-free medium in wells of four different microtiter plates. These plates were incubated in the dark for 20 hr. The wells were then exposed to white light ($30 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 0, 60, 90, or 240 min. At the end of the illumination period, competent mating partners were added and incubation continued in the dark for another 20 hr. The presence or absence of a zygote pellicle, indicating the conversion of pregametes to gametes during the period of illumination, was then recorded. *lrg2* strains form gametes only after 240 min of illumination. Due to a low mating efficiency of diploid strains (always *mt-*), an alternative protocol was devised to test these strains for gamete formation. By this protocol, the number of mating competent gametes is assessed by the number of zygotes formed after mating with an excess of mating partner. To induce germination of zygotes, generated as described below, the plates were transferred into the light. Every colony visible after 1 week of incubation represented a germinated zygote and thus a mating competent gamete.

Generation of zygotes: After the generation of gametes according to our standard protocol, cells after one hr of mating were plated on TAP plates containing 4% agar. These plates were incubated with illumination for 24 hr and then in the dark for 5 days. The bulk of vegetative cells were then removed with a razor blade. To kill remaining vegetative cells, the plates were treated with chloroform vapours for 30 sec.

Genetic analyses: For tetrad analyses, the standard protocol was used (HARRIS 1989). Diploid strains were constructed by using complementing wild-type and mutant alleles of *arg7-3* and *pab1*. Gametes of opposite mating types, harboring mutations that confer auxotrophic phenotypes, were mated. After cell fusion, the cells were plated on minimal medium lacking the necessary supplements (HARRIS 1989). Diploid clones were recovered and tested.

Northern blot analysis: Total RNA was isolated and processed for RNA blot analysis as described previously (VON GROMOFF *et al.* 1989). Labeling of probes, hybridization conditions, and autoradiography were performed as described (WEGENER and BECK 1991).

RESULTS

Isolation and characterization of mutants: Sexual differentiation in *C. reinhardtii*, *i.e.*, the formation of gametes from vegetative cells, may be divided into two consecutive steps controlled by the extrinsic cues ammonium limitation and light. While nitrogen starvation induces the first step that results in pregametes, light

controls the second step, *i.e.*, the conversion of pregametes to gametes. Four mutants affected in this light-dependent step have been identified. They all are the result of spontaneous mutation events that must have occurred while the strains were propagated. Thus, strains harboring mutations *lrg1* and *lrg4* were present in wild-type strains CC-1010 and CC-1009, respectively, while the *lrg2* mutation was present in our stock of wild-type strain 137c *mt+*. The *lrg3* mutation was isolated as a spontaneous event in the 137c *mt-* wild-type strain. As outlined below, these mutants are specifically affected in the conversion of pregametes to gametes, *i.e.*, the light-dependent step in sexual differentiation. In Figure 1 the phenotypes of the *lrg3* and *lrg4* mutants are compared with that of the wild-type strain. When vegetative cells of the wild-type strain 137c *mt-*, grown in liquid media, were transferred into nitrogen-free medium and incubated with continuous illumination, cells differentiated and gametes accumulated with the kinetics shown. When instead cells were incubated in the dark, no gametes were detected (TREIER *et al.* 1989). Mutant *lrg3* exhibited gamete formation even in the dark, and the level of gametes formed was similar to that observed in the light. However, gametes in the dark accumulated with a delay of ~ 2 hr. The restriction in the availability of energy due to the use of acetate as sole source of energy and carbon in the dark may be responsible for this delay. The *lrg4* mutant also exhibited gamete formation in the dark (Figure 1). The level of gametes observed in the dark was only $\sim 25\%$ when compared to the number of gametes generated with illumination. This partial light dependence may possibly reflect the nature of the mutation, *e.g.*, the mutant gene product may be partially functional (see DISCUSSION). Here again, a delay was observed when the kinetics of gamete formation in the dark were compared to those in the light. Previously we have shown that mutant *lrg1* is capable of light-independent gamete formation, and that mutant *lrg2* exhibits a delay in the conversion of pregametes to gametes (BUERKLE *et al.* 1993). Since in the *lrg1*, *lrg2*, *lrg3*, and *lrg4* mutants gametes are formed only after a period of nitrogen starvation, we conclude that the products of the mutated genes act after the first part of the program of differentiation and specifically control the second light-dependent step in gametogenesis, *i.e.*, the conversion of pregametes to gametes.

This conclusion is supported by studies in which a molecular indicator for the formation of mature gametes was employed. The mRNA of gene *gas28* has previously been shown to accumulate in the last phase of gametogenesis; specifically, when pregametes are exposed to light (VON GROMOFF and BECK 1993). Mutants *lrg3* and *lrg4*, but not wild-type cells, accumulated *gas28* mRNA in the dark (Figure 2). In agreement with the differences in the kinetics and in the extent of gamete formation observed with the mutants in the dark (Fig-

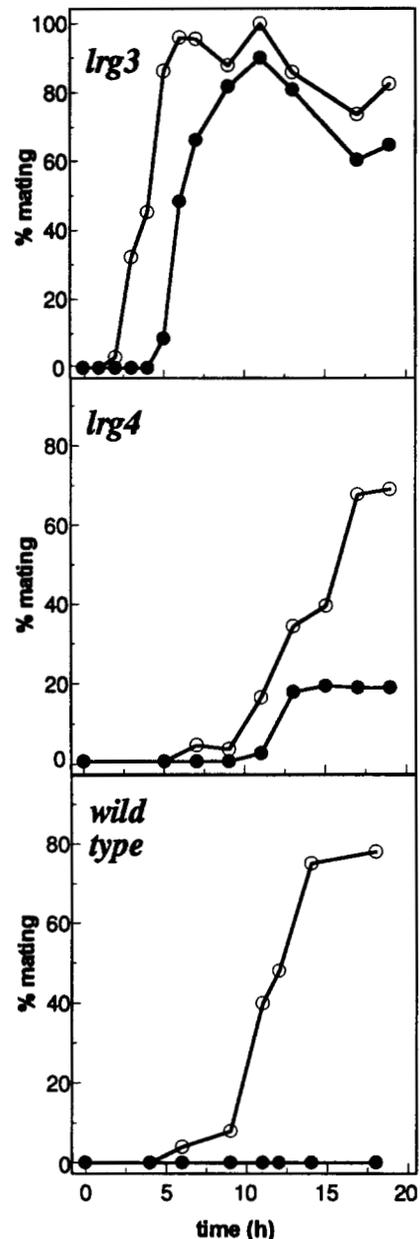


FIGURE 1.—Kinetics of gametic differentiation of *lrg3* and *lrg4* mutant strains and wild type. The nitrogen source was removed at time 0. Incubations were performed either with continuous irradiation ($30 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of white light) (○) or in the dark (●). At the times indicated, cells were removed, mixed with an excess of sexually competent mating partner, and incubated in the dark. After 60 min, the cells were fixed by addition of glutaraldehyde (final concentration 1%) and the percentage of mating was determined.

ure 1), *gas28* mRNA accumulated more rapidly and to higher levels in the dark in the *lrg3* than in the *lrg4* mutant (Figure 2). These results suggest that the *lrg3* and *lrg4* mutations abolish the control over *gas28* expression and the light-dependent step in gametic differentiation.

Mapping of *lrg* loci: Since mutants *lrg1*, *lrg3*, and *lrg4* exhibited the same phenotype, *i.e.*, light independence of gametogenesis, it was essential to test whether the

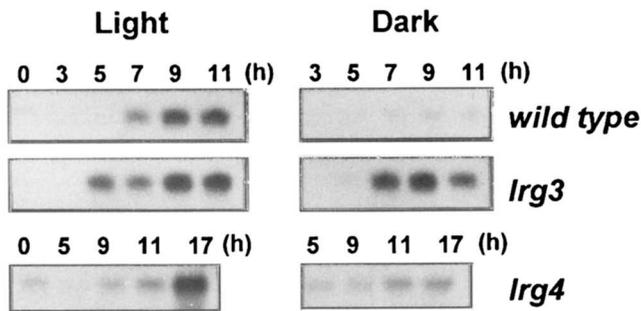


FIGURE 2.—Accumulation of *gas28* mRNA in the wild-type strain and in the *lrg3* and *lrg4* mutants during gametogenesis. For RNA blot analyses, 10 μ g of total RNA was applied to each slot. The nitrogen source was removed at time 0, and cells were incubated either in the light or in the dark. Samples for RNA isolation were removed at the times indicated.

mutations were allelic or, alternatively, represent different loci. Crosses of the *lrg1* mutant with strains harboring the *lrg3* mutation showed that the mutant loci *lrg1* and *lrg3* are different though linked (Table 1). Also, the locus of the *lrg4* mutation was shown to be linked to the *lrg1* mutation. The distance of *lrg3* as well as *lrg4* to *lrg1* is ~ 9 cM. The difference in the mutant loci of the *lrg3* and *lrg4* mutants was demonstrated by the distance of 20 cM between the *lrg3* and *lrg4* loci. This placed the *lrg1* mutation in the middle between these two loci. By crosses with the *lrg2* mutant, previously shown to be weakly linked to *lrg1* (BUERKLE *et al.* 1993), the *lrg4* locus was mapped between *lrg1* and *lrg2*. The *lrg3* locus thus must be located on the *lrg2* distal side of *lrg1*. The phenotypes of the mutants were the same whether they were in a *mt+* or a *mt-* background. The mapping data are summarized in Figure 3.

Recessiveness of *lrg3* and *lrg4* mutations: Heterozygous diploids harboring wild-type and mutant alleles of *lrg3* or *lrg4* did not form gametes in the dark (Table

TABLE 1

Linkage data for *lrg1*, *lrg2*, *lrg3*, and *lrg4*

Mutations ^a	Segregation		Map distance (cM)
	PD:NPD:T ^b		
<i>lrg1/lrg2</i>	21:3:28		32.7
<i>lrg1/lrg3</i>	40:1:7		9.4
<i>lrg1/lrg4</i>	35:0:8		9.3
<i>lrg3/lrg4</i>	22:1:12		20
<i>lrg2/lrg4</i>	16:2:11		25.8

^a Zygotes from crosses between strains harboring *lrg2* and *lrg3* often failed to germinate and those that germinated contained aberrant numbers of progeny, *i.e.*, not four or eight. Therefore, data from these crosses were not included in the table.

^b PD, parental ditype; NPD, nonparental ditype; T, tetraply. In crosses between *lrg1*, *lrg3*, and *lrg4* harboring strains, a 4:0 segregation was considered as PD, a 3:1 segregation as T, and a 2:2 segregation as NPD. No evidence for a synthetic phenotype of the double mutants was observed.

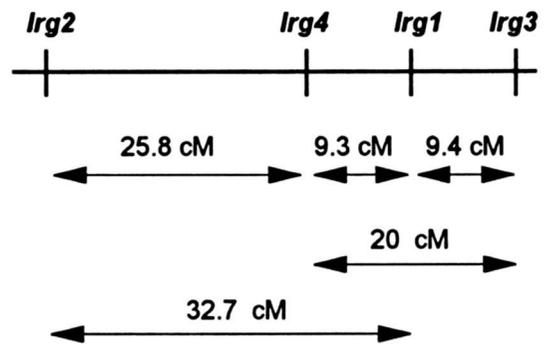


FIGURE 3.—Linkage map of the *lrg* loci.

2), demonstrating that the two mutations are recessive to their wild-type alleles. Also, the *lrg1* mutation is recessive to its wild-type allele. In diploids, combinations of two different heterozygous mutant/wild-type *lrg* loci were constructed and tested for their ability to form gametes in the dark (Table 2). The combination of the *lrg1* and *lrg3* mutant/wild-type loci resulted in the expected wild-type phenotype. However, a combination of mutant/wild-type loci of *lrg3* with *lrg4* or *lrg1* with *lrg4* resulted in an unexpected light independent gamete formation. A wild-type phenotype was demonstrated for the heterozygous pair of *lrg2* and *lrg4* (data not shown).

Control of zygote germination by light: When zygotes generated by our standard protocol were kept in the dark, none of the zygotes germinated and no colonies were formed. To determine the duration of illumination needed to induce germinations in these zygotes, plates with mature zygotes were exposed to white light

TABLE 2

Phenotypes of various combinations of *lrg* mutations in diploids

<i>lrg</i> mutations in diploids ^a	Gamete formation ^b	
	Light	Dark
+/ <i>lrg1</i>	+++	—
<i>lrg1/lrg1</i>	+++	+++
+/ <i>lrg3</i>	+++	—
<i>lrg3/lrg3</i>	+++	+++
+/ <i>lrg4</i>	+++	—
<i>lrg4/lrg4</i>	+++	+++
+/ <i>lrg1</i> , <i>lrg3</i> /+	+++	—
+/ <i>lrg1</i> , <i>lrg4</i> /+	+++	++
+/ <i>lrg3</i> , <i>lrg4</i> /+	+++	+++

Diploids were constructed as described in MATERIALS AND METHODS. Of each combination, at least 10 independently derived diploids were tested.

^a + indicates wild-type.

^b The formation of gametes in the light or in the dark was assayed by two independent methods (i) by our standard protocol for gametogenesis, subsequent plating of the mated cells, and determination of the number of zygotes, and (ii) by screening for the formation of a zygote pellicle in microtiter plates. Details of these procedures are given in MATERIALS AND METHODS.

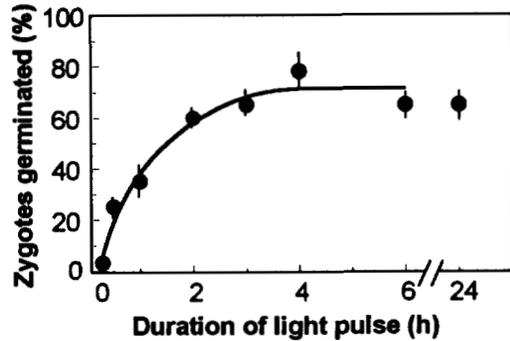


FIGURE 4.—Light induction of zygote germination. Zygotes were generated following the standard protocol. After treatment with chloroform vapors, the plates were transferred back into the dark. For the 0 time control, the pretreatments were performed in the dark. After 11 days in the dark, the number of zygotes germinated and nongerminated was counted. The number of nongerminated zygotes was determined using a dissecting microscope. The percentage of germination was calculated by dividing the number of zygotes germinated by that of the total number of zygotes (germinated and nongerminated). Bars represent the standard deviation of data obtained in six independent experiments.

($30 \mu\text{mol} \cdot \text{m}^2 \cdot \text{s}^{-1}$) for time intervals between 30 min and 24 hr. After the light treatment, the plates were again incubated in the dark for another 11 days. The percentage of zygotes that germinated was then determined. The results (Figure 4) show clearly that in the absence of light, no germinated zygotes were observed. However, 2–3 hr of illumination were sufficient to induce germination in all zygotes able to germinate. We conclude that light is required to induce meiosis, the subsequent events, *i.e.*, germination and colony formation, may occur in the dark.

Influence of *lrg* mutations on zygote germination: To investigate a possible involvement of the *lrg* mutations in zygote germination, we tested whether zygotes homozygous for mutations *lrg1*, *lrg3*, and *lrg4* would germinate in the dark. While mutations *lrg1* and *lrg3* had no effect, the homozygous combination of the *lrg4* loci resulted in germination of zygotes independent from light induction (Table 3). As expected from the results with diploid cells (Table 2), the heterozygous combination of mutant/wild-type loci in all cases did not allow light independent germination (data not shown). This result was confirmed by the analysis of progeny from two tetrads (from a cross of the *lrg4* mutant with a wild-type strain) in which the formation of gametes in the dark cosegregated with germination in the dark, suggesting that both are phenotypes of *lrg4*.

Since some combinations of the heterozygous mutant/wild-type *lrg* loci resulted in gamete formation in the dark (Table 2), we also tested whether zygotes that harbor various allele combinations were affected in their light dependence of germination. As observed for gametogenesis, the combination of the *lrg4* alleles with *lrg1* or *lrg3* resulted in a light independent phenotype, *i.e.*, germination in the dark (Table 3).

TABLE 3

Influence of *lrg* mutations on zygote germination

<i>lrg</i> mutations in zygotes	Zygote germination ^a	
	Dark	Light
+/+	—	+++
<i>lrg1/lrg1</i>	—	+++
<i>lrg3/lrg3</i>	—	+++
<i>lrg4/lrg4</i> ^b	+++	+++
+/ <i>lrg1</i> , <i>lrg3</i> /+	—	+++
+/ <i>lrg1</i> , <i>lrg4</i> /+	++	+++
+/ <i>lrg3</i> , <i>lrg4</i> /+	++	+++

^a Zygotes were generated as described in MATERIALS AND METHODS. After maturation the plates with zygotes were cleared of vegetative cells and treated with chloroform vapors for 30 sec. These procedures were performed in the dark. After an additional incubation for 12 days in the dark, the plates were screened for zygotes that had germinated and had formed colonies. The plates were then incubated in the light and after an additional 10-day incubation, the total number of germinated zygotes was determined.

^b The vast majority of zygotes after maturation by incubation in the light for 24 hr and then in the dark for 5 days had not germinated as confirmed by microscopic inspection. A cross of *lrg4*, *pab1* × *lrg4*, *arg7* gave rise to meiotic recombinants that grew on plates lacking p-aminobenzoic acid and arginine, confirming that colonies that appeared in the dark were derived from zygotes.

DISCUSSION

Here we describe the identification and characterization of two mutants that are affected in the light-dependent conversion of pregametes to gametes. A possible involvement of these loci in an earlier step of gametogenesis, *i.e.*, the generation of pregametes from vegetative cells, is unlikely since the mutant phenotypes were obvious only after the nitrogen starvation step has been completed. This conclusion is based on the similarity in the kinetics of gamete formation by the mutants in the light and in the dark (Figure 1). It is corroborated by the light-independent and late accumulation of *gas28* mRNA, shown to be a marker for pregamete to gamete conversion (VON GROMOFF and BECK 1993). We have shown previously that the light-dependent step in gametogenesis requires gene expression (TREIER *et al.* 1989). A signal chain activated by light may thus control gene expression, ultimately mediating the differentiation of pregametes to sexually mature gametes. The phenotypes of mutants *lrg3* and *lrg4* (Figures 1 and 2) suggest that mutational alterations can activate this signal chain in a constitutive manner. One can envision that the gene products either have a function in signal perception or in the signal transduction chain by which blue light controls the differentiation of pregametes to gametes. Pleiotropic effects of these mutations on, *e.g.*, phototaxis were not detected.

The *lrg3* and *lrg4* mutants differ in their degree of light independence during gamete formation: While

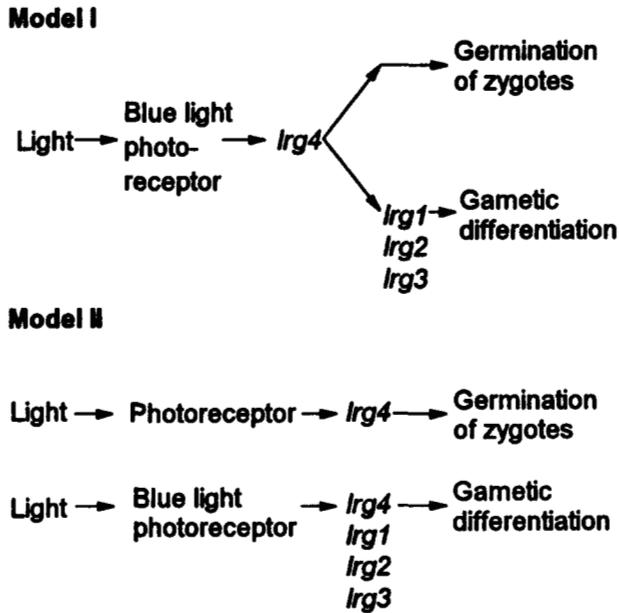


FIGURE 5.—Two possible models representing the signal pathways by which light controls gamete formation and zygote germination.

the *lrg3* mutation caused complete light independence of pregamete to gamete conversion, in strains with the *lrg4* mutation not all cells differentiated into gametes (Figure 1). This partial light independence may be accounted for by a residual activity of the mutated gene product. Alternatively, a second gene with a similar function to that of *lrg4* may exist in *C. reinhardtii*. In this case only double mutants would be expected to exhibit complete light independence of gamete formation.

For genetic analyses, these two mutants were crossed with the *lrg1* and *lrg2* mutants already described (BUERKLE *et al.* 1993). By tetrad analysis a linkage map of the four loci was elaborated (Figure 3). Attempts to map these loci on any of the established linkage groups have so far not been successful.

The recessiveness/dominance of the mutant alleles was assessed in heterozygous diploids (HARRIS 1989). The *lrg1*, *lrg3*, and *lrg4* mutations were all recessive to their wild-type alleles (Table 2). These results suggest that the wild-type gene products normally act to repress gamete formation in darkness and that light reverses this repression. An unexpected phenotype was observed when heterozygous wild-type/mutant alleles were combined. The combination of the heterozygous loci of $[+ / lrg4, lrg1 / +]$ and $[+ / lrg4, lrg3 / +]$ resulted in light independence of gamete formation, *i.e.*, the mutant phenotype dominated (Table 2). In contrast, the heterozygous $[+ / lrg1, lrg3 / +]$ loci exhibited wild-type phenotype. Thus, while the individual mutations do not exhibit a dominant phenotype, the combination of two recessive mutant alleles together with their wild-type alleles gave rise to a mutant phenotype. For an explanation of these results we assume that the mutant loci *lrg1*, *lrg3*, and *lrg4*

encode nonfunctional or partially functional proteins and that these proteins may be assembled into homo- or hetero-multimeric complexes. Based on this assumption, we offer the following explanations for the phenotypes observed. (1) If homo-multimeric complexes are formed, the mutant gene product may result in a reduced activity of the wild-type protein. The combination of two different reduced activities in a single cell may be insufficient to ensure the repression of gamete formation. (2) The products of genes *lrg1*, *lrg3*, and *lrg4* may assemble into a single multimeric complex. The observation that gene pairs $[+ / lrg1, lrg3 / +]$ exhibited a wild-type phenotype while combinations that contained the $+ / lrg4$ loci, *i.e.*, $[+ / lrg4, lrg1 / +]$ and $[+ / lrg4, lrg3 / +]$, resulted in mutant phenotypes, may point to a special role of the *lrg4* product. We speculate that the *lrg4* protein in this complex may interact directly with the *lrg1* and *lrg3* proteins while these latter two proteins do not interact with each other. We envision that the assembly of, *e.g.*, defective and functional proteins, encoded by the *lrg1* and *lrg4* loci, into a single multimeric complex may in a negative synergistic manner render the whole complex inactive. Evidence for the assembly of signal transduction chain proteins into a multimeric complex has been obtained recently in *Saccharomyces cerevisiae*. One of these proteins (Ste5) was demonstrated to serve as a scaffold for three other proteins that exhibit protein kinase activities. (CHOI *et al.* 1994; MARCUS *et al.* 1994; PRINTEN and SPRAGUE 1994).

To test for epistatic effects, the *lrg1*, *lrg3*, and *lrg4* mutations were crossed into a strain with the *lrg2* mutation. In all cases, the phenotype of the mutation that resulted in light independent gamete formation was observed (data not shown). This indicated that these alleles are epistatic to the *lrg2* allele. This could mean that the *lrg1*, *lrg3*, and *lrg4* proteins function downstream from the *lrg2* protein. However, since we do not know whether individual mutations that cause the constitutive phenotype could overrule expression of the *lrg2* phenotype, we cannot exclude other possibilities.

Zygote germination is another light-dependent step in the life cycle of *C. reinhardtii*. The data presented in Figure 4 indicate that illumination for ~ 2 hr is sufficient to induce germination. Since germination is initiated only several hours after zygotes were illuminated (LEVINE and FOLSOME 1959; WEGENER and BECK 1991), light is needed as a signal to initiate germination rather than as an energy source. In analogy to the situation in gametes, a signal transduction chain may be postulated to mediate light induction of zygote germination. This raises the question whether the two signal chains may share common elements. The data presented in Table 3 suggest that the *lrg4* mutation influences the transduction of the light signal that initiates germination. Since homozygous combinations of the *lrg1* and *lrg3* mutations had no effect on zygote germination, these genes appear not to play a role in the signal pathway by which

light controls germination. However, the observation that *lrg1* or *lrg3*, in combination with the *lrg4* allele, resulted in light independence of zygote germination (Table 3) appears to be contradicting. This contradiction, though, may be resolved when a central role is postulated for the *lrg4* gene product in the light control of gametogenesis. According to our model, light independence of germination caused by these gene combinations is a consequence of the partial functional inactivation of the *lrg4* protein due to its interaction with mutant *lrg1* or *lrg3* proteins. Thus, in these zygotes the amount of functional *lrg4* protein remaining would be insufficient to prevent germination in the absence of light. We propose two models to account for the observation that *lrg4* mutants are affected in light control of both gamete formation and zygote germination (Figure 5). According to model I, the early steps of the signal pathway are shared for gametogenesis and zygote germination. The product of the *lrg4* locus may function in this common pathway before a point of divergence into a gamete specific and a zygote specific fork. Alternatively, as illustrated in model II, the two signal pathways are different but the *lrg4* protein has a function in both. In any case, a molecular characterization of the signal pathway by which light controls gamete formation should also provide information on the mode of light control of meiosis and germination.

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