Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 6981–6985, August 1993
Plant Biology

Chlamydomonas mutants affected in the light-dependent step of sexual differentiation

(gametogenesis/blue light/signal transduction chains)

SOLVEIG BUERKLE, GERNOT GLOECKNER, AND CHRISTOPH F. BECK*

Institut für Biologie III, Albert-Ludwigs-Universität, Schaenzlestrasse 1, D-79104 Freiburg, Federal Republic of Germany

Communicated by Winslow R. Briggs, April 19, 1993

ABSTRACT **Sexual differentiation of *Chlamydomonas reinhardtii* is induced by the consecutive action of two extrinsic cues—nitrogen deprivation and blue light. The definition of a blue light-dependent step in gamete formation provided a basis for the isolation of mutants altered in the signal transduction pathway by which light controls sexual differentiation. In one mutant (*lrg1*), gamete formation has become light independent. In the other mutant (*lrg2*), perception or transduction of the light signal appears to be partially impaired. In both mutants, the expression of genes activated by light in the late phase of gamete formation is affected. Genetic analyses showed that genes *LRG1* and *LRG2* are linked. The recessive nature of the *lrg1-1* mutation implies that the gene encodes a negative factor or a protein that controls the activity of a negative factor. In the case of *lrg2-1*, neither wild-type nor mutant allele was dominant. Rather, two copies of the *lrg2-1* gene simulate a wild-type phenotype. The identification of genetic loci in the pathway for blue light-mediated differentiation provides a basis for the isolation of signal transduction genes in *Chlamydomonas*.**

Light is an environmental factor of particular importance for plants since it controls growth and development (1–3). In contrast to the abundance of information about light-induced responses, our knowledge about the molecular basis for the perception and transduction of the light signal remains fragmentary. For red light-induced responses, progress has been made in the molecular characterization of the phytochrome-based photoreceptor system (2, 4). An initial step in the molecular definition of blue light photoreceptors has been made only recently (5–7). Very little is known about the transduction chains by which the light signal perceived by the photoreceptors is transmitted to the sites of action, where the signal is ultimately converted into responses at the physiological or genetic level.

For an elucidation of the signal transduction chain(s) by which light controls plant development, a genetic approach, which permits the dissection of the chain, appears to be well suited. Such an approach has been used successfully in bacterial, fungal, and animal systems. Recently, it has been introduced for the elucidation of the chains by which a light signal is transduced in plant cells. In *Arabidopsis*, this genetic approach has resulted in the isolation of mutants that exhibit altered responses to light or do not require light for greening (8–16). One of the genes marked by mutation has recently been cloned and shown to encode a protein that combines a guanine nucleotide-binding protein-related domain and a putative DNA-binding motif (17).

Mutants with defects in photoreceptors or a connected chain for signal transmission have also been identified in fungal systems such as *Aspergillus* (18), phycomyces (19), *Neurospora* (20), and *Trichoderma* (21). Thus, the *veA1* mutation of *Aspergillus* confers independence of conidiation from illumination with red light (18). The site of action and a molecular characterization of the *veA* gene product remain to be elucidated.

For a molecular analysis of light signal transduction chains in a plant organism, the unicellular and haploid green alga *Chlamydomonas reinhardtii* is ideally suited in many respects. In this alga, blue light is an essential signal for the differentiation of vegetative cells to gametes (22). This sexual differentiation may proceed in two steps. The first step is induced by withdrawal of the nitrogen source. If incubation without nitrogen is performed in the dark, cells differentiate not into gametes but rather into sexually noncompetent pregametes. In a second step, these pregametes may be converted into gametes by irradiation with blue light (22–24). This conversion was shown to be dependent on the light-induced expression of nuclear genes (23). Since the differentiation of pregametes to gametes is well defined by physiological and molecular criteria (23, 25), a solid basis for a genetic analysis of the transduction chain(s) by which light controls sexual differentiation is available.

Here we report on the characterization of two mutants; one can form gametes in the absence of light and the other exhibits a delay in pregamete to gamete conversion. The mutant phenotypes are caused by mutations in genes *LRG1* and *LRG2*, respectively. Our analyses suggest that these genes encode components of the signal transduction chain through which light controls the differentiation of pregametes to gametes.

MATERIALS AND METHODS

Strains and Culture Conditions. *C. reinhardtii* strains 137c *nit1*, *nit2* of mating types *mt+* and *mt−* (wild type), 38 (*pab2*, *ac14*, SM1), and 105 (*arg7-3*) were kindly provided by R. Matagne (University of Liège, Belgium); strains CC-1705 (*pab1*) and CC-1010 (wild type) were from the *Chlamydomonas* Genetics Center (Duke University, Durham, NC). Mutants *lrg1* and *lrg2* were single cell clones of strains 137c *mt+* and CC-1010, respectively. We assume that they represent spontaneous mutations that occurred while the strains were propagated for the culture collection. Cells were grown photomixotrophically with aeration in acetate-containing TAP medium (26). When necessary, the medium was supplemented with L-arginine (100 mg/liter) or *p*-aminobenzoic acid (1 mg/liter). Gametogenesis was performed as described (23). White light at 0.14 $mmol \cdot m^{-2} \cdot s^{-1}$ was provided by fluorescent tubes (Osram L36W/35, F.R.G.). For irradiation with blue light, an interference filter (Jenaer Glaswerke, Schott und Genossen, Mainz, F.R.G.) with a maximal transmission at 459 nm and a bandwidth of 19.4 nm was used. To test for the Lrg1 phenotypes of progeny from tetrads, gametogenesis was performed in 96-well microtiter plates. Prior to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed.

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 $\approx 5 \times 10^6$ cells per ml. One microtiter plate was incubated with continuous illumination (30 μmol·m^{-2}·s^{-1}); the other plate was kept in the dark. After 20 h, an approximately equal number of competent gametes of the opposite mating type were added. To cells incubated in the dark, the gametes were added in a dark room illuminated very weakly by a green light. After an additional incubation for 12–24 h in the dark, the presence or absence of a zygote pellicle in individual wells was scored under a dissecting microscope. 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 Lrg1$^+$ phenotype. Presence of zygote pellicles in wells of both dark- and light-incubated cells indicated a Lrg1$^-$ phenotype. A similar protocol was used to test for the Lrg2 phenotype. 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 h. The wells were then exposed to white light (30 μmol·m^{-2}·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 h. The presence or absence of a zygote pellicle, indicating the conversion of pregametes to gametes during the period of illumination, was then recorded.

To assay diploid strains (always *mt*−) for mating competence, gametes were generated according to the standard protocol (23) and tested by the agglutinin-based dried-spot bioassay (27). The agglutinin for this test was isolated as described (27).

Genetic Analyses. For tetrad analyses, the standard protocol was used (28). Diploid strains were constructed by using complementing wild-type and mutant alleles of *arg7-3*, *pab1*, *pab2*, *nit1*, *nit2*, and *ac14*. 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 (28) or, in the case of Nit$^-$ mutants, NO_3^- was used as sole nitrogen source. Diploid clones were recovered and tested.

Northern Blot Analysis. Total RNA was isolated and processed for RNA blot analysis as described (29). Labeling of probes, hybridization conditions, and autoradiography were performed as described (30). The probes used were *gas28* (E. D. von Gromoff and C.F.B., unpublished data) and *gle4* (31). Clone gas28 represents a gene specifically expressed in the late phase of gametic differentiation—i.e., the conversion of pregametes to gametes. It was isolated by differential screening of a genomic DNA library with labeled single-stranded DNAs reverse transcribed from poly(A)$^+$ RNA of gametes and vegetative cells.

RESULTS

Mutants and Their Phenotypes. In the induction of sexual differentiation of *C. reinhardtii* the two extrinsic signals, nitrogen deprivation and blue light, act in sequence. Only pregametes, generated from vegetative cells by incubation in the dark without a utilizable nitrogen source, are competent for the second, light-induced step. This provided a basis for the isolation of mutants in which the light-dependent step is altered. Among such mutants, we expect to find those carrying mutations in genes for the photoreceptor as well as for the connected signal transduction chain(s).

Two mutants that met these expectations were identified. The affected genes were named *LRG1* and *LRG2* and the mutated alleles were designated *lrg1-1* and *lrg2-1*, respectively (*lrg* for light regulation of gametogenesis). The *lrg1* mutant forms gametes in the absence of light. We assume that it is based on a spontaneous mutation in strain CC-1010. The kinetics of gamete formation in the light and in the dark are shown in Fig. 1. While the standard wild-type strain did not form gametes in the absence of light, the *lrg1* mutant produced gametes in the dark at a level comparable to that observed when gametogenesis was performed in the light. In the mutant, gametes appeared in the dark 8 h after gametogenesis was induced by withdrawal of the nitrogen source. This suggested that in the mutant, as in wild type, the program of differentiation induced by nitrogen withdrawal has to be completed before the second step (for which, in wild-type cells, light is required) may be executed.

Since in the *lrg1* mutant the light-dependent step in gametogenesis—i.e., the conversion of pregametes to gametes—occurred in the absence of light, it was of interest to test the expression of genes shown to be induced by light treatment of pregametes. Such genes are *gas28*—a gamete-specific gene (E. D. von Gromoff and C.F.B., unpublished data)—and *gle*—a gene encoding gametic autolysin (31). During gametogenesis in the light, *gas28* and *gle* mRNAs accumulated in the late phase (Fig. 2). When gametogenesis of wild-type cells was performed in the dark, mRNA levels of both genes were low. In the *lrg1* mutant, we observed an increase in *gas28* and *gle* mRNA levels in the late phase during gametogenesis in the dark. The expression of both genes in the mutant has thus become light independent. This result suggests that the product of the *LRG1* gene controls expression of both genes; mutant allele *lrg1-1* permitted their expression in the absence of light once competence for this step has been reached in the differentiation program.

A second mutant was detected when the kinetics of pregamete to gamete conversion were analyzed in various *C. reinhardtii* strains. These kinetics for wild type and the *lrg2* mutant at three different fluence rates of blue light are shown in Fig. 3. The mutant exhibited a delay in the light-dependent conversion of pregametes to gametes. This delay could not be reduced to wild-type level by an increase in the light inten-

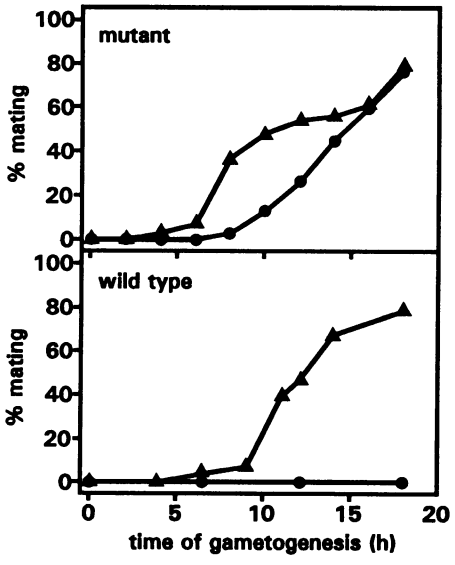

FIG. 1. Kinetics of gametic differentiation of *lrg1* mutant strain and wild type. The nitrogen source was removed at time 0. Incubations were performed either with continuous irradiation (0.1 mmol·m^{-2}·s^{-1} of white light) (▲) or in the dark (●). At the times indicated, cells were removed, mixed with an excess of sexually competent mating partner, and mated in the dark. After 60 min, the cells were fixed by the addition of glutaraldehyde (final concentration, 1%) and the percentage of mating was determined as described (24).

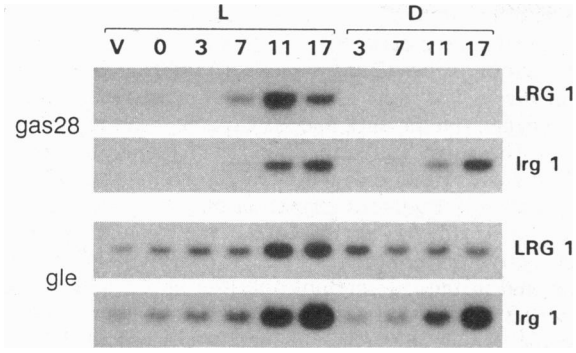

FIG. 2. Accumulation of *gas28* and *gle* mRNAs in *lrg1* and wild-type strains during gametogenesis. For Northern blot analyses, 10 μg of total RNA was applied to each slot. The nitrogen source was removed at time 0 and cells were incubated either in the light (L) or in the dark (D). Samples for RNA isolation were removed at the times (h) indicated. For a control, RNA from vegetative cells (V) was included. Autoradiography from blots of mutant and wild-type RNAs was performed for the same length of time.

sity. However, extended duration of illumination of mutant pregametes resulted in gamete levels comparable to those observed with wild-type cells (data not shown). A different and extended illustration of the *lrg2* mutant phenotype is shown in Fig. 4. Here, the percentage of gametes generated from pregametes after an irradiation with blue light for 90 min is plotted against the fluence applied, which ranged (in photons) from 10^{-1} to 10^{-11} mol/m^2 (32). In the *lrg2* mutant, the overall pattern of fluence dependence was similar to that of wild-type cells. However, as a consequence of the delay in the light-dependent step, it exhibited a reduced level of pregamete to gamete differentiation over the whole range of fluences applied. To test whether the mating reaction itself may account for the mutant phenotype, we tested the kinetics of this reaction and observed no difference to wild type (data not shown). Also, the kinetics of differentiation of vegetative cells to pregametes were not influenced by the mutation (data

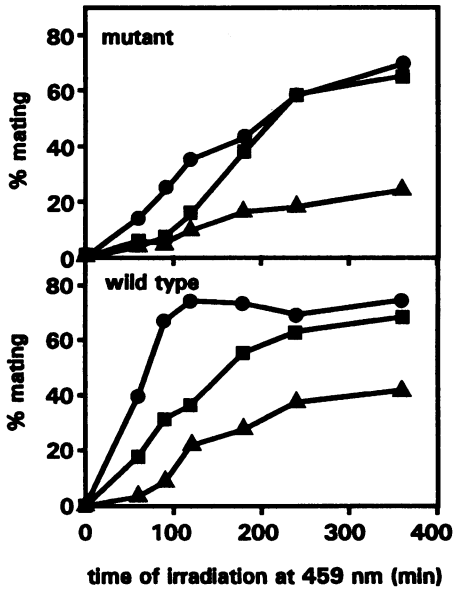

FIG. 3. Kinetics of pregamete to gamete differentiation of *lrg2* mutant strain and wild type. Prior to time 0, pregametes were generated by incubation of cells without a nitrogen source in the dark for 20 h. Starting at 0 min, pregametes were irradiated with blue light (459 nm). Fluence rates applied were 3.8×10^{-9} μmol·m^{-2}·s^{-1} (▲), 3.8×10^{-5} μmol·m^{-2}·s^{-1} (■), and 5.7 μmol·m^{-2}·s^{-1} (●). Cells were removed and assayed for gametes as described in the legend of Fig. 1.

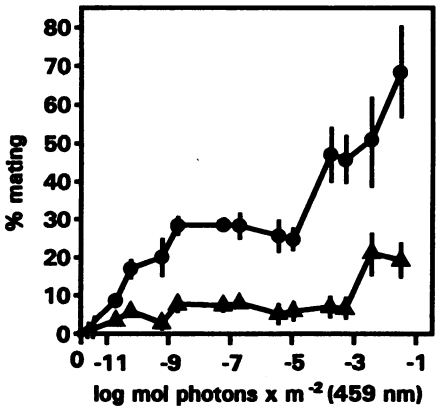

FIG. 4. Fluence response curves for pregamete to gamete conversion. Pregametes of wild type (●) and the *lrg2* mutant (▲) were irradiated for 90 min with blue light (459 nm) of the fluences indicated on the abscissa. Generation of pregametes and assay for gametes after irradiation were done as described in the legend of Fig. 1.

not shown). The only defect of the mutant at the physiological level thus appears to be a delayed response of pregametes to the extrinsic signal light. This conclusion is supported by data on the expression of genes *gas28* and *gle* (Fig. 5). While in wild-type pregametes *gas28* mRNA levels started to increase within 30 min after start of irradiation, *lrg2* pregametes exhibited a delay and reduction in *gas28* mRNA accumulation. Fluctuations in *gas28* mRNA levels after induction were observed in both strains. The *gle* mRNA levels in the mutant were lower and did not remain at an induced level as in wild-type cells. Rather, 1 h after start of irradiation, the mRNA level began to decrease. We conclude that gene *LRG2* exerts control over the expression of genes *gas28* and *gle*.

Genetic Characterization of Mutants. First we performed crosses to determine whether the mutant phenotypes were caused by mutations in single or multiple genes. To answer this question, progeny from crosses between the *lrg1* mutant and wild type and between the *lrg2* mutant and wild type were analyzed. More than 50 complete tetrads from each cross exhibited a 2:2 segregation of mutant and wild-type phenotypes (Table 1). These results suggest that the mutant phenotypes are based on mutations in single genes—although the possibility that more than one mutation in closely linked genes is the cause for the mutant phenotypes cannot rigorously be excluded. Crosses between the *lrg1* and *lrg2* mutants indicated that the two *LRG* genes are located on the same

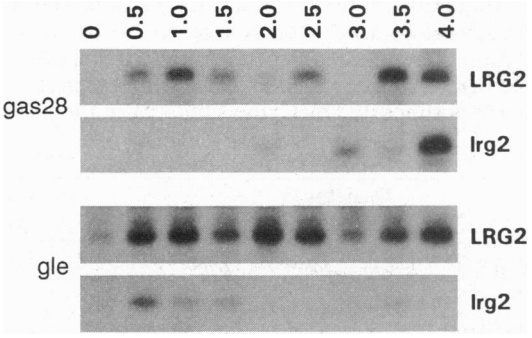

FIG. 5. Changes in the amount of *gas28* and *gle* mRNAs during pregamete to gamete conversion. Pregametes of the *lrg2* mutant or wild type were generated by starvation for nitrogen in the dark for 20 h. Starting at time 0, pregametes were irradiated (white light) and samples for RNA isolation were removed at the times (h) indicated. Total RNA (10 μg) was applied to each slot. Northern blot analyses were performed as described (29). Autoradiography from blots of mutant and wild-type RNAs was performed for the same length of time.

Table 1. Genetic characterization of *lrg* mutations

Cross	Tetrads	Segregation
lrg1-1 × *LRG1*	53	2:2
lrg2-1 × *LRG2*	64	2:2
lrg1-1 × *lrg2-1*	52	21:3:28*

*Types of tetrads, parental ditype, nonparental ditype, and tetratype.

chromosome, separated by 32.7 map units. No segregation of the mutant alleles with the mating-type locus on chromosome VI was observed (data not shown). From this result, we conclude that the *mt* locus has no effect on the phenotype of either *lrg* mutant. Double-mutant strains carrying *lrg1-1* and *lrg2-1* exhibited the phenotype of the *lrg1* mutant, indicating that *lrg1-1* is epistatic to allele *lrg2-1*.

We also tested the dominance of mutant versus wild-type alleles. For these tests, *C. reinhardtii* diploid cells were constructed (28). For an assay of diploid gametes, we tested their ability to bind to agglutinin of *mt+* gametes immobilized on glass microscope slides (27). The analyses indicated a dominance of the wild-type *LRG1* allele over the *lrg1-1* mutant allele; heterozygous diploid cells formed gametes in the light but failed to form gametes in the dark (Table 2). Analyses of cells with various combinations of *LRG2* alleles gave the surprising result that diploids harboring two mutant alleles (*lrg2-1/lrg2-1*) exhibited a phenotype similar to wild type—i.e., they did not exhibit a delay in the differentiation of pregametes to gametes in the light (Table 2). In contrast, diploid strains harboring the *LRG2/LRG2* or the *LRG2/lrg2-1* alleles formed gametes from pregametes only with a delay. Thus, the combination of two wild-type gene copies or a wild-type gene combined with the *lrg2-1* allele may cause a mutant phenotype (see *Discussion*). Detailed kinetic analyses of pregamete to gamete conversion showed that the outcome of these experiments was not dependent on the genetic background of the strains used (data not shown). This confirms the observation that in diploids two mutant *lrg2-1* alleles give a phenotype similar to that of haploid wild-type cells.

DISCUSSION

For the differentiation of pregametes to gametes both nitrogen deprivation and blue light are necessary (23). Light has previously been shown to be required for the execution of the second step in gametogenesis—i.e., the conversion of pregametes to gametes. We postulate that in the *lrg1* mutant, this step is specifically affected. Before the phase when light becomes effective, a program of differentiation induced by the withdrawal of a nitrogen source has to be completed. Completion of this first step of differentiation appears to be required before the gene product of *LRG1* may function. This conclusion is supported by (*i*) the similarity in the kinetics of gametic differentiation of wild-type cells in the light and *lrg1* mutant cells in the dark (Fig. 1) and by (*ii*) the pattern of the accumulation of *gas28* and *gle* mRNAs during gametogenesis. In the *lrg1* mutant, these mRNAs accumulated during gametogenesis in the dark and, as expected, in the late phase (Fig. 2). These results together indicate that in the *lrg1* mutant solely the conversion of pregametes to gametes is affected.

Table 2. Test of *lrg1-1* and *lrg2-1* alleles for dominance*

LRG1 genotype	Gametogenesis† Light	Gametogenesis† Dark	*LRG2* genotype	Irradiation,‡ min 0	60	240
lrg1-1/lrg1-1	+	+	*lrg2-1/lrg2-1*	−	+	+
lrg1-1/LRG1	+	−	*lrg2-1/LRG2*	−	−	+
LRG1/LRG1	+	−	*LRG2/LRG2*	−	−	+
LRG1	+	−	*LRG2*	−	+	+
lrg1-1	+	+	*lrg2-1*	−	−	+

*Formation of gametes was assayed by reaction with immobilized agglutinin (27).
†Gametogenesis was performed as described in the legend of Fig. 1. Cells were assayed 20 h after start of gametogenesis.
‡Pregametes used for the tests were generated as described in the legend of Fig. 3.

The control of gamete formation may be explained by a model that assumes a convergence of the signal transduction chains activated by the two extrinsic cues (24). The observation that in the *lrg1* mutant only the light signal is indispensable suggests that gene *LRG1* encodes a component of the signal transduction chain located between (and including) the photoreceptor and the point of convergence of the two signal transduction chains. By a mutation in gene *LRG1*, this component of the signal transduction chain may have been inactivated or altered, resulting in generation of a constitutive signal. The recessiveness of the *lrg1-1* allele suggests that the *LRG1* gene product is a negative factor or affects the activity of a negative factor.

In the *lrg2* mutant as well, the differentiation of pregametes to gametes appears to be specifically affected, although its phenotype is clearly distinct from that of the *lrg1* mutant. The delay in the conversion of pregametes to gametes could not be abolished by an increase in fluence rate (Figs. 3 and 4). However, the kinetics of pregamete to gamete differentiation in the *lrg2* mutant were influenced by the fluence rate (Fig. 3). Also, the expression of genes *gas28* and *gle* upon illumination of pregametes was reduced, albeit not abolished (Fig. 5). We have shown previously that the rate of pregamete to gamete conversion may be limited by the fluence rate of blue light, suggesting that the end product of the signal transduction chain accumulates in a fluence rate-dependent manner (24). In the context of these observations, the results may best be explained by assuming that the mutation in *LRG2* caused either a less active gene product or, alternatively, a lower abundance of the gene product. The *LRG2* gene product may be part of the photoreceptor or the connected signal transduction chain. In view of the responsiveness of the *lrg2* mutant to an increase in fluence rate, we consider it unlikely that the *LRG2* gene product has an enzymatic or structural function in a later step—i.e., the differentiation of pregametes to gametes.

Surprisingly, in diploid strains, two *lrg2-1* mutant alleles restored a wild-type phenotype, while two wild-type alleles or a mutant and a wild-type allele together caused a delayed conversion of pregametes to gametes—i.e., a mutant phenotype (Table 2). A doubled dosage of two partially defective genes may best match the situation in haploid cells. An advantage of two partially defective mutant alleles over two wild-type genes has also been observed in another system (33). In diploid cells (an artificial situation for *C. reinhardtii*), the gene dosis of *LRG2/LRG2* or *LRG2/lrg2-1* may be too high. The elevated gene dosis may result in an excess of *LRG2* gene product. Overexpression of genes encoding components of signal transduction pathways has been shown to cause aberrant regulatory effects (34). These data thus point to a very sensitive role of the *LRG2* gene product in transduction of the light signal.

In plants, the identification of individual components of signal transduction pathways and their classification into distinct signal pathways remains a major challenge (35). Mutants will be of crucial importance in establishing a causal relationship between a signal pathway component and the biological response elicited by the cue that triggers transduction of the signal. For an analysis at the molecular level, the isolation and characterization of the mutated genes is crucial. To this end, experiments to clone genes *LRG1* and *LRG2* need to be done.

The help of S. Masleša with the *gas28* expression experiments is gratefully acknowledged. We thank Dr. Y. Matsuda for providing the *gle* gene and Dr. E. Harris for advice on an easy screening system for sexually competent gametes. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 206).

1. Shropshire, W., Jr., & Mohr, H., eds. (1983) *Photomorphogenesis: Encyclopedia of Plant Physiology* (Springer, Berlin), New Series, Vol. 16A, pp. 1–456.
2. Kendrick, R. E. & Kronenberg, G. H. M., eds. (1986) *Photomorphogenesis in Plants* (Kluwer, Dordrecht, The Netherlands), pp. 1–580.
3. Senger, H., ed. (1987) *Blue Light Responses: Phenomena and Occurrence in Plants and Microorganisms* (CRC, Boca Raton, FL), Vol. 1.
4. Sharrock, R. A. & Quail, P. H. (1989) *Genes Dev.* **3,** 1745–1757.
5. Short, T. W. & Briggs, W. R. (1990) *Plant Physiol.* **92,** 179–185.
6. Warpeha, K. M. F., Hamm, H. E., Rasenick, M. M. & Kaufman, L. S. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 8925–8929.
7. Reymond, P., Short, T. W., Briggs, W. R. & Poff, K. L. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4718–4721.
8. Khurana, J. P. & Poff, K. L. (1989) *Planta* **178,** 400–406.
9. Koorneef, M., Rolff, E. & Spruit, C. J. P. (1980) *Z. Pflanzenphysiol.* **100,** 147–160.
10. Liscum, E. & Hangarter, R. P. (1991) *Plant Cell* **3,** 685–694.
11. Chory, J., Peto, C. A., Feinbaum, R., Pratt, L. & Ausubel, F. (1989) *Cell* **58,** 991–999.
12. Chory, J., Nagpal, P. & Peto, C. A. (1991) *Plant Cell* **3,** 445–459.
13. Deng, X.-W., Caspar, T. & Quail, P. H. (1991) *Genes Dev.* **5,** 1172–1182.
14. Chory, J. (1992) *Development* **115,** 337–354.
15. Chory, J. & Peto, C. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8776–8780.
16. Deng, X.-W. & Quail, P. H. (1992) *Plant J.* **1,** 83–95.
17. Deng, X.-W., Matsui, M., Wei, N., Wagner, D., Chu, A. M., Feldmann, K. A. & Quail, P. H. (1992) *Cell* **71,** 791–801.
18. Mooney, J. L. & Yager, L. N. (1990) *Genes Dev.* **4,** 1473–1482.
19. Corrochano, L. M. & Cerdá-Olmedo, E. (1992) *Trends Genet.* **8,** 268–274.
20. Degli-Innocenti, F. & Russo, V. E. A. (1984) *J. Bacteriol.* **159,** 757–761.
21. Horwitz, B. A., Gressel, J., Malkin, S. & Epel, B. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 2736–2740.
22. Weissig, H. & Beck, C. F. (1991) *Plant Physiol.* **97,** 118–121.
23. Treier, U., Fuchs, S., Weber, M., Wakarchuk, W. W. & Beck, C. F. (1989) *Arch. Microbiol.* **152,** 572–577.
24. Beck, C. F. & Acker, A. (1992) *Plant Physiol.* **98,** 822–826.
25. Treier, U. & Beck, C. F. (1991) *Physiol. Plant.* **83,** 633–639.
26. Gorman, D. S. & Levine, R. P. (1965) *Proc. Natl. Acad. Sci. USA* **54,** 1665–1669.
27. Adair, W. S., Monk, B. C., Cohen, R., Hwang, C. & Goodenough, U. W. (1982) *J. Biol. Chem.* **257,** 4593–4602.
28. Harris, E. H. (1989) *The Chlamydomonas Sourcebook* (Academic, San Diego).
29. von Gromoff, E. D., Treier, U. & Beck, C. F. (1989) *Mol. Cell. Biol.* **9,** 3911–3918.
30. Wegener, D. & Beck, C. F. (1991) *Plant Mol. Biol.* **16,** 937–946.
31. Kinoshita, T., Fukuzawa, H., Shimada, T., Saito, T. & Matsuda, Y. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4693–4697.
32. Zimmerman, B. K. & Briggs, W. R. (1963) *Plant Physiol.* **38,** 248–253.
33. McPherson, C. E. & Singleton, C. K. (1992) *Dev. Biol.* **150,** 231–242.
34. Choi, P. M., Tchou-Wong, K.-M. & Weinstein, I. B. (1990) *Mol. Cell. Biol.* **10,** 4650–4657.
35. Trewavas, A. & Gilroy, S. (1991) *Trends Genet.* **7,** 356–361.