37. T. Newman *et al.*, *Plant Physiol.* **106**, 1241 (1994).
38. J. L. Brewster, T. de Valoir, N. D. Dwyer, E. Winter, M. C. Gustin, *Science* **259**, 1760 (1994).
39. T. Maeda, S. M. Wurgler-Murphy, H. Saito, *Nature* **369**, 242 (1994).
40. I. M. Ota and A. Varshavsky, *Science* **262**, 566 (1993).
41. C. Chang and E. M. Meyerowitz, *Res. Microbiol.* **145**, 481 (1994).
42. L. A. Alex and M. I. Simon, *Trends Genet.* **10**, 133 (1994).
43. S. L. Weinstein, J. S. Sanghera, K. Lemke, A. L. DeFranco, S. L. Pelech, *J. Biol. Chem.* **267**, 14955 (1992).
44. J. Han, J. D. Lee, L. Bibbs, R. J. Ulevitch, *Science* **265**, 808 (1994).
45. Z. Galcheva-Gargova, B. Dérijard, I.-H. Wu, R. J. Davis, *ibid.*, p. 806.
46. J. Rouse *et al.*, *Cell* **23**, 1027 (1994).
47. B. Dérijard *et al.*, *Science* **267**, 682 (1995).
48. G. Roman, M. Rothenberg, J. R. Ecker, in preparation.
49. M. B. Lanahan, H.-C. Yen, J. J. Giovannoni, H. J. Klee, *Plant Cell* **6**, 521 (1994).
50. D. W. Fujino, D. W. Burger, K. J. Bradford, *J. Plant Growth Regul.* **8**, 53 (1989).
51. H.-C. Yen *et al.*, *Plant Physiol.* **107**, 1343 (1995).
52. R. G. H. Cormack, *New Phytol.* **34**, 19 (1935).
53. L. Dolan *et al.*, *Development* **120**, 2465 (1994).
54. B. Scheres *et al.*, *ibid.*, p. 2475.
55. J. W. Schiefelbein and P. N. Benfey, in (*6*), pp. 335–354.
56. E. Bunning, *Planta* **39**, 126 (1951).
57. J. D. Masucci and J. W. Schiefelbein, *Plant Physiol.* **106**, 1335 (1994).
58. W. K. Silk and R. O. Erickson, *Am. J. Bot.* **65**, 310 (1978).
59. A. Schwark and J. J. Schierle, *Plant Physiol.* **142**, 562 (1993).
60. A. Schwark and M. J. Bopp, *ibid.*, p. 585.
61. A. J. Bent, R. W. Innes, J. R. Ecker, B. J. Staskawicz, *Mol. Plant Microbe Interact.* **5**, 372 (1992).
62. A. F. Ross, *Virology* **14**, 329 (1961).
63. V. Raz and R. Fluhr, *Plant Cell* **5**, 523 (1993).
64. N. T. Keen, *Ann. Rev. Genet.* **24**, 447 (1990).
65. B. J. Staskawicz *et al.*, *Science* **268**, 661 (1995).
66. J. R. Ecker and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 5202 (1987).
67. K. E. Broglie, P. Biddle, R. Cressman, R. Broglie, *Plant Cell* **1**, 599 (1989).
68. M. Ohme-Takagi and H. Shinshi, *Plant Mol. Biol.* **15**, 941 (1990).
69. D. A. Samac and D. M. Shah, *Plant Cell* **3**, 1063 (1991).
70. Y. Eyal, Y. Meller, S. Lev-Yadun, R. Fluhr, *Plant J.* **4**, 225 (1993).
71. Y. Meller, G. Sessa, Y. Eyal, R. Fluhr, *Plant Mol. Biol.* **23**, 453 (1993).
72. C. M. Hart, F. Nagy, F. Meins, *ibid.* **21**, 121 (1993).
73. E. Alonso *et al.*, *Plant J.* **7**, 309 (1995).
74. M. Ohme-Takagi and H. Shinshi, *Plant Cell* **7**, 173 (1995).
75. K. D. Jofuku, B. G. W. den Boer, M. Van Montagu, J. K. Okamuro, *ibid.* **6**, 1211 (1994).
76. G. N. Drews, J. L. Bowman, E. M. Meyerowitz, *Cell* **65**, 991 (1991).
77. J. Marx, *Science* **260**, 1588 (1993).
78. L. Van Aelst, M. Barr, S. Marcus, A. Polverino, M. Wigler, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 6213 (1993).
79. A. B. Vojtek, S. M. Hollenberg, J. A. Cooper, *Cell* **74**, 205 (1993).
80. P. H. Warne and P. R. Viciana, *Nature* **364**, 352 (1993).
81. S.-F. Zhang *et al.*, *ibid.*, p. 308.
82. V. Raz and R. Fluhr, *Plant Cell* **4**, 1123 (1992).
83. W. Su and S. H. Howell, *Plant Physiol.* **99**, 1569 (1992).
84. G. Roman and J. R. Ecker, *Philos. Trans R. Soc. London Ser. B*, in preparation.
85. Y. Hou, A. G. von Arnim, X.-W. Deng, *Plant Cell* **5**, 329 (1993).
86. I thank G. Roman, M. Rothenberg, A. Lehman, M. Ohme-Takagi, H. Sinshi, H. Klee, L. Dolan, C. Chang, and E. Meyerowitz for preprints of manuscripts or communication of results before publication. I also thank H. Klee for providing photographs of the tomato *Nr* mutant; L. Dolan and K. Roberts for permission to reproduce the photograph of *ctr1* root hairs; L. Klimczak for assistance in preparing the sequence alignments; D. Marks, G. Ahlstrand, and the Electron Microscopy Optical Facility at the Minnesota Agricultural Experimental Station, University of Minnesota, for assistance in scanning electron microscopy analysis and A. Theologis for stimulating discussions. Supported by grants from the National Science Foundation (IBN) and the Department of Energy to J.R.E.

Phytochromes: Photosensory Perception and Signal Transduction

Peter H. Quail,* Margaret T. Boylan, Brian M. Parks, Timothy W. Short, Yong Xu, Doris Wagner

The phytochrome family of photoreceptors monitors the light environment and dictates patterns of gene expression that enable the plant to optimize growth and development in accordance with prevailing conditions. The enduring challenge is to define the biochemical mechanism of phytochrome action and to dissect the signaling circuitry by which the photoreceptor molecules relay sensory information to the genes they regulate. Evidence indicates that individual phytochromes have specialized photosensory functions. The amino-terminal domain of the molecule determines this photosensory specificity, whereas a short segment in the carboxyl-terminal domain is critical for signal transfer to downstream components. Heterotrimeric GTP–binding proteins, calcium-calmodulin, cyclic guanosine 5′-phosphate, and the COP-DET-FUS class of master regulators are implicated as signaling intermediates in phototransduction.

Light is a critical environmental factor for plants. It provides not only the radiant energy for photosynthesis, but also the informational signals that plants use to adapt and optimize growth and development in response to the ambient conditions (*1*). Perception, interpretation, and transduction of these light signals is accomplished with the use of regulatory photoreceptors: the phytochromes [responsive to red (R) and far-red (FR) light], the blue-light (B) receptors, the ultraviolet A (UV-A) receptor or receptors, and the UV-B receptor or receptors (*2*). This article focuses on recent developments regarding the phytochromes (*2–5*).

Phytochromes are cytosolically localized dimers composed of two ~125-kD polypeptides, each carrying a covalently linked tetrapyrrole chromophore in the NH_2-terminal domain and dimerization determinants in the COOH-terminal domain. The photosensory function of the molecule is based on its capacity for reversible interconversion between the R-absorbing Pr form and the FR-absorbing Pfr form upon sequential absorption of R and FR light. Photosignal perception by the receptor activates signaling pathways leading to the changes in gene expression that underlie the physiological and developmental responses to light (*2, 3*). These responses occur throughout the life of the plant and range from seed germination, seedling deetiolation, and shade avoidance to flowering (*1*). The molecular nature of the primary transduction processes by which the photoreceptors relay their sensory information to the cell is unknown. However, various analytical approaches have converged in recent years to provide insights into possible mechanisms.

Phytochrome genes encode a small family of photoreceptors (*6*). In *Arabidopsis*, the apoprotein is encoded by five genes, designated *PHYA*, -*B*, -*C*, -*D*, and -*E* (*7, 8*). Sequences related to these genes have been found in species ranging from algae to angiosperms (*6, 9, 10*). Evidence indicates that the phytochrome variants have distinct photosensory functions, but their regulatory mechanisms of action remain unclear. In this discussion, the distinction is made between the photosensory function of the molecule, defined as perception and interpretation of the incoming light signal, and the regulatory function, defined as induction of changes in downstream transduction components by the activated photoreceptor molecule. Investigations of the mechanism of action and the downstream signaling pathways focus on three broad areas: the photoreceptor molecule itself,

The authors are in the Department of Plant Biology, University of California, Berkeley, CA 94720, USA, and at the Plant Gene Expression Center, Agricultural Research Service, U.S. Department of Agriculture, Albany, CA 94710, USA.

*To whom correspondence should be addressed.

potential signaling components, and light-responsive gene expression. Phytochrome-regulated gene expression has been recently reviewed (*11*, *12*) and will not be examined further here.

Photosensory Functions of Different Phytochromes

The diversity of responses attributed to the phytochrome system became increasingly difficult to reconcile with the action of a single molecular species of the photoreceptor (*13*). However, genetic and reverse genetic studies to dissect the deetiolation process (manifested as hypocotyl suppression, hook straightening, cotyledon separation, and expansion, and chloroplast development) in *Arabidopsis* seedlings are revealing photosensory specialization among the multiple family members that may permit rationalization of previous paradoxes.

Phytochromes A (phyA) and B (phyB) exhibit contrasting roles in controlling hypocotyl elongation in etiolated *Arabidopsis* seedlings (*14*, *15*) (Fig. 1). PhyB is necessary for continuous red light (Rc) perception, phyA is neither necessary nor sufficient for Rc perception, and neither phyC, -D, nor -E is sufficient for Rc perception. Conversely, phyA is necessary for continuous far-red light (FRc) perception [the so-called FR–high irradiance response (FR-HIR) (*16*, *17*)], phyB is neither necessary nor sufficient for FRc perception, and neither phyC, -D nor -E is sufficient for FRc perception. Thus, although phyA and phyB each absorb R and FR light, and although the morphogenic response of wild-type seedlings both to Rc and to FRc is similar in regard to the deetiolation process, the two phytochromes monitor distinct facets of the light environment.

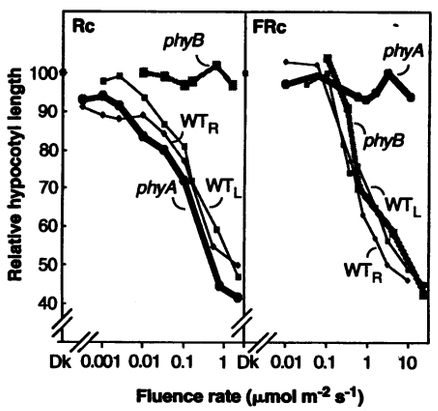

Fig. 1. Phytochromes phyA and phyB have discrete photosensory functions. Hypocotyl lengths of etiolated wild-type or mutant *Arabidopsis* seedlings in response to increasing fluence rates of either continuous red light (Rc) or continuous far-red light (FRc). Because the data are compiled from different experiments (*14*, *15*), hypocotyl lengths have been normalized to the respective dark control (Dk) seedling values (set at 100) for each experiment. Mutants deficient in either phyA (*phyA*) or phyB (*phyB*) are compared with their respective wild-type parental lines in ecotype RLD (WT$_R$) for *phyA*, or in ecotype Landsberg erecta (WT$_L$) for *phyB*. Seedlings were exposed to Rc or FRc for 3 or 5 days after germination until measurement.

Indeed, phyA and phyB transduce mutually antagonistic signals to the seedling in response to Rc or FRc light enrichment, in a yin-yang type of relationship (Fig. 2A). Rc absorbed by phyB induces deetiolation (inhibits hypocotyl elongation), but this response is suppressed by simultaneous irradiation with FRc absorbed by phyB (*18*). This suppression of deetiolation by FRc enrichment (observed as acceleration of hypocotyl elongation) is a manifestation of the "shade-avoidance" response (*18*). Conversely, FRc absorbed by phyA induces deetiolation, but this response is suppressed by simultaneous irradiation with Rc absorbed by phyA (*19*), as can be predicted from the photobiological experiments of Hartmann (*16*). Thus, Rc enrichment both elicits deetiolation through phyB and supresses deetiolation through phyA (Fig. 2A). Conversely, FRc enrichment elicits deetiolation through phyA and suppresses deetiolation through phyB. However, in FRc-rich light the balance in this mutual antagonism between phyA and phyB shifts rapidly during deetiolation, from phyA dominance initially, to phyB dominance in fully deetiolated (green) seedlings. This shift is due to the rapid, 99%, light-induced decline in the amount of the photolabile phyA molecule, coupled with sustained expression of the photostable phyB molecule (Fig. 2B) (*20*). This reduced amount of phyA cannot transduce a strong inductive

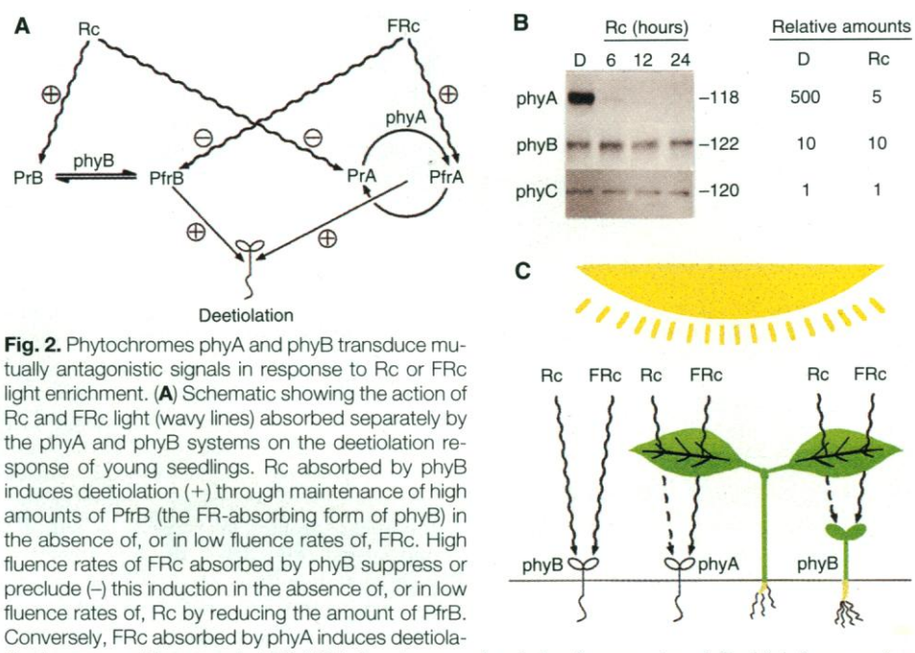

Fig. 2. Phytochromes phyA and phyB transduce mutually antagonistic signals in response to Rc or FRc light enrichment. (**A**) Schematic showing the action of Rc and FRc light (wavy lines) absorbed separately by the phyA and phyB systems on the deetiolation response of young seedlings. Rc absorbed by phyB induces deetiolation (+) through maintenance of high amounts of PfrB (the FR-absorbing form of phyB) in the absence of, or in low fluence rates of, FRc. High fluence rates of FRc absorbed by phyB suppress or preclude (–) this induction in the absence of, or in low fluence rates of, Rc by reducing the amount of PfrB. Conversely, FRc absorbed by phyA induces deetiolation by means of the so-called FR-HIR in the absence of, or in low fluence rates of, Rc. High fluence rates of Rc absorbed by phyA suppress or preclude this induction in the absence of, or in low fluence rates of, FRc by displacing the photoequilibrium toward PfrA (the FR-absorbing form of phyA) (*16*). PrB and PrA: R-absorbing forms of phyB and phyA, respectively. (**B**) Levels of phyA, -B, and -C in wild-type *Arabidopsis* seedlings that either were grown for 6 days from germination in darkness (D) or were exposed to Rc for 6, 12, or 24 hours after this dark growth period as indicated. Immunoblots of seedling extracts probed with monoclonal antibodies selective for the respective phytochromes are shown on the left (sizes shown to the right in kilodaltons). Because blot development times were different for each phytochrome to optimize band visibility, quantitative comparisons between the photoreceptor species cannot be made directly from these blots. On the right are quantitative estimates of the amounts of phyA, -B, and -C polypeptides relative to phyC in dark-grown seedlings set at an arbitrary value of 1. Values are shown for dark-grown seedlings (D) and seedlings exposed to Rc for 24 hours after the dark-growth period (Rc). [Adapted from (*20*)] (**C**) Simplified schematic of apparent photosensory functions of phyA and phyB in seedling development in the natural environment. In open sunlight, which is Rc-rich compared with vegetational shade, an emergent seedling is induced to deetiolate primarily through the phyB system (left). The light environment under a vegetational canopy is selectively depleted of Rc because of strong chlorophyll absorbance by the overlying leaves. A seedling emerging into this FRc-enriched environment is induced to deetiolate primarily through the phyA system (center). Rapid depletion of the photolabile phyA in the light relieves the inhibition of hypocotyl elongation imposed by FRc through the phyA system, leaving phyB to dominate growth regulation in the deetiolated seedling (right). Because FRc enrichment negates the inhibitory effects of phyB on elongation, the seedlings then exhibit accelerated extension growth as part of the shade-avoidance syndrome (*18*).

effect of FRc on the deetiolation process. Under phyB dominance, fully green plants then exhibit the shade-avoidance response elicited by FRc enrichment (*18*).

Open sunlight is Rc-rich compared with the light under vegetational shade, because the latter is selectively depleted in Rc as a result of strong chlorophyll absorbance by the overlying canopy (Fig. 2C). Thus, seedlings emerging from soil darkness into open sunlight appear to use primarily the phyB system for deetiolation. Seedlings emerging into FRc-enriched vegetational shade appear to use primarily the phyA system for initial deetiolation, and then later the phyB system to signal the shade-avoidance response, presumed to enable shaded plants to strive to reach the top of the canopy rapidly (Fig. 2C). Mutant *Arabidopsis* seedlings null for phyA are strongly retarded in their capacity for deetiolation when grown under natural vegetational shade (*21*). Moreover, these mutants have reduced survival rates under vegetation, indicating that phyA confers a significant competitive advantage for seedling establishment under these conditions. Because it seems likely that most seedlings in the natural environment will emerge into a world already substantially occupied by other green plants, the phyA signaling system is apparently important in early seedling establishment and survival. In contrast, phyB appears to have a complementary role in later growth and development. The two phytochromes also seem to have differential photosensory roles in regulating seed germination and flowering (*22–25*). However, the mechanism underlying this photosensory specialization is unknown. The functions of phyC, -D, and -E are also yet to be determined.

The Phytochrome Molecule

Various biochemical mechanisms of action have been proposed for phytochrome, but definitive evidence has been lacking (*3, 26*). Although it has been proposed that the phytochromes may function as light-activated protein kinases, either of the eukaryotic Ser-Thr-Tyr class (*27*) or the prokaryotic two-component His class (*28*), evidence now argues against this hypothesis (*2*). Together with the lack of any striking sequence similarities to other proteins in the databases, these studies leave open the possibility that the phytochromes may use a previously unknown mechanism of primary signal transduction.

In the absence of molecular models for phytochrome action, many laboratories have attempted to understand function by investigating structure. Strategies have included defining domains and individual residues involved in the assembly, photochemical activity, and regulatory action. Recombinant phyA, -B, and -C polypeptides autocatalytically attach the tetrapyrrole chromophore to the target cysteine in the NH_2-terminal domain in vitro (*29–32*) as well as in living yeast cells (phyA) (*33*). Subdomains within the NH_2-terminal domain containing the information necessary and sufficient for this process, and for fidelity of photoperception and reversible interconversion between the Pr and Pfr forms of the molecule, have been defined by using the expression of deletion derivatives of phyA in transgenic plants, yeast, and *Escherichia coli* (*34–38*). Site-directed substitution of Ser for Cys at the chromophore attachment site eliminates ligation and photosensory activity of both phyA (*34*) and phyB (*39*). Separate substitutions for five conserved amino acids surrounding the chromophore attachment site variously affect in vitro ligation efficiencies and photochemical properties of recombinant phyA (*40*), but no tests on in vivo photoregulatory activity have yet been done. One region near the center of the molecule and one near the COOH-terminus may be involved in dimerization of the polypeptide (*38, 41*).

Mutagenesis of phyA and phyB has defined sequences necessary for the regulatory activity of the photoreceptor in the living cell. The activity of various deletion derivatives of phyA or phyB in transgenic plants indicates that sequences necessary, directly or indirectly, for normal activity reside at both ends of the polypeptides within the terminal 110 residues or less (*34–36, 38, 39, 42*). A striking, light-dependent, dominant-negative phenotype induced by certain deletion derivatives of oat phyA overexpressed in transgenic *Arabidopsis* suggests that the mutant molecules interact with endogenous transduction pathway components in a nonproductive manner that prevents subsequent access to these components by the wild-type *Arabidopsis* phyA molecules (*34*). A subdomain within the chromophore-bearing NH_2-terminal domain between residues 53 and 616 is apparently responsible for this aberrant activity and therefore presumably carries contact sites for interaction with downstream signaling molecules.

Domain swapping experiments in transgenic *Arabidopsis* have established that the NH_2-terminal domain of phyA is sufficient, when fused to the COOH-terminal domain of phyB, to confer FRc photosensory activity on the chimeric molecule (*43*). The COOH-terminal domain of phyA, in contrast, cannot confer FRc photosensory activity on the NH_2-terminal domain of phyB, suggesting, therefore, that the determinants of the photosensory specificities of phyA and phyB toward Rc and FRc reside in the NH_2-terminal

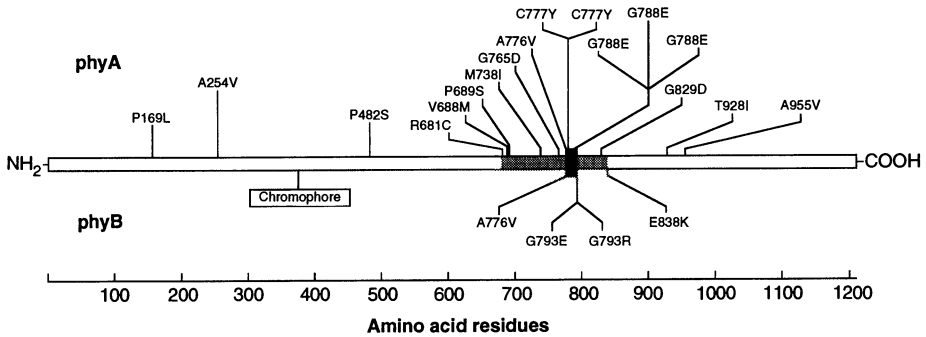

Fig. 3. Regulatory-function mutations in phyA and phyB cluster in a restricted segment of the COOH-terminal domain. The locations of single amino acid substitutions detected in individual mutant phytochromes in *Arabidopsis* are depicted for phyA and phyB above and below the schematic representation of the molecule, respectively. Residue positions are numbered according to the alignment of Mathews *et al.* (*9*). The location of the single tetrapyrrole chromophore is indicated. A 160-residue segment (positions 681 to 840) showing a high frequency of mutations is stippled. A short, 18-residue segment (positions 776 to 793) showing mutations that occurred multiple, independent times at four positions is solid black. All the mutant molecules depicted are expressed at parental levels, fully photochemically active, and dimers, but they are defective in regulatory activity in the cell [with the exception of V688M (V at position 688 mutated to M) where dimerization data are not available]. Lines carrying these mutant proteins were selected in four separate screens for *Arabidopsis* mutants defective in phytochrome-regulated suppression of hypocotyl elongation. Screen 1: M_2 seedlings of EMS-mutagenized ecotype RLD grown in FRc yielded phyA mutants P689S, C777Y, G788E (three times), G829D, and A995V (*14, 86*). Screen 2: M_2 seedlings of EMS-mutagenized Landsberg erecta grown in FRc yielded phyA mutant V688M (*22, 46*). Screen 3: M_2 seedlings of EMS-mutagenized transgenic *Arabidopsis* overexpressing oat phyA grown in Rc or FRc yielded P169L, A254V, P482S, R681C, M738I, G765D, A776V, C777Y, and T928I in the transgene-encoded oat phyA molecule (*47*). Screen 4: M_2 seedlings from EMS-mutagenized transgenic *Arabidopsis* overexpressing *Arabidopsis* phyB grown in Rc yielded all four phyB mutants in the transgene-encoded sequence (*39*). In all cases, the sequence of the target genes was determined after polymerase chain reaction amplification directly from genomic DNA (*14, 22*). Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

domains. Deletion analysis suggests that the 52 residues at the NH_2-terminus of phyA are necessary for its FRc-specific photosensory activity (*34*). Multiple Ser-to-Ala substitutions engineered at the extreme NH_2-terminus of phyA enhanced photoactivity in transgenic plants by an unknown mechanism (*44*). On the other hand, because the NH_2-terminal domains alone lack normal regulatory activity for both phyA and phyB (*34*, *39*), it appears that the COOH-terminal domains carry determinants, either structural or otherwise, necessary for execution of phytochrome regulatory action. These determinants appear to be common to phyA and phyB because either COOH-terminal domain functions in conjunction with either NH_2-terminal domain. Thus, the primary biochemical mechanism of action may be the same for phyA and phyB.

Sequence analysis of *phyA* and *phyB* genes carrying ethylmethane sulfonate (EMS)–induced point mutations has begun to reveal an intriguing pattern. Photochemically active, missense mutants (Fig. 3) were selected in screens for long hypocotyls under various irradiation regimes, either from mutagenized populations of wild-type *Arabidopsis* (*22*, *32*, *45*, *46*), or from mutagenized populations of transgenic *Arabidopsis* lines overexpressing either phyA (*47*) or phyB (*39*). A series of secondary genetic, photobiological and immunoblot screens was then used to select those lines carrying mutations in one or the other of the target *PHY* genes themselves but expressing parental amounts of fully photoactive phyA or phyB holoproteins encoded by those genes. In this manner, plants producing normal amounts of mutant phytochromes, normal in their photoperception function, but defective in their regulatory function, were selected. Thus, it may be concluded that the amino acid residues identified are necessary for effective communication of perceived light signals to downstream transduction components.

Although amino acid substitutions were detected throughout the polypeptide, all four of the phyB mutations obtained, and 12 out of the 17 phyA mutations (76% of the total), are located in a 160-residue region (Fig. 3, stippled area). More remarkable is the cluster of mutations between positions 776 and 793 in both phytochromes, where 9 out of 21 mutations occur in only four residues. Three independent mutations occur in phyA at G788, which is conserved in all sequenced phytochromes (9). Residue C777, which is conserved in all sequenced phytochromes, was found to be mutated to Y once in endogenous *Arabidopsis* phyA and once in overexpressed oat phyA. A mutation of A776 to V (A776V) was detected in both phyA and phyB. A776 is conserved in all phytochromes other than phyC and -E. G793 is mutated once to E and once to R in phyB. This residue is conserved as G in all non-phyA phytochromes and as A in all phyA sequences thus far published. The majority of the molecules with mutations in the region between positions 680 and 840 have been examined for the ability to dimerize, and all retain this capacity.

This region of the phytochrome molecule therefore seems critical for productive interaction with the signal transduction circuitry, possibly as part of the "active site" of the photoreceptor. The observations that the highest density of mutations is clustered in the same limited polypeptide segment for both phyA and phyB, that one mutation is common to both molecules, and that four residues are multiply mutated is consistent with the previously mentioned notion that the regulatory activity of the photoreceptors requires COOH-terminal domain sequences common to both. A search of current sequence databases has suggested no obvious sequence similarity to the 680-to-840 region. Thus, if this region is indeed involved in the biochemical mechanism of phytochrome action, the nature of this mechanism may prove to be one not previously detected. A composite model depicts the spatially separate photosensory-specificity and regulatory functions within the phytochrome molecule (Fig. 4).

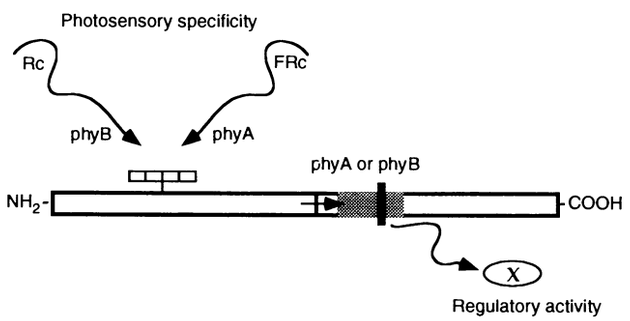

Fig. 4. Sequences determining the photosensory specificity and regulatory activity of phyA and phyB are spatially separate within the polypeptide. Sequence differences between phyA and phyB in the chromophore-bearing NH_2-terminal domain (chromophore indicated by series of four rectangles) result in contrasting interpretations of the same incoming Rc and FRc light signals (wavy lines) by the two photoreceptors in early seedling development. Intramolecular informational transfer (→) and successful transmission of the perceived signal to downstream transduction components (X) require a 160-residue COOH-terminal polypeptide segment (stippled) with indications that a subregion of 18 residues (solid) is particularly critical to this process. The activity specified by the COOH-terminal domain is common to both phyA and phyB, suggestive of a similar biochemical basis for the regulatory activity of the two photoreceptors.

Potential Signaling Molecules

Evidence suggests the photoactivated phytochrome molecules themselves do not translocate to the nucleus to bind to target gene promoters (*3*). Thus, other signaling intermediates must relay the information from photoreceptor to genome. Despite considerable effort, more traditional biochemical, immunocytochemical, and cell fractionation procedures have thus far failed to identify any molecules that interact directly with phytochromes in a functionally meaningful manner (*26*, *48*). However, genetic and new biochemical and cell biological approaches have begun to provide evidence of the nature of potential signaling pathways.

Genetic approaches. In *Arabidopsis*, two contrasting classes of photomorphogenic

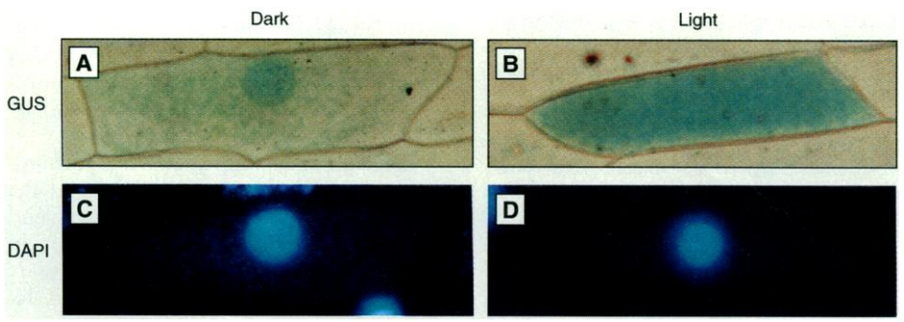

Fig. 5. Light induces partitioning of the COP1 protein from nucleus to cytoplasm. A chimeric construct encoding a β-glucuronidase (GUS)–COP1 fusion protein was expressed transiently in onion cells after microprojectile-mediated transfection. Cells were maintained either in darkness (**A** and **C**) or exposed to white light (**B** and **D**) for 16 hours before assay for GUS subcellular localization (GUS) [(A) and (B)]. The DNA stain 4′,6′-diamidino-2-phenylindole (DAPI) was also used to monitor nuclear location in the same cells (DAPI) [(C) and (D)]. [Adapted from (*71*)]

mutants carrying lesions in components downstream of phytochrome have been identified. One, the group of mutants including *cop*, *det*, and *fus*, exhibits seedling development in complete darkness that mimicks that normally induced by light (*49–53*). The other class of mutants, including *hy5*, *fhy1*, and *fhy3*, exhibits normal seedling development in darkness, but has a reduced sensitivity to light like the photoreceptor mutants (*54*, *55*).

Because the *cop*, *det*, and *fus* mutations are recessive and their effects pleiotropic, it can be concluded that the wild-type gene products act negatively in darkness early in the signaling cascade to repress photomorphogenesis, and that this repression is reversed by light (*51*, *56*, *57*). Genetic epistasis tests indicate that of those members of this mutant class thus far examined (*det1*, *-2*, *-3*, and *cop1*, *-2*, *-3*, *-4*, *-8*, *-9*, *-10*, *-11*), all are either at or downstream of the convergence of the phytochrome and blue-light photoreceptor signaling pathways (*56*, *58–64*). The COP1, -8, -9, -10, -11, and DET1 molecules are necessary for dark-imposed repression of a battery of light-inducible genes and of light-induced structural changes associated with plastid differentiation (*52*, *56*, *60*, *63–65*).

Therefore, at least a subset of the COP-DET-FUS class of gene products may function as components of one or more light-responsive master switches at the nexus between input signals from multiple upstream photoreceptors and the downstream cascade of gene expression that dictates seedling photomorphogenesis. Evidence suggests a regulatory hierarchy placing DET1 and HY5 upstream of COP1, -8, -9, -10, and -11, with possible direct interaction between COP1 and HY5 (*56*, *58*). Whether these molecules function as direct targets of, or as participants in, the signal transduction process, or have a structural or permissive function, is not established by these data. However, because overexpression of COP1 in transgenic *Arabidopsis* leads to reduced sensitivity to light (*57*), it appears that COP1 at least is rate-limiting for photosignal transduction. Additional mutant loci, *cop2*, *-3*, *-4*, *det2*, *-3*, and *doc1*, indicate that the signaling pathway branches downstream of COP1 and DET1 (*59*, *61*, *62*).

The sequencing of *COP1* (*66*), *COP9* (*67*), *COP11* (*FUS6*) (*49*), and *DET1* (*68*) suggests potential functions. The COP1 polypeptide contains a ring-finger class zinc-binding domain (*69*), a coiled-coil region, and a set of WD-40 repeats homologous to those of trimeric GTP-binding protein (G protein) β subunits (*66*). The functional importance of these domains is suggested by analysis of an allelic series of *cop1* mutants (*70*).

The zinc-binding domain of COP1 suggests DNA binding activity. Although there is no direct evidence to this effect, studies with chimeric fusions between COP1 and the reporter protein β-glucuronidase (GUS) show that COP1 is localized to the nucleus in the dark (*71*). Exposure to light causes an apparent relocation of the fusion protein to the cytoplasm (Fig. 5). These results are consistent with COP1 acting in the nucleus to repress transcription in the dark, an activity reversed by light. It has been proposed, based on the presence of the zinc-binding domain in COP1 and the sequence similarity between COP1 and *Drosophila* $dTAF_{II}80$, a component of the TFIID complex (*72*), that COP1 might impose transcriptional repression on target promoters in darkness by binding to an upstream DNA sequence motif and interfering with normal $TAF_{II}80$ assembly into the TFIID complex (*51*, *73*). Light-triggered derepression would then involve deactivation of COP1 and instatement of normal TFIID complex assembly at the target promoters.

The proteins encoded by *COP9* (*67*), *COP11* (*FUS6*) (*49*), and *DET1* (*68*) have no obviously informative sequence motifs and no compelling similarities to published protein sequences. However, DET1 is localized to the nucleus, suggesting a possible function in controlling light-regulated promoters (*68*). The relatively small COP9 protein (197 amino acids) is found in *Arabidopsis* extracts in a large (>560 kD) complex. This complex appears to be light-modulated and to require both COP8 and COP11 (FUS6) for its integrity (*67*). Such complexes may be functionally active units in the regulatory pathway.

The *hy5* mutant of *Arabidopsis* has impaired responsiveness to multiple wavelengths of light (B, R, and FR) (*54*). Thus, the *HY5* gene product would appear to be necessary for an activity at or downstream of the convergence of the phytochrome and blue-light receptor transduction pathways. In contrast, the *fhy1* and *fhy2* mutants are selectively impaired in responsiveness only to FRc (*55*). The *FHY1* and *FHY2* gene products would appear, therefore, to lie upstream in the transduction pathway specific to phyA. None of these loci have yet been characterized molecularly.

Biochemical and cell biological approaches. Numerous attempts have been made to determine whether the various second-messenger systems discovered in animal systems might be operative in phytochrome signal transduction (*3*). Previously, however, there was little if any compelling evidence in favor of this possibility (*3*, *5*). In a novel approach, N.-H. Chua and colleagues have used microinjection into hypocotyl cells of the phytochrome-deficient *aurea* mutant of tomato to assay the activity of introduced phytochrome molecules, pharmacological agents, and putative signaling intermediates (*5*, *74–76*). The *aurea* mutant carries a lesion, possibly in chromophore biosynthesis, that causes a deficiency in all phytochromes (*2*, *77*, *78*). In consequence, the hypocotyl cells of *aurea* fail to develop normal chloroplasts or to synthesize anthocyanin in response to light (*79*, *80*). Wild-type photoresponsiveness is restored upon microinjection of purified oat phyA into the mutant tomato cells.

Microinjections of various signaling molecules, agonists, and antagonists indicate that heterotrimeric G proteins, Ca^{2+}-calmodulin, and cyclic guanosine 5′-phosphate (cGMP) may mediate the phytochrome-induced responses (*75*, *76*). The data suggest that phyA may activate one or more trimeric G proteins, which stimulate increases in cellular levels of cGMP and Ca^{2+} (acting through calmodulin), which in turn activates parallel and partially convergent signaling pathways that induce the changes in gene expression necessary to account for the observed cellular phenotypes (*5*, *74*). Direct involvement of phytochrome in the transduction process is apparently restricted to steps upstream of the putative G protein. The data lead to the provocative conclusion that the specific course of differentiation followed by these cells is directed by a preprogrammed ("hard-wired") configuration of signaling circuitry that is poised to respond fully to only two signaling intermediates, Ca^{2+} and cGMP, which carry only minimal intrinsic informational specificity.

Protein phosphorylation cascades have long been proposed as transduction mechanisms for phytochromes (*81*). Although there are several reports of photomodulation of the phosphorylation of various anonymous proteins (*82–84*), conclusive evidence that this is involved in phytochrome signaling is lacking. On the other hand, evidence that changes in the DNA binding characteristics and nuclear translocation of a G-box binding factor might result from light-induced phosphorylation of the factor (*85*) is potentially consistent with a role for phosphorylation in phytochrome-regulated gene expression.

Conclusions

Research into photosensory perception and signal transduction in plants has unveiled both unanticipated complexity and simplicity in phytochrome-mediated phototransduction as well as similarities to, and possible differences from, known signaling systems in other organisms. The discovery of multiple phytochromes has

provided a satisfying conceptual framework for rationalizing the multiple, and sometimes seemingly incompatible, photosensory activities of the phytochrome system. The demonstration that phyA and phyB have mutually antagonist photosensory functions in young seedlings suggests that similar photosensory specialization may be anticipated for phyC, -D, and -E. In contrast, the similar regulatory activities of phyA-phyB chimeric fusions and the coincidence of amino acid substitutions in a restricted COOH-terminal region of mutant forms of the two proteins defective in regulatory function both suggest that the biochemical mechanism by which the photoreceptors communicate with downstream signaling molecules might be similar if not identical for these two family members.

The COP-DET-FUS class of molecules represents apparent complexity in terms of the relatively large number of loci with similar phenotypes and as yet undefined roles in light signal transduction. On the other hand, the discovery of these gene products and HY5 has simplified the larger picture by delimiting the boundary between the signals emanating from the photoreceptor systems and the downstream cascade of gene expression responsible for normal light-induced seedling development. Thus, interest in the primary signal transduction processes is likely to be focused upstream of HY5 and the COP-DET-FUS complex. The products of the *FHY1* and *FHY2* loci may be components specific to the phyA system, and may indicate branching in the transduction pathway upstream of the COP-DET-FUS complex.

That elevation of cytosolic Ca^{2+} and cGMP concentrations alone seems sufficient to trigger the developmental program normally induced by phytochrome in the target cells examined has resounding implications. These observations raise the prospect that other phytochrome-induced processes, from growth modulation to floral induction, also represent preprogrammed responses of participant cells dictated by a cell-specific set of transduction components poised to respond completely to just Ca^{2+} and cGMP.

Much remains to be learned about phytochrome phototransduction mechanisms relative to some other signaling systems in yeast and animals. On the one hand, the evidence for G protein, Ca^{2+}, and cGMP involvement may indicate basic similarities with known pathways. On the other hand, this photoreceptor family appears to be unique to plants, exceptional in its cytosolic subcellular localization, and lacking any compelling sequence similarity with other known receptors. The biochemical mechanism of phytochrome action, and possibly early steps in the signaling process, may therefore be novel.

REFERENCES AND NOTES

1. R. E. Kendrick and G. H. M. Kronenberg, Eds., *Photomorphogenesis in Plants* (Kluwer, Dordrecht, Netherlands, ed. 2, 1994).
2. P. H. Quail, *Curr. Opin. Genet. Dev.* **4**, 652 (1994).
3. ———, *Annu. Rev. Genet.* **25**, 389 (1991).
4. M. Furuya, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44**, 617 (1993).
5. A. J. Millar, R. B. McGrath, N. H. Chua, *Annu. Rev. Genet.* **28**, 325 (1994).
6. P. H. Quail, in (*1*), pp. 71–104.
7. R. A. Sharrock and P. H. Quail, *Genes Dev.* **3**, 1745 (1989).
8. T. Clack, S. Mathews, R. A. Sharrock, *Plant Mol. Biol.* **25**, 413 (1994).
9. S. Mathews, M. Lavin, R. A. Sharrock, *Anal. Missouri Bot. Garden*, in press.
10. H. A. W. Schneider-Poetsch, S. Marx, H. U. Koluk-isaoglu, S. Hanelt, B. Braun, *Physiol. Plant.* **91**, 241 (1994).
11. A. Batschauer, P. M. Gilmartin, F. Nagy, E. Schäfer, in (*1*), pp. 559–599.
12. E. Tobin and D. M. Kehoe, in *Seminars in Cell Biology*, (Academic Press, New York, 1994), pp. 1–12.
13. H. Smith and G. C. Whitelam, *Plant Cell Environ.* **13**, 695 (1990).
14. K. Dehesh *et al.*, *Plant Cell* **5**, 1081 (1993).
15. A. C. McCormac *et al.*, *Plant J.* **4**, 19 (1993).
16. K. M. Hartmann, *Photochem. Photobiol.* **5**, 349 (1966).
17. A. L. Mancinelli, in (*1*), pp. 211–269.
18. H. Smith, *ibid.*, pp. 377–416.
19. ——— and P. Quail, unpublished data.
20. D. E. Somers, R. A. Sharrock, J. M. Tepperman, P. H. Quail, *Plant Cell* **3**, 1263 (1991).
21. M. J. Yanovsky, J. J. Casal, G. C. Whitelam, *Plant Cell Environ.*, in press.
22. J. W. Reed, A. Nagatani, T. D. Elich, M. Fagan, J. Chory, *Plant Physiol.* **104**, 1139 (1994).
23. K. J. Halliday, M. Koornneef, G. C. Whitelam, *ibid.*, p. 1311.
24. E. Johnson, M. Bradley, N. P. Harberd, G. C. Whitelam, *ibid.* **105**, 141 (1994).
25. T. Shinomura, A. Nagatani, J. Chory, M. Furuya, *ibid.* **104**, 363 (1994).
26. P. H. Quail, in *Trends in Photobiology*, C. Hélène, M. Charlier, T. L. Montenay-Garestier, Eds. (Plenum, New York, 1982), pp. 485–500.
27. R. W. McMichael Jr. and J. C. Lagarias, in *Proceedings of the Ninth Annual Plant Biochemistry and Physiology Symposium*, University of Missouri–Columbia, 4 to 7 April 1990, P. D. Randall and D. G. Blevins, Eds. (Interdisciplinary Plant Biochemistry and Physiology Program, Univ. of Missouri, Columbia, MO, 1990), pp. 259–270.
28. H. A. W. Schneider-Poetsch, B. Braun, S. Marx, A. Schaumburg, *FEBS Lett.* **281**, 245 (1991).
29. J. A. Wahleithner, L. Li, J. C. Lagarias, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10387 (1991).
30. J. Cornejo, S. I. Beale, M. J. Terry, J. C. Lagarias, *J. Biol. Chem.* **267**, 14790 (1992).
31. T. Kunkel *et al.*, *Eur. J. Biochem.* **215**, 587 (1993).
32. B. Parks and P. Quail, unpublished data.
33. L. Li and J. C. Lagarias, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12535 (1994).
34. M. Boylan, N. Douglas, P. H. Quail, *Plant Cell* **6**, 449 (1994).
35. J. R. Cherry *et al.*, *ibid.* **5**, 565 (1993).
36. J. R. Cherry and R. D. Vierstra, in (*1*), pp. 271–300.
37. L. Deforce *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10392 (1991).
38. R. D. Vierstra, *Plant Physiol.* **103**, 679 (1993).
39. D. Wagner and P. Quail, unpublished data.
40. L. Deforce, M. Furuya, P. S. Song, *Biochemistry* **32**, 14165 (1993).
41. M. D. Edgerton and A. M. Jones, *ibid.*, p. 8239.
42. J. R. Cherry, D. Hondred, J. M. Walker, R. D. Vierstra, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5039 (1992).
43. D. Wagner, R. Kuhn, P. Quail, unpublished data.
44. J. Stockhaus *et al.*, *Genes Dev.* **6**, 2364 (1992).
45. B. M. Parks and P. H. Quail, *Plant Cell* **5**, 39 (1993).
46. T. D. Elich and J. Chory, *Plant. Mol. Biol.* **26**, 1315 (1994).
47. M. Boylan and P. Quail, unpublished data.
48. L. H. Pratt, in (*1*), pp. 163–185.
49. L. A. Castle and D. W. Meinke, *Plant Cell* **6**, 25 (1994).
50. J. Chory, C. Peto, R. Feinbaum, L. Pratt, F. Ausubel, *Cell* **58**, 991 (1989).
51. X. W. Deng, *ibid.* **76**, 423 (1994).
52. ———, T. Caspar, P. H. Quail, *Genes Dev.* **5**, 1172 (1991).
53. S. Miséra, A. J. Müller, U. Weiland-Heidecker, G. Jürgens, *Mol. Gen. Genet.* **244**, 242 (1994).
54. M. Koornneef, E. Rolff, C. Spruit, *Z. Pflanzenphysiol.* **100**, 147 (1980).
55. G. Whitelam *et al.*, *Plant Cell* **5**, 757 (1993).
56. J. Chory, *Trends Genet.* **9**, 167 (1993).
57. T. W. McNellis, A. G. von Arnim, X. W. Deng, *Plant Cell* **6**, 1391 (1994b).
58. L. H. Ang and X. W. Deng, *ibid.*, p. 613.
59. H. L. Cabrera y Poch, C. A. Peto, J. Chory, *Plant J.* **4**, 671 (1993).
60. J. Chory, *Development* **115**, 337 (1992).
61. Y. Hou, A. G. von Arnim, X. W. Deng, *Plant Cell* **5**, 329 (1993).
62. H. Li, L. Altschmied, J. Chory, *Genes Dev.* **8**, 339 (1994).
63. N. Wei and X. W. Deng, *Plant Cell* **4**, 1507 (1992).
64. N. W. Wei *et al.*, *ibid.* **6**, 629 (1994).
65. H. Li, T. Washburn, J. Chory, *Curr. Opin. Cell Biol.* **5**, 455 (1993).
66. X. W. Deng *et al.*, *Cell* **71**, 791 (1992).
67. N. Wei, D. A. Chamovitz, X. W. Deng, *ibid.* **78**, 117 (1994).
68. A. Pepper, T. Delaney, T. Washburn, D. Poole, J. Chory, *ibid.*, p. 109.
69. A. G. von Arnim and X. W. Deng, *J. Biol. Chem.* **268**, 19626 (1993).
70. T. W. McNellis *et al.*, *Plant Cell* **6**, 487 (1994a).
71. A. G. von Arnim and X. W. Deng, *Cell* **79**, 1035 (1994).
72. B. D. Dynlacht, R. O. J. Weinzierl, A. Admon, R. Tjian, *Nature* **363**, 176 (1993).
73. P. H. Quail, in *The Past, Present and Future of Plant Biology, A Symposium to Honor Joseph E. Varner*, T. D. Ho and H. Pakrasi, Eds. (Washington University, St. Louis, MO, 1993), pp. 87–90.
74. C. Bowler and N. H. Chua, *Plant Cell* **6**, 1529 (1994).
75. C. Bowler, G. Neuhaus, H. Yamagat, N. H. Chua, *Cell* **77**, 73 (1994).
76. G. Neuhaus, C. Bowler, R. Kern, N. H. Chua, *ibid.* **73**, 937 (1993).
77. A. van Tuinen, L. H. J. Kerckhoffs, A. Nagatani, R. E. Kendrick, M. Koornneef, *Mol. Gen. Genet.* **246**, 133 (1995).
78. G. C. Whitelam and N. P. Harberd, *Plant Cell Environ.* **17**, 615 (1994).
79. R. E. Kendrick and A. Nagatani, *Plant J.* **1**, 133 (1991).
80. M. Koornneef and R. E. Kendrick, in (*1*), pp. 601–630.
81. B. R. Singh and P. S. Song, *Photochem. Photobiol.* **52**, 249 (1990).
82. K. M. Fallon and A. J. Trewavas, *Plant Physiol.* **105**, 253 (1994).
83. K. M. Fallon, P. S. Shacklock, A. J. Trewavas, *ibid.* **101**, 1039 (1993).
84. K. Harter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 5038 (1994).
85. K. Harter *et al.*, *Plant Cell* **6**, 545 (1994).
86. T. Short, B. Parks, Y. Xu, P. Quail, unpublished data.

87. We are grateful to X.-W. Deng for Fig. 4 and to the members of our laboratory for fruitful discussions. Supported by grants from NIH (GM47475), U.S. Department of Energy (PRO3-92ER13742), U.S. Department of Agriculture (USDA) National Research Initiative Competitive Grants Program (92-37301-7678), National Science Foundation (MCB 9220161), and USDA–Agricultural Research Service Current Research Information Service (5335-21000-006-00D).