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Mutations of Arabidopsis in Potential Transduction and Response Components of the Phototropic Signaling Pathway[1,2]

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Four genetic loci were recently identified by mutations that affect phototropism in *Arabidopsis thaliana* (L.) Heyhn. seedlings. It was hypothesized that one of these loci, *NPH1*, encodes the apoprotein for a phototropic photoreceptor. All of the alleles at the other three mutant loci (*nph2*, *nph3*, and *nph4*) contained wild-type levels of the putative NPH1 protein and exhibited normal blue-light-dependent phosphorylation of the NPH1 protein. This indicated that the NPH2, NPH3, and NPH4 proteins likely function downstream of NPH1 photoactivation. We show here that, although the *nph2*, *nph3*, and *nph4* mutants are all altered with respect to their phototropic responses, only the *nph4* mutants are also altered in their gravitropic responsiveness. Thus, NPH2 and NPH3 appear to act as signal carriers in a phototropism-specific pathway, whereas NPH4 is required for both phototropism and gravitropism and thus may function directly in the differential growth response. Despite their altered phototropic responses in blue and green light as etiolated seedlings, the *nph2* and *nph4* mutants exhibited less dramatic mutant phenotypes as de-etiolated seedlings and when etiolated seedlings were irradiated with unilateral ultraviolet-A (UV-A) light. Examination of the phototropic responses of a mutant deficient in biologically active phytochromes, *hy1-100*, indicated that phytochrome transformation by UV-A light mediates an increase in phototropic responsiveness, accounting for the greater phototropic curvature of the *nph2* and *nph4* mutants to UV-A light than to blue light.

Plants need to respond rapidly to changes in their environment because they are sessile organisms. One manner in which plants do this is by modifying their growth pattern(s) in ways that ultimately lead to changes in morphology and development. Light is arguably one of the most important environmental factors impinging on a plant because it supplies energy for photosynthesis. The phototropic response, or the directional bending of plant organs induced by the detection of lateral differences in light intensity and/or quality, is one mechanism by which the plasticity of plant growth and development is expressed in changing light environments such that photosynthetic light capture can be maximized. Despite the fact that phototropism was first documented more than a century ago (Darwin, 1880) and a vast phenomenological knowledge base has been accumulated from detailed photophysiological studies of phototropism in a number of species (for reviews, see Dennison, 1979; Iino, 1990; Firn, 1994), little is known about the molecular basis for how the light signals are perceived and transduced or how those transduced signals result in differential growth responses in higher plants.

Most of the advances in our understanding of the possible molecular regulation of phototropism in higher plants come from the biochemical and genetic studies carried out during the past decade (for reviews, see Koornneef and Kendrick, 1994; Liscum and Hangarter, 1994; Short and Briggs, 1994; Briggs et al., 1996). Two recent advances are the isolation and photophysiological characterization of the mutants of *Arabidopsis thaliana* (L.) Heyhn. with altered phototropic responses (Khurana and Poff, 1989; Khurana et al., 1989; Okada and Shimura, 1992; Liscum and Briggs, 1995) and the biochemical characterization of a rapid blue-light-potentiated phosphorylation event at the plasma membrane. The latter phenomenon has been both physiologically (Short and Briggs, 1994; Briggs et al., 1996) and genetically (Reymond et al., 1992; Liscum and Briggs, 1995) correlated with phototropism. Furthermore, analyses of multiple mutant alleles at a single locus of Arabidopsis, namely the *NPH1* locus, indicate that the phosphoprotein mentioned above and the NPH1 protein are the same. They have also led to the hypothesis that the NPH1 phosphoprotein is the apoprotein for a dual- or multichromophoric holoprotein photoreceptor that mediates phototropic response to UV-A, blue, and green light (Liscum and Briggs, 1995).

Although no other biochemical events or molecules have been definitively linked to the phototropic response, several additional loci have been identified by mutations in Arabidopsis that appear to play a role in the transduction and/or response to phototropic stimuli downstream of NPH1 (Liscum and Briggs, 1995; E. Liscum, unpublished results). Here we report the characterization of mutants at three such loci. The results suggest that the proteins encoded by the *NPH2* and *NPH3* loci likely act as carriers of the phototropic signal, whereas the protein product of the *NPH4* locus is involved both in phototropism and in grav-

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itropism and thus may function directly in the establishment of differential growth. In addition we provide evidence that phytochrome is capable of acting as a positive regulator of phototropic curvatures that are induced by a nonphytochrome photoreceptor, likely encoded by *NPH1*, in etiolated and de-etiolated seedlings.

MATERIALS AND METHODS

The *nph1-1*, *nph2-1*, *nph3-1*, *nph3-2*, *nph4-1*, and *nph4-2* phototropism mutants of *Arabidopsis thaliana* (L.) Heynh. were isolated previously by Liscum and Briggs (1995). A third *nph4* allele (*nph4-3*) was isolated during the course of these studies by methods described previously (Liscum and Briggs, 1995). The *nph3-3* mutant was isolated previously by Khurana and Poff (1989). The phytochrome-deficient long-hypocotyl mutant, *hy1-100*, was described previously by Chory et al. (1989), and the CRY1-deficient long-hypocotyl mutant, *hy4-101* (previously designated *blu1-1* [R.P. Hangarter, unpublished data]) was described previously by Liscum and Hangarter (1991). The *nph3-1* and *nph3-2* alleles are in the Wassilenskija ecotype, whereas the *nph3-3* allele is in the Estland ecotype. All other mutant lines are in the Columbia ecotype.

Measurement of Tropic Responses

For all experiments, sterilization, sowing, and induction of the germination of seeds were as described previously by Liscum and Briggs (1995). For phototropism and gravitropism experiments, seedlings were handled as described previously for the characterization of phototropic and gravitropic responses of mutant Arabidopsis seedlings (Liscum and Briggs, 1995), with the noted exceptions. In experiments designed to assess the ability of various genotypes to establish large (second-positive) phototropic curvatures in the hypocotyl, 71.5-h-old etiolated seedlings were exposed to 10 h of continuous unilateral light of a particular quality and fluence rate. In experiments in which the function of a second photosensory system was assessed, 61.5-h-old etiolated seedlings were irradiated from above for 10 h with either red or UV-A light and then transferred to unilateral blue light for 10 h. Phototropic and gravitropic curvatures were determined as described by Liscum and Briggs (1995).

Light Sources

Red light for the induction of germination and for all phototropism experiments was as described by Short and Briggs (1990). Blue, green, and UV-A light was as described previously for the physiological characterization of *nph1* mutants (Liscum and Briggs, 1995). White light for de-etiolation (50 μmol m^{-2} s^{-1}) was as described previously (Liscum and Briggs, 1995). Far-red light was obtained by filtering light from one 60-W incandescent bulb (Phillips, Eindhoven, The Netherlands) through 6.5 cm of water and one layer of Plexiglas (Rohm and Haas FRF 700 far-red, AIN Plastics, San Jose, CA). This source resulted in light of wavelengths greater than 700 nm, with a wavelength maximum at 822 nm. Because phytochrome in its far-red-absorbing form has an absorption maximum at 730 nm, the fluence rate of this source was measured between 710 and 750 nm. Although considerable light was produced above 750 nm, it was considered inactive in the experiments presented here. Spectral qualities were measured with a portable spectroradiometer (Li1800, Li-Cor, Lincoln, NE). Fluence rates of the blue, green, and red light sources were measured with a quantum photometer (Li185A, Li-Cor), and the fluence rates of the UV-A and far-red light sources were measured with a bismuth-silver thermopile (Eppley Laboratory, Newport, RI) and the Li1800 spectroradiometer, respectively.

RESULTS AND DISCUSSION

Characterization of the Tropic Responses of the Transduction/Response Mutants

Recently, Liscum and Briggs (1995) described the isolation and genetic characterization of eight mutants of Arabidopsis, representing four genetic loci (*nph1*, *nph2*, *nph3*, and *nph4*) that lacked or had severely impaired phototropic responses. It was demonstrated that the *nph2*, *nph3*, and *nph4* mutants contained wild-type amounts of the putative NPH1 photoreceptor protein. Furthermore, the *nph2*, *nph3*, and *nph4* mutants all exhibited normal levels of blue-light-dependent phosphorylation of the NPH1 protein, which is presumed to be an autophosphorylation (Short and Briggs, 1994), indicating that a function of the NPH1 protein is not dramatically affected by the lesions in these mutants. Based on those physiological and biochemical results it is hypothesized that the *NPH2*, *NPH3*, and *NPH4* gene products act downstream of the NPH1 protein, either as signal carriers or as regulators of the differential growth response. Therefore, we have characterized the tropic responses of the *nph2*, *nph3*, and *nph4* mutants in an attempt to better understand the possible role(s) of the wild-type proteins encoded by these loci as signal transducers and/or response regulators.

When etiolated *nph3* seedlings were exposed to 10 h of unilateral UV-A, blue, or green light, no phototropic response was observed in the hypocotyl, whereas hypocotyls of wild-type seedlings grown under the same light conditions exhibited approximately 50, 40, and 35° curvature, respectively (Table I). Etiolated *nph2* seedlings were partially responsive in each of these light conditions, exhibiting about 25% of the wild-type response in blue and green light and about 75% of the wild-type response in UV-A light (Table I). Hypocotyls of the *nph4* mutants were nonphototropic in blue and green light but were about 25% as responsive as wild-type in UV-A light (Table I).

As shown in Table II, the *nph3* mutants were phototropic nulls with respect both to hypocotyl and root phototropism in de-etiolated seedlings when exposed to unilateral blue light, as observed for strong *nph1* alleles (Liscum and Briggs, 1995). By contrast, de-etiolated *nph2* and *nph4* seedlings showed partial responsiveness of the hypocotyl and root to unilateral blue light (Table II). The phototropic response of the root was about 55 to 60% of that observed for wild type in all of the *nph2* and *nph4* mutants examined,

Table I. *Second-positive hypocotyl phototropism in etiolated Arabidopsis wild-type, nph2, nph3, and nph4 seedlings*

Seedlings were grown in darkness for 71.5 h and then transferred to continuous unilateral light of the indicated quality. After 10 h of growth in the light, the phototropic response of the hypocotyl was measured, as described by Liscum and Briggs (1995). Data represent the means ± SE from a minimum of two replicate experiments. Numbers of seedlings are given in parentheses.

Genotype	Curvature (°)				
	UV-A Light[a]	Blue Light[b]	Green Light[a]	Red Light[c]	Far-Red Light[d]
Col[e]	59.3 ± 3.4 (26)	43.9 ± 2.5 (25)	42.2 ± 1.8 (44)	1.3 ± 0.8 (47)	0.1 ± 1.0 (51)
nph2-1	44.6 ± 2.9 (33)	12.1 ± 2.3 (38)	14.1 ± 2.4 (46)	nd[f]	nd
nph4-1	17.6 ± 1.7 (67)	1.1 ± 1.4 (28)	0.5 ± 0.8 (52)	nd	nd
nph4-2	13.5 ± 2.2 (18)	1.7 ± 0.7 (27)	−0.2 ± 0.6 (33)	nd	nd
nph4-3	10.9 ± 1.4 (47)	2.3 ± 1.4 (26)	2.0 ± 1.1 (43)	nd	nd
WS[g]	50.3 ± 2.8 (29)	38.9 ± 2.4 (38)	35.2 ± 4.6 (27)	nd	nd
nph3-1	1.4 ± 1.2 (42)	0.2 ± 0.9 (26)	1.1 ± 1.2 (27)	nd	nd
nph3-2	−0.9 ± 1.2 (31)	0.6 ± 0.7 (34)	0.0 ± 0.6 (55)	nd	nd
Est[h]	55.0 ± 2.7 (32)	49.1 ± 2.5 (36)	39.0 ± 2.0 (45)	nd	nd
nph3-3[i]	−1.3 ± 0.9 (18)	−0.3 ± 1.6 (39)	−0.5 ± 0.7 (41)	nd	nd

[a] UV-A and green light were given at a fluence rate of 0.2 μmol m^{-2} s^{-1}. [b] Blue light was given at 0.1 μmol m^{-2} s^{-1}. [c] Red light was given at 0.3 μmol m^{-2} s^{-1}. [d] Far-red light was given at 0.6 μmol m^{-2} s^{-1}. [e] Col, Wild-type Columbia ecotype. [f] nd, Not determined. [g] WS, Wild-type Wassilewskija ecotype. [h] Est, Wild-type Estland ecotype. [i] This mutant was previously designated as strain JK218 (Khurana and Poff, 1989).

whereas the response of the hypocotyl ranged from 25% of wild type in *nph4-2* to 50% of wild type in *nph2*. The results presented in Tables I and II indicate that the NPH2, NPH3, and NPH4 proteins probably function during the establishment of phototropic curvatures under laboratory (as etiolated seedlings) and natural (as green seedlings) growth conditions.

Because phototropism results from the differential growth of cells in the opposing flanks of the organ in question, molecules directly involved in regulating the growth of those cells might be common among various tropistic responses, even if those responses have unique stimulus receptors and even signal carriers. One tropistic response that clearly has been genetically separated from phototropism, but that also has been shown to have common genetic elements with phototropism, is the gravitropic response (Khurana and Poff, 1989; Khurana et al., 1989; Okada and Shimura, 1992; Liscum and Briggs, 1995). As shown in Figure 1, *nph2* and *nph3* seedlings exhibited tropic curvature in response to an altered gravity stimulus in a manner similar to that of wild-type and *nph1* seedlings (Liscum and Briggs, 1995), whereas the *nph4* mutants had a dramatically impaired gravitropic response. Although hypocotyl gravitropism is altered in the *nph4* mutants, root gravitropism is apparently not affected (data not shown). Similarly, root gravitropism is normal in the *nph2* and *nph3* mutants (data not shown).

Table II. *Second-positive hypocotyl and root phototropism in de-etiolated Arabidopsis wild-type and nph seedlings*

Seedlings were grown on plates oriented on edge. After 23.5 h in darkness, plates were transferred to continuous white light (50 μmol m^{-2} s^{-1}) from above for 48 h and then given continuous unilateral blue light (0.1 μmol m^{-2} s^{-1}) for 10 h. Curvatures were then determined as described by Liscum and Briggs (1995). Data represent the means ± SE from a minimum of three replicate experiments. Because hypocotyl and root measurements were made for each seedling, the number of seedlings analyzed are given in parentheses for hypocotyl data only.

Genotype	Curvature (°)	
	Hypocotyl	Root
Col[a]	51.6 ± 3.9 (48)	−15.0 ± 1.1
nph2-1	28.2 ± 3.9 (60)	−8.7 ± 1.3
nph4-1	18.5 ± 3.1 (43)	−8.8 ± 1.2
nph4-2	13.1 ± 3.2 (48)	−8.1 ± 1.3
WS[b]	54.3 ± 3.4 (46)	−17.0 ± 1.9
nph3-1	0.0 ± 3.8 (48)	−0.1 ± 1.2
nph3-2	−1.5 ± 2.6 (41)	−1.3 ± 1.3
Est[c]	58.6 ± 3.5 (46)	−21.1 ± 1.6
nph3-3	2.8 ± 1.8 (59)	2.6 ± 1.7

[a] Col, Wild-type Columbia ecotype. [b] WS, Wild-type Wassilewskija ecotype. [c] Est, Wild-type Estland ecotype.

Phytochrome-Mediated Enhancement of Phototropism

As previously mentioned, mutations in the *NPH2*, *NPH3*, and *NPH4* loci do not affect the abundance or the light-dependent phosphorylation of the putative NPH1 photoreceptor protein (Liscum and Briggs, 1995), suggesting that their gene products act downstream of a photoperception event. If this hypothesis is correct, the greater phototropic sensitivities of the *nph2* and *nph4* mutants to UV-A light versus blue and green light (Table I) probably resulted from the activation of a second photosensory system. This photoreceptor, although not capable of initiating the transduction of directional light signals, and hence not capable of inducing phototropic curvatures, might either modify or enhance the transduction of signals that are stimulated by the activation of NPH1 during unilateral UV-A light irradiations, or it might independently enhance a differential growth response capable of leading to curvature.

We have tested whether a second photosensory system is "enhancing" the primary phototropic response of *nph2* and *nph4* mutants by using sequential irradiations. Specifically,

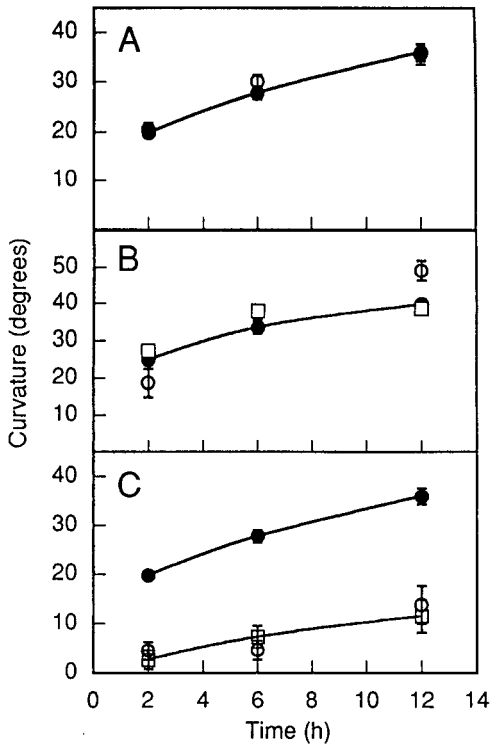

Figure 1. Time course for hypocotyl gravitropism in etiolated Arabidopsis wild-type, *nph2*, *nph3*, and *nph4* seedlings. Seedlings were grown on plates oriented on edge. After 71.5 h in darkness, plates were rotated 90° (still on edge) under dim green safelight (Liscum and Briggs, 1995) and returned to darkness. At the indicated times, plates were removed and curvatures were determined as described by Liscum and Briggs (1995). A, Responses of wild-type Columbia ecotype (●) and *nph2-1* (○). B, Responses of wild-type Wassilewskija ecotype (●), *nph3-1* (○), and *nph3-2* (□). C, Responses of wild-type Columbia ecotype (●), *nph4-1* (○), and *nph4-2* (□). Each data point represents the mean response of a minimum of 25 seedlings from at least three replicate experiments. Vertical error bars represent the SES. Because the symbols often overlap and the SES are small, some individual symbols and error bars are not readily visible.

etiolated seedlings were preirradiated with UV-A light in a manner that would not stimulate phototropic curvature (i.e. from above) prior to exposure to unilateral blue light that would stimulate phototropic signaling. To avoid possible problems with data interpretation because of differences in the developmental age of seedlings, preirradiation and unilateral light exposures were each given for 10 h, such that the total irradiation time was 20 h but the total growth period was kept to 82 h, as in previous experiments (Table I). As shown in Table III, the responses of wild-type, *nph2*, and *nph4-1* seedlings to unilateral blue light after a preirradiation with UV-A light given from above (Table III) were remarkably similar to the responses observed with these genotypes after irradiation with unilateral UV-A light alone (Table I). When *nph1-1* seedlings were treated with sequential UV-A and blue-light irradiations, no phototropic response was observed (Table III). This is consistent with the hypothesis that NPH1 is required for the perception of directional light cues (Liscum and Briggs, 1995). In addition, *nph3-1*, which is a phototropic null under all conditions tested thus far (Tables I and II) but is hypothesized to have a functional phototropic photoreceptor (NPH1) and thus should be able to sense directional light, also was not responsive to the UV-A light preirradiation (Table III). Thus, it is unlikely that the response in the *nph2* and *nph4* mutants represented some artifact of the sequential irradiation conditions used. It more likely resulted from the activation of a second photosensory system that could possibly lead to increased phototropic curvature.

Although the above data show that a second photosensory system can modify the phototropic response initiated by NPH1, they do not provide any insights into the nature of the UV-A-sensitive second photosensor. Such a photosensor could be a non-NPH1 blue/UV-A-absorbing system, such as CRY1 (HY4) (Lin et al., 1995a, 1995b; Malhotra et al., 1995), or one or more phytochromes, which have strong UV-A absorbance as Pr, the form that predominates in etiolated tissue (Butler et al., 1964; Pratt and Briggs, 1966; Vierstra and Quail, 1983). As shown in Table III, seedlings preirradiated with red light exhibited responses to subsequent unilateral blue light similar to those of seedlings preirradiated with UV-A light, suggesting that phytochrome may mediate this effect. Furthermore, the UV-A- and red-light-dependent enhancement of the phototropic response observed in wild-type seedlings was eliminated in *hy1-100* (Table III), a mutant that contains dysfunctional phytochromes because of an apparent deficiency in the biosynthesis of phytochromobilin (Parks and Quail, 1991; Nagatani et al., 1993), the phytochrome chromophore. In contrast, etiolated *hy4-101* seedlings responded like wild-type seedlings to sequential UV-A- and blue-light irradiations (Table III). Two additional strong *hy4* alleles also were tested, and each responded in a similar fashion (data not shown). These results are not surprising, since it has been

Table III. *Enhancement of second-positive hypocotyl phototropism in etiolated wild-type and nph seedlings*

Seedlings were grown as described in Table I, except that after 62 h of growth seedlings were irradiated from above (or mock irradiated) with the light of the indicated quality for 10 h, followed by exposure to 10 h of continuous unilateral blue light (0.1 μmol m^{-2} s^{-1}). Curvatures were determined as described by Liscum and Briggs (1995). Data represent the means ± SE from a minimum of two replicate experiments. Numbers of seedlings are given in parentheses.

Genotype	Curvature (°)		
	Darkness	UV-A Light[a]	Red Light[b]
Col[c]	39.6 ± 1.8 (58)	51.9 ± 2.5 (58)	54.3 ± 1.7 (59)
nph1-1	1.5 ± 1.7 (60)	−0.4 ± 3.1 (60)	−2.9 ± 5.8 (60)
nph3-1	nd[d]	−0.1 ± 0.8 (41)	nd
nph2-1	22.3 ± 2.5 (58)	53.0 ± 2.0 (55)	41.8 ± 1.9 (65)
nph4-1	0.4 ± 1.6 (55)	13.2 ± 1.2 (59)	17.0 ± 2.1 (56)
hy4-101	35.1 ± 2.3 (32)	50.3 ± 1.3 (36)	nd
hy1-100	36.3 ± 1.6 (48)	36.1 ± 1.6 (70)	33.1 ± 1.6 (54)

[a] UV-A light was given at a fluence rate of 0.2 μmol m^{-2} s^{-1}. [b] Red light was given at a fluence rate of 0.3 μmol m^{-2} s^{-1}. [c] Col, Wild-type Columbia ecotype. [d] nd, Not determined.

shown that mutations in *HY4* appear not to affect phototropic responsiveness over a wide range of fluences (Liscum et al., 1992; Liscum and Briggs, 1995). Therefore, phytochrome transformation is necessary and sufficient for UV-A and red-light enhancement of phototropism, whereas no function for the CRY1 protein was apparent with regard to phototropic responses.

Phytochrome has previously been implicated as a primary photoreceptor for phototropism in dark-adapted maize (Iino et al., 1984a, 1984b; Kunzelmann and Schäfer, 1985), etiolated pea (Parker et al., 1989) and in selected light-grown monocot and dicot seedlings (Atkins, 1936; Shuttleworth and Black, 1977). However, unilateral red or far-red light given for 10 h to etiolated wild-type Arabidopsis seedlings did not result in phototropic curvature (Table I). Similarly, Steinitz et al. (1985) noted that etiolated Arabidopsis seedlings exhibited no bending of the hypocotyl when irradiated unilaterally with light at wavelengths at or above 560 nm and at different fluences varying by as much as 6 orders of magnitude. Therefore, it appears that the directional light cues that induce phototropic curvature in etiolated Arabidopsis seedlings are processed via activation of the putative UV-A-/blue-/green-light-sensitive photoreceptor exclusively (Liscum and Briggs, 1995). It also appears that the induction of a phytochrome-activated signal somehow modifies either the transduction of signals initiated through the activation of the photoreceptor for phototropism or the differential growth response itself, such that enhanced phototropic curvatures are observed.

Parks et al. (1996) have recently shown that phytochrome A is required for the red-light-induced enhancement of first-positive phototropism in etiolated Arabidopsis seedlings. The results presented here indicate that phytochrome phototransformation is necessary for the enhancement of second-positive curvature in Arabidopsis as well. Although this might appear to be a fairly trivial point, it suggests that phototropic curvatures established under natural conditions (long-term irradiations of green seedlings) may be modulated by phytochrome photoconversion.

Possible Physiological Roles for NPH2, NPH3, and NPH4

Although it is clear that phytochrome phototransformation to Pfr is necessary for the UV-A-light-dependent enhancement of phototropism in wild-type Arabidopsis seedlings (Tables I and III), the results presented here are not sufficient to address how that phytochrome photoconversion results in enhanced curvatures in the *nph2* and *nph4* mutant backgrounds (Tables I and III). Specifically, it is not currently known whether the *nph2* and *nph4* mutants represent biochemically leaky alleles, and thus the phytochrome-induced signal possibly enhances the function of mutated proteins in these genetic backgrounds, or whether the phytochrome signal acts at a physiologically redundant step(s) independently of NPH2 and NPH4 in null mutant backgrounds.

Results from all of the physiological and genetic analyses of *nph2-1* suggest that this mutant is a leaky allele. For example, it represents the only locus affecting phototropism for which we have identified only a single mutant allele (Liscum and Briggs, 1995; E. Liscum, unpublished results). Furthermore, this single allele exhibits a partial response under all conditions tested (Tables I–III; Liscum and Briggs, 1995). The *NPH2* locus may encode a protein with an additional function(s) that is indispensable for seedling survival, thus precluding the isolation of a knockout (null) allele at this locus in screens designed to identify homozygous phenotypic nulls (Liscum and Briggs, 1995). Alternatively the *nph2-1* mutant may be a molecular null, in which case the NPH2 protein is not likely to be the primary signal carrier at that step in the transduction cascade, since the phenotype of the allele is so leaky. The probability of finding a mutant of this type should be fairly low, again consistent with the observed frequency of hits at this locus to date.

In contrast to the situation with the *NPH2* locus, three mutant alleles of the *NPH4* locus exist and each was isolated from fast neutron-mutagenized seed lots. This provides the strongest circumstantial evidence that these mutants might in fact be molecular nulls and not leaky alleles, since fast neutrons usually induce deletions or other gross chromosomal changes (Rédei and Koncz, 1992). These types of genetic lesions would be expected to have a high probability of resulting in a severely dysfunctional protein product encoded by the mutated gene or a lack of that protein altogether. In addition, as mentioned above, with the exception of the UV-A-light-dependent phototropism of etiolated hypocotyls, which clearly requires phytochrome action (Tables I and III), the *nph4* alleles exhibit a "leaky" phenotype only with respect to the gravitropic response of the hypocotyl (Fig. 1C) and the blue-light-induced phototropic responses of de-etiolated seedlings (Table II). Since the differential growth responses induced by tropistic stimuli likely result from the coordinated action of several cellular components acting directly on cell elongation processes, and different stimuli might only induce a subset of these molecules, it might be difficult to isolate mutants completely lacking multiple differential growth responses. It then follows that molecular nulls could be isolated for any given single component of the several that regulate cell elongation (i.e. NPH4), but cellular elongation processes would not be eliminated entirely. To our knowledge, no mutants exist that are true nulls for any two tropistic responses. Furthermore, because phytochrome activity is necessary for the UV-A-light-dependent phototropic curvatures observed in etiolated *nph4* seedlings (Table I and III), it is likely that the blue-light-induced phototropic curvatures observed in de-etiolated *nph4* seedlings (Table II) resulted from a similar phytochrome-stimulated mechanism(s). Thus, if several proteins are directly involved in the differential growth response giving rise to phototropic curvature, the phytochrome-mediated signal could enhance the function of any of these proteins such that one or more could provide a redundant function in the absence of NPH4 protein.

The studies presented here and elsewhere (Liscum and Briggs, 1995) indicate that three loci identified by mutation (*NPH2*, *NPH3*, and *NPH4*) encode proteins that act down-

stream of the putative NPH1 phototropism photoreceptor. It appears that NPH2 and NPH3 proteins act as signal carriers, whereas the NPH4 protein may act close to or directly on the differential growth response giving rise to tropistic curvature. However, the molecular nature of the lesions in these mutant loci, the relationship of the proteins encoded by the wild-type *NPH* loci to the primary phototropic signal transduction pathway (especially with respect to NPH2), and the true function of the proteins encoded by these loci require additional analysis of these mutants. Definitive answers to these questions may not be available until these genes are cloned.

It is probable that further studies of the *nph* mutants and interactions of phytochromes with the phototropic signaling pathway will lead to a more cohesive understanding of not only how phototropism is regulated but also how plants are able to integrate multiple signal inputs and regulate complex morphological and developmental processes in a coordinated adaptive fashion.

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