Plant Physiol. (1996) 112: 337–342

Phytochrome B Affects Responsiveness to Gibberellins in Arabidopsis[1]

Jason W. Reed[2], Kenneth R. Foster[3], Page W. Morgan, and Joanne Chory*

Plant Biology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, California 92186–5800 (J.W.R., J.C.); and Department of Soil and Crop Sciences, Texas A&M University, College Station, Texas 77843–2474 (K.R.F., P.W.M.)

Plant responses to red and far-red light are mediated by a family of photoreceptors called phytochromes. *Arabidopsis thaliana* seedlings lacking one of the phytochromes, phyB, have elongated hypocotyls and other tissues, suggesting that they may have an alteration in hormone physiology. We have studied the possibility that *phyB* mutations affect seedling gibberellin (GA) perception and metabolism by testing the responsiveness of wild-type and *phyB* seedlings to exogenous GAs. The *phyB* mutant elongates more than the wild type in response to the same exogenous concentrations of GA_3 or GA_4, showing that the mutation causes an increase in responsiveness to GAs. Among GAs that we were able to detect, we found no significant difference in endogenous levels between wild-type and *phyB* mutant seedlings. However, GA_4 levels were below our limit of detectability, and the concentration of that active GA could have varied between wild-type and *phyB* mutant seedlings. These results suggest that, although GAs are required for hypocotyl cell elongation, phyB does not act primarily by changing total seedling GA levels but rather by decreasing seedling responsiveness to GAs.

Phytochromes are a family of light-sensing proteins that mediate plant developmental responses to red and far-red light (for review, see Furuya, 1993). They consist of an apoprotein with a covalently attached linear tetrapyrrole chromophore and are interconverted by light between red- and far-red-light-absorbing forms. Although the far-red-light-absorbing form, Pfr, is generally thought to be the active form, recent data have suggested that the Pr form may also have an activity (Liscum and Hangarter, 1993; Reed et al., 1994; Shinomura et al., 1994). Light responses that are known to be mediated by phytochromes include seed germination, chloroplast development, leaf expansion, regulation of gene expression, inhibition of cell elongation, and photoperiodic control of flowering (Mullet, 1988; Chory, 1991; Thompson and White, 1991; Cosgrove, 1994).

Arabidopsis thaliana has five genes encoding phytochrome apoproteins, called *PHYA-PHYE* (Sharrock and Quail, 1989). *Arabidopsis* lines carrying mutations in *PHYB* (originally called *hy3*, for the long hypocotyl phenotype by which they were recognized) germinate poorly in red light, have elongated hypocotyls, stems, petioles, and root hairs, accumulate less chlorophyll than the wild type, and flower early (Koornneef et al., 1980; Goto et al., 1991; Reed et al., 1993, 1994; Shinomura et al., 1994). They also elongate to a lesser extent than the wild type in response to supplementary far-red light, implicating phyB in control of the shade avoidance response (Nagatani et al., 1991; Whitelam and Smith, 1991). The elongated hypocotyls in these mutants arise primarily from increased cell elongation (Reed et al., 1993). In sorghum, the ma_3^R (maturity) allele causes a phenotype similar to *phyB* in Arabidopsis and a deficiency in a light-stable phytochrome (Childs et al., 1992), and was recently shown to be a *phyB* mutation. Ma_3 and *PHYB* were found to map to the same location, and the *PHYB* gene from ma_3^R plants has a single base-pair deletion that results in a stop codon in the coding sequence (K.L. Childs, F.R. Miller, M.M. Cordonnier-Pratt, L.H. Pratt, P.W. Morgan, and J.E. Mullet, unpublished data). The *lh* (long hypocotyl) mutant of cucumber, the *lv* mutant of pea, and the *ein* (elongated internode) mutant of *Brassica rapa* may also be *phyB* mutants (Devlin et al., 1992; López-Juez et al., 1992; Weller and Reid, 1993; Weller et al., 1994, 1995). Although these mutants have not been shown to have mutations in a *PHYB* cognate gene, they are each missing a light-stable protein recognized by anti-phytochrome antibodies and have morphological and physiological phenotypes analogous to those of the *phyB* mutants in Arabidopsis.

In several plants, alterations in GA metabolism can cause phenotypes that resemble the elongation and flowering phenotypes of the *phyB* mutants. A slender mutant of pea overproduces GAs and has elongated internodes (Reid et al., 1992). The barley *slender* mutants and pea *la cry*^{s} mutant are GA-insensitive lines that behave as if constitutively

[1] Supported by grants to J.C. from the National Science Foundation, the U.S. Department of Energy, and the International Human Frontier Science Program and to P.W.M. from the U.S. Department of Agriculture (competitive grant no. 91–37304–6582). J.W.R. was a Department of Energy-Energy Biosciences fellow of the Life Sciences Research Foundation (Baltimore, MD). K.R.F. was supported in part by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada.

[2] Present address: University of North Carolina, Department of Biology, CB #3280, Coker Hall, Chapel Hill, NC 27599–3280.

[3] Present address: Alberta Environmental Protection, Environmental Assessment Division, 2938 11th St., NE, Calgary, Alberta, Canada T2E 7L7.

* Corresponding author; e-mail joanne_chory@qm.salk.edu; fax 1–619–558–6379.

Abbreviations: phyB, phytochrome B apoprotein; *PHYB*, gene encoding phyB apoprotein; *phyB*, mutation in the *PHYB* gene.

activated for GA pathways, and they also elongate excessively and flower early (Potts et al., 1985; Lanahan and Ho, 1988). Moreover, in GA-deficient dwarf varieties of several species, exogenously applied GAs can promote stem elongation, proving that they are required for these responses (Ross, 1994). Induction of flowering by long days in rosette plants has been correlated with increased GA levels (Talon et al., 1991; Zeevaart and Gage, 1993). GA-deficient (*ga*) mutants of Arabidopsis flower late in long days and fail to flower at all in short days (Wilson et al., 1992).

The connection between light and GA has also been studied by testing how mutations in light physiology affect GA metabolism. For example, the ma_3^R mutant of sorghum and the *ein* mutant of *Brassica*, which are deficient in phyB, have elevated levels of GAs, suggesting that in these species phyB controls GA levels (Rood et al., 1990a; Beall et al., 1991; Foster et al., 1994). However, more recently it was found that levels of some GAs in sorghum vary according to a diurnal rhythm, and that the ma_3^R mutant was phase-shifted in this rhythm (Foster and Morgan, 1995). Therefore, the increase in GA levels in the ma_3^R mutant may be an indirect consequence of the shifted diurnal rhythm, and average GA levels may not actually be appreciably increased. Similarly, the *ein* mutant has elevated GA levels only under some physiological conditions (Rood et al., 1990b). The cucumber *lh* mutant and the pea *lv* mutant also lack phyB. These mutants have near wild-type levels of GAs (Weller et al., 1994; López-Juez et al., 1995) but show enhanced elongation responses to exogenous GAs (Weller et al., 1994; López-Juez et al., 1995). Moreover, red and far-red light affected the responsiveness of wild-type cucumber seedlings in a manner analogous to the *lh* mutation (López-Juez et al., 1995). Finally, transgenic tobacco lines overexpressing oat phyA have a dwarfed phenotype and lower levels of several GAs (Jordan et al., 1995), suggesting that phyA can inhibit GA biosynthesis when overproduced.

These results suggest that the elongated hypocotyl phenotype of Arabidopsis *phyB* mutants might arise from an increased level of endogenous GAs, or from greater responsiveness to GA. To test these possibilities, we have compared the hypocotyl elongation responses of wild-type and *phyB* seedlings to an inhibitor of GA biosynthesis and to exogenous GAs. We also measured GA levels in *PHYB* and *phyB* Arabidopsis seedlings. Our results suggest that, in Arabidopsis seedlings (as for pea and cucumber), phyB-signaling affects responsiveness to GAs.

MATERIALS AND METHODS

Genetic Material

Arabidopsis thaliana mutations *phyB-1* and *phyB-5* are both mutations that are presumed null and have stop codons in the *PHYB* gene (Reed et al., 1993). *phyB-1* was previously called *phyB-Bo64*, and *phyB-5* was called *phyB-8-36* (Quail et al., 1994). The *ga1-3* mutation causes a deletion of most of the *GA1* coding sequence, which causes an almost complete absence of GA (Koornneef et al., 1983; Sun et al., 1992; Zeevaart and Talon, 1992). This line is maintained by exogenous application of GA_3. All of these mutations are in the ecotype Landsberg and carry the *erecta* mutation. A *ga1-3 phyB-5* double mutant was constructed by crossing the two single mutants, allowing the F_1 progeny to self-fertilize, and then picking GA-requiring F_3 progeny of a tall (*phyB/phyB*) F_2 plant.

Growth of Seedlings

Seeds were surface-sterilized and plated on Murashige-Skoog plates (1× Murashige-Skoog salts [GIBCO], 0.8% phytagar [GIBCO], 1× Gamborg's B5 vitamin mixture [Sigma]), stored overnight at 4°C, and grown in light chambers (16-h day/8-h night) kept at 22°C. Light was from six cool-white fluorescent bulbs (F24T12/CW/HO, Sylvania) and two incandescent bulbs (T10481C, Sylvania) and had a fluence rate of 3×10^2 to 5×10^2 μmol m^{-2} s^{-1}. In some cases, Murashige-Skoog plates were supplemented with 2% Suc. Paclobutrazol (PP333; ICI Americas, Goldsboro, NC) and GA_3 (Sigma) or GA_4 (Kyowa Hakko Kogyo, Tokyo, Japan) were added to the agar immediately before pouring, at the indicated concentrations. Hypocotyl lengths were measured to within 0.2 mm. For GA measurements, seedlings were harvested during the first 4 h of the light period, on d 7 or d 8 after sowing.

Measurement of GA Levels

Seedlings were grown on Murashige-Skoog plates, harvested 7 or 8 d after sowing, and lyophilized. Extraction, purification, and GC-MS analysis of GAs was as described previously (Foster et al., 1994). For GC-MS quantitation, 25 ng each of [17,17-^{2}H]GA_1, GA_8, GA_{19}, and GA_{20}; 20 ng each of [17,17-^{2}H]GA_{12} and GA_{53}; and 50 ng of [17,17-^{2}H]GA_3 were added as internal standards. Amounts of standards to be added were initially chosen based on the GA concentration in adult Arabidopsis reported by Talon et al. (1990b) and were used in all subsequent extractions for consistency. All data were corrected for the enrichment of the endogenous GAs by the internal standards. HPLC fractions thought to contain GA_4, GA_5, GA_9, GA_{17}, GA_{24}, GA_{25}, GA_{29}, GA_{34}, GA_{36}, and GA_{44} (Hedden, 1987) were analyzed by monitoring the three largest ions from each GA (Hedden, 1987). Deuterated standards were not available to aid in analysis of these GAs. Since we detected GA_4, GA_{29}, and GA_{44} in previous studies (Mattheussen et al., 1991), the procedure used (Foster et al., 1994) is adequate for GAs other than those detected. [^{3}H]GA_1 and [^{3}H]GA_4 were added to samples before extraction to allow pre-GC-MS calculations of recovery; ^{3}H from both GAs was routinely recovered in HPLC fractions at the appropriate retention times at recoveries of approximately 70% for GA_1 and 60% for GA_4. In a preliminary experiment with older plants and a 2-fold larger sample than available for the analyses reported in Table I, we detected a weak signal at the proper retention time and Kovat's retention index for GA_4-methyl-trimethyl silyl. In a few assays, we detected a peak at a position corresponding to the GA_3 standard. However, subsequent analysis revealed this peak to be a contaminant.

RESULTS AND DISCUSSION

To determine the extent to which phyB affects GA-dependent hypocotyl elongation, we assessed the ability of various concentrations of the GA biosynthesis inhibitor paclobutrazol to inhibit hypocotyl elongation of wild-type and *phyB* seedlings. Paclobutrazol prevents GA synthesis by inhibiting mono-oxygenases involved in converting *ent*-kaurene to *ent*-kaurenoic acid, an early step in GA biosynthesis (Rademacher, 1989, 1991; Jacobsen and Olszewski, 1993). Figure 1 shows that paclobutrazol inhibited elongation of both wild-type and *phyB* seedling hypocotyls starting at a concentration of about 10^{-9} M paclobutrazol. Higher concentrations of paclobutrazol inhibited elongation of *phyB* hypocotyls to a greater extent than wild-type hypocotyls, but at no concentration were *phyB* seedlings as short as wild-type seedlings. For both genotypes, the concentration of paclobutrazol required to inhibit hypocotyl length to one-half that achieved in the absence of paclobutrazol was about 2×10^{-8} M. Concentrations of paclobutrazol greater than 10^{-6} M inhibited germination of both wild-type and *phyB* mutant seeds, precluding measurement of hypocotyl lengths at this concentration. Thus, the curve for *phyB* is an amplification of the curve for the wild type, and the *phyB* mutation does not shift the curve to higher or lower paclobutrazol concentrations. We were able to restore the full hypocotyl lengths to both wild-type and *phyB* seedlings by including GA_4 in the medium, showing that paclobutrazol inhibited hypocotyl elongation by decreasing GA synthesis (data not shown).

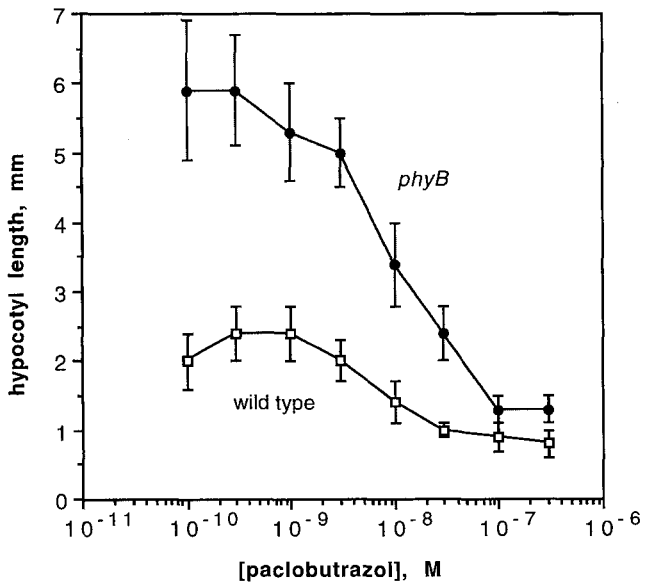

Figure 1. Hypocotyl elongation of wild-type and *phyB-5* Arabidopsis seedlings in the presence of exogenous paclobutrazol. Seeds were germinated on Murashige-Skoog/Suc plates containing the indicated concentrations of paclobutrazol, and hypocotyl length measurements were taken 6 d after germination. □, Wild-type (Landsberg *erecta*) seedlings; ●, *phyB-5* seedlings. Error bars indicate SDs of measurements. Between 14 and 30 seedlings were measured for each point.

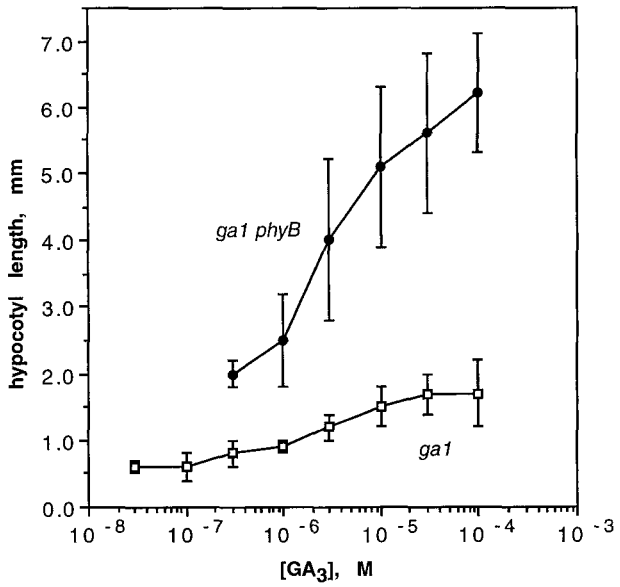

Figure 2. Hypocotyl elongation of *ga1-3* and *ga1-3 phyB-5* Arabidopsis seedlings in response to exogenous GA_3. Seeds were plated on Murashige-Skoog plates containing the indicated concentrations of GA_3, and hypocotyl length measurements were taken 7 d after germination. □, *ga1-3* seedlings; ●, *ga1-3 phyB-5* seedlings. Error bars indicate SDs of measurements. Between 6 and 29 seedlings were measured for each point.

Having established that GA is required for hypocotyl elongation in both wild-type and *phyB* seedlings, we wished to determine whether seedlings of the two genotypes responded differently to equal amounts of exogenous GAs. The *ga1-3* mutation (Koornneef et al., 1983; Sun et al., 1992) prevents GA biosynthesis almost entirely (Zeevaart and Talon, 1992). Figure 2 shows that a *ga1-3 phyB-5* double mutant elongated approximately 3 times as much as a *ga1-3* single mutant in response to exogenous GA_3. Both lines became taller as GA_3 concentrations in the medium increased from 3×10^{-7} to 10^{-4} M. Below 3×10^{-7} M GA_3, *ga1-3 phyB-5* seeds germinated poorly, preventing accurate measurement of hypocotyl lengths. We did not test medium concentrations of GA_3 above 10^{-4} M because of limited solubility of GA_3. We obtained similar results on plates containing Suc (data not shown).

We found that, as for the induction of seed germination (Derkx et al., 1994), GA_4 acted at much lower concentrations than GA_3 for stimulating hypocotyl elongation. As shown in Figure 3, for both genotypes hypocotyl lengths increased as GA_4 concentration in the medium increased from 10^{-9} to 3×10^{-8} M. At 3×10^{-8} M GA_4, the response for both genotypes appeared to saturate, because higher GA_4 concentrations caused no additional increase in hypocotyl length. These data show that the *phyB* mutation amplifies the elongation response of seedlings to exogenous GAs.

Since the *ga1-3* single-mutant hypocotyl is shorter than the *ga1-3 phyB-5* double-mutant hypocotyl at saturating concentrations of GA_4, the greater elongation of the *ga1-3 phyB-5* mutant probably does not arise from a higher level

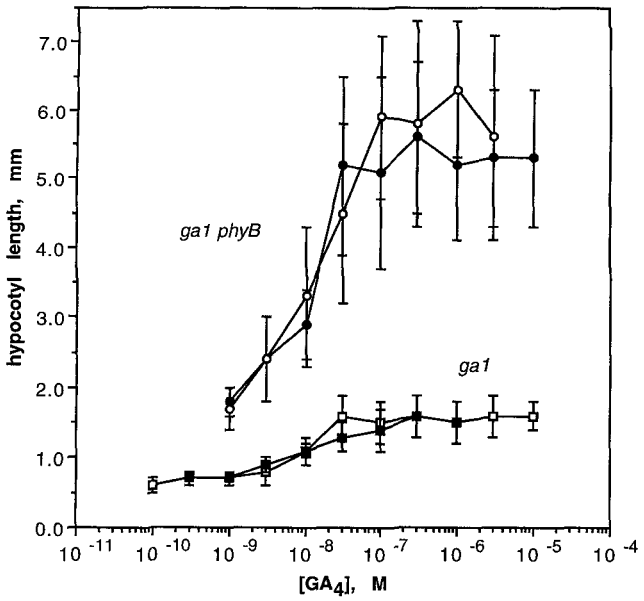

Figure 3. Hypocotyl elongation of *ga1-3* and *ga1-3 phyB-5* Arabidopsis seedlings in response to exogenous GA_4. Seeds were plated on Murashige-Skoog plates containing the indicated concentrations of GA_4, and hypocotyl length measurements were taken 7 d after germination. Results of two separate experiments are shown. Squares, *ga1-3* seedlings; circles, *ga1-3 phyB-5* seedlings. Error bars indicate SDs of measurements. Between 8 and 48 seedlings were measured for each point.

of active GA. Similarly, we have been unable to cause wild-type seedlings to elongate to the same extent as *phyB* mutant seedlings, even with high exogenous concentrations of GA (data not shown). Taken together, the paclobutrazol inhibition and GA feeding experiments show that GA responses are amplified in the *phyB* mutant background. These data suggest a model whereby GA is required for hypocotyl elongation and phyB acts to change the degree to which the hypocotyl elongates in response to active GA. In this model, phyB action might change a component of a GA-signaling pathway or, more likely, affect a distinct, non-GA-limited step in hypocotyl elongation.

Consistent with this model, we failed to detect significant differences in endogenous GA levels between wild-type and *phyB* seedlings. We previously found that the increased hypocotyl length of *phyB* mutant seedlings relative to that of wild-type seedlings arises primarily from increased cell elongation (Reed et al., 1993). Observation of hypocotyl lengths at various times after sowing revealed that the hypocotyls of *phyB* mutant seedlings elongate more quickly than those of the wild type for approximately 7 d after germination, after which time elongation of both strains slows markedly and the difference in hypocotyl elongation rate between wild-type and *phyB* seedlings becomes insignificant (data not shown). Therefore, to maximize the amount of tissue available for GA extractions, we measured GA levels after 7 to 8 d of growth, at the end of the period during which *phyB* seedlings elongate faster than wild-type seedlings.

In both wild-type and *phyB* seedlings we detected GAs in the early 13-hydroxylation pathway of GA synthesis: GA_{12}, GA_{53}, GA_{19}, GA_{20}, GA_1, and GA_8. Levels of all of these were similar in the two genotypes (Table I). The slightly higher GA_{20} and GA_8 levels in *phyB* than in wild-type seedlings were not statistically significant (Table I). Moreover, we found no difference in the level of the active GA of this pathway, GA_1.

GAs from the early C-3 hydroxylation pathway and the non-3,13-hydroxylation pathway have also been detected in samples of adult tissue of Arabidopsis (Talon et al., 1990a, 1990b). We attempted but failed to detect some of these in our seedling extracts, namely GA_4, GA_9, GA_{24}, GA_{25}, GA_{29}, GA_{34}, and GA_{36}. We also failed to detect GA_{17}

Table I. *GA levels in wild-type and phyB Arabidopsis seedlings*

GA	Experiment[b]	GA Level[a]				P[c]
		Wild type		*phyB*		
GA_1	1	2.5		1.3		
	2	1.3	1.6 ± 0.5	3.5	1.9 ± 0.5	0.82
	3	1.0		0.8		
GA_8	1	2.4		3.1		
	2	2.2	2.2 ± 0.1	4.6	3.8 ± 0.4	0.08
	3	1.9		3.8		
GA_{12}	1	14.5		15.6		
GA_{19}	1	2.1		2.3		
	2	3.9	3.0 ± 0.5	4.6	3.4 ± 0.7	0.10
	3	3.0		3.4		
GA_{20}	1	1.1		2.3		
	2	1.9	1.6 ± 0.3	5.2	3.5 ± 0.8	0.14
	3	1.9		2.8		
GA_{53}	1	0.5		0.4		

[a] Shown are measured levels of GAs (in ng g^{-1} dry weight), and means ± SE from the separate experiments. [b] Data from three separate experiments are shown. Tissue was harvested 7 d after germination in experiment 1 and 8 d after germination in experiments 2 and 3. [c] Probability values were calculated using a paired *t* test (df = 2). P represents the probability that the means of wild type and *phyB* measurements would differ by the amount that they do or more if the actual levels for wild-type and mutant tissue were the same. A P value of less than 0.05 would indicate a statistically significant difference.

and GA_{44} from the early 13-hydroxylation pathway. These differences from previous reports might reflect differences in GA metabolism between adult and seedling tissue or might be a consequence of the small amounts of tissue we extracted: 0.6 to 2.0 g dry weight compared with 42 to 84 g in the two previous studied (Talon et al., 1990a, 1990b).

Thus, we have found no evidence that the levels of GAs we did detect differ between wild-type and *phyB* seedlings. However, considering that we did not detect GA_4, and given the low level of exogenous GA_4 needed to induce hypocotyl elongation (see above), it is possible that phyB causes significant changes in GA_4 concentrations that we could not detect. We also did not investigate the possibility that phyB action might change the distribution of active GAs in seedlings. More detailed studies, perhaps with a larger plant than Arabidopsis, might answer these questions more definitively.

As discussed above, the simplest model to explain our data is that phyB affects responsiveness to GA. In this model, GA is required for hypocotyl elongation, and phyB action modulates the degree of GA-dependent elongation of the hypocotyl. This same model has also been deduced for the interaction of phyB-like phytochromes and GAs in pea (Weller et al., 1994) and in cucumber (López-Juez et al., 1995). Raskin and Kende (1984) proposed a similar model for the internode elongation response of submerged deepwater rice. These workers found that GA mediated this elongation and that ethylene (and light) affected the degree to which the stems elongated in response to GAs. In *Thlaspi arvense*, GA and light had distinct effects on petiole elongation: light conditions affected the degree of cell elongation, whereas GAs affected cell division but not cell elongation (Metzger, 1988). It will be interesting to learn whether, in the experiments in *Brassica*, sorghum, and tobacco in which changes in phytochrome activity affected GA levels (Rood et al., 1990a; Beall et al., 1991; Foster et al., 1994; Foster and Morgan, 1995; Jordan et al., 1995), the phenotypic consequences of altered phytochrome activity reflect changes in cell elongation as in the above cases.

The question remains whether other hormones mediate light responses. Both auxins (Law and Davies, 1990; Jones et al., 1991; Behringer and Davies, 1992) and brassinosteroids (Chory et al., 1991; Li et al., 1996; Szekeres et al., 1996) have been implicated in mediating phytochrome responses.

ACKNOWLEDGMENTS

We thank N. Olszewski for the gift of paclobutrazol, K. Oshima of Kyowa Hakko Kogyo for the gift of GA_4, and M. Chrisman for assisting with the GA analyses.

Received February 15, 1996; accepted May 25, 1996.
Copyright Clearance Center: 0032–0889/96/112/0337/06.

LITERATURE CITED

Beall FD, Morgan PW, Mander LN, Miller FR, Babb KH (1991) Genetic regulation of development in *Sorghum bicolor*. V. The ma_3^R allele results in gibberellin enrichment. Plant Physiol **95:** 116–125

Behringer FJ, Davies PJ (1992) Indole-3-acetic acid levels after phytochrome-mediated changes in the stem elongation rate of dark- and light-grown *Pisum* seedlings. Planta **188:** 85–92

Childs KL, Cordonnier-Pratt M-M, Pratt LH, Morgan PW (1992) Genetic regulation of development in *Sorghum bicolor*. VII. ma_3^R mutant lacks a phytochrome that predominates in green tissue. Plant Physiol **99:** 765–770

Chory J (1991) Light signals in leaf and chloroplast development: photoreceptors and downstream responses in search of a transduction pathway. New Biologist **3:** 538–548

Chory J, Nagpal P, Peto CA (1991) Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. Plant Cell **3:** 445–459

Cosgrove DJ (1994) Photomodulation of growth. *In* RE Kendrick, GHM Kronenberg, eds, Photomorphogenesis in Plants, Ed 2. Kluwer Academic, Dordrecht, The Netherlands, pp 631–658

Derkx MPM, Vermeer E, Karssen CM (1994) Gibberellins in seeds of *Arabidopsis thaliana*: biological activities, identification and effects of light and chilling on endogenous levels. Plant Growth Regul **15:** 223–234

Devlin PF, Rood SB, Somers DE, Quail PH, Whitelam GC (1992) Photophysiology of the elongated internode (*ein*) mutant of *Brassica rapa*. The *ein* mutant lacks a detectable phytochrome B-like polypeptide. Plant Physiol **100:** 1442–1447

Foster KR, Miller FR, Childs KL, Morgan PW (1994) Genetic regulation of development in *Sorghum bicolor*. VIII. Shoot growth, tillering, flowering, gibberellin biosynthesis and phytochrome levels are differentially affected by dosage of the ma_3^R allele. Plant Physiol **105:** 941–948

Foster KR, Morgan PW (1995) Genetic regulation of development in *Sorghum bicolor*. IX. The ma_3^R allele disrupts diurnal control of gibberellin biosynthesis. Plant Physiol **108:** 337–343

Goto N, Kumagai T, Koornneef M (1991) Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. Physiol Plant **83:** 209–215

Hedden P (1987) Gibberellins. *In* L Rivier, A Crozier, eds, Principles and Practice of Plant Hormone Analysis. Academic Press, Orlando, FL, pp 9–110

Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* locus of Arabidopsis alter gibberellin signal transduction. Plant Cell **5:** 887–896

Jones AM, Cochran DS, Lamerson PM, Evans ML, Cohen JD (1991) Red light regulated growth. I. Changes in the abundance of indoleacetic acid and a 22-kilodalton auxin-binding protein in the maize mesocotyl. Plant Physiol **97:** 352–358

Jordan ET, Hatfield PM, Hondred D, Talon M, Zeevaart JAD, Vierstra RD (1995) Phytochrome A overexpression in transgenic tobacco. Correlation of dwarf phenotype with high concentrations of phytochrome in vascular tissue and attenuated gibberellin levels. Plant Physiol **107:** 797–805

Koornneef M, Rolff E, Spruit CJP (1980) Genetic control of light-inhibited hypocotyl elongation in *Arabidopsis thaliana* (L.) Heynh. Z Pflanzenphysiol **100:** 147–160

Koornneef M, Van Eden J, Hanhart CJ, De Jongh AMM (1983) Genetic fine-structure of the *GA-1* locus in the higher plant *Arabidopsis thaliana* (L.) Heynh. Genet Res **41:** 57–68

Lanahan MB, Ho DTH (1988) *Slender* barley: a constitutive gibberellin-response mutant. Planta **175:** 107–114

Law DM, Davies PJ (1990) Comparative indole-3-acetic acid levels in the slender pea and other pea phenotypes. Plant Physiol **93:** 1539–1543

Li J, Nagpal P, Vitart V, McMorris T, Chory J (1996) A role for brassinosteroids in light-dependent development of Arabidopsis. Science **272:** 398–401

Liscum E, Hangarter RP (1993) Genetic evidence that the Pr form of phytochrome B mediates gravitropism in *Arabidopsis thaliana*. Plant Physiol **103:** 15–19

López-Juez E, Kobayashi M, Sakurai A, Kamiya Y, Kendrick RE (1995) Phytochrome, gibberellins, and hypocotyl growth. A study using the cucumber (*Cucumis sativus* L.) *long hypocotyl* mutant. Plant Physiol **107:** 131–140

López-Juez E, Nagatani A, Tomizawa KI, Deak M, Kern R, Kendrick RE, Furuya M (1992) The cucumber long hypocotyl mu-

tant lacks a light-stable PHYB-like phytochrome. Plant Cell **4:** 241–251

Mattheussen AM, Morgan PW, Frederiksen RA (1991) Implication of gibberellins in head smut (*Sporisorium reilianum*) of *Sorghum bicolor*. Plant Physiol **96:** 537–544

Metzger JD (1988) Gibberellins and light regulated petiole growth in *Thlaspi arvense* L. Plant Physiol **86:** 237–240

Mullet JE (1988) Chloroplast development and gene expression. Annu Rev Plant Physiol **39:** 475–502

Nagatani A, Chory J, Furuya M (1991) Phytochrome B is not detectable in the *hy3* mutant of *Arabidopsis*, which is deficient in responding to end-of-day far-red light treatments. Plant Cell Physiol **32:** 1119–1122

Potts WC, Reid JB, Murfet IC (1985) Internode length in *Pisum*. Gibberellins and the slender phenotype. Physiol Plant **63:** 357–364

Quail PH, Briggs WR, Chory J, Hangarter RP, Harberd NP, Kendrick RE, Koornneef M, Parks B, Sharrock RA, Schäfer E, Thompson WF, Whitelam GC (1994) Spotlight on phytochrome nomenclature. Plant Cell **6:** 468–471

Rademacher W (1989) Gibberellins: metabolic pathways and inhibitors of biosynthesis. *In* P Boger, G Sandmann, eds, Target Sites of Herbicide Action. CRC Press, Boca Raton, FL, pp 128–140

Rademacher W (1991) Inhibitors of gibberellin biosynthesis: applications in agriculture and horticulture. *In* N Takahashi, B Phinney, J MacMillan, eds, Gibberellins. Springer-Verlag, New York, pp 296–310

Raskin I, Kende H (1984) Role of gibberellin in the growth response of submerged deep water rice. Plant Physiol **76:** 947–950

Reed JW, Nagatani A, Elich TD, Fagan M, Chory J (1994) Phytochrome A and phytochrome B have overlapping but distinct functions in *Arabidopsis* development. Plant Physiol **104:** 1139–1149

Reed JW, Nagpal P, Poole DS, Furuya M, Chory J (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. Plant Cell **5:** 147–157

Reid JB, Ross JJ, Swain M (1992) Internode length in *Pisum*. A new, slender mutant with elevated levels of C_{19} gibberellins. Planta **188:** 462–467

Rood SB, Williams PH, Pearce D, Murofushi N, Mander LN, Pharis RP (1990a) A mutant gene that increases gibberellin production in *Brassica*. Plant Physiol **93:** 1168–1174

Rood SB, Zanewich KP, Bray DF (1990b) Growth and development of *Brassica* genotypes differing in endogenous gibberellin content. II. Gibberellin content, growth analyses and cell size. Physiol Plant **79:** 679–685

Ross JJ (1994) Recent advances in the study of gibberellin mutants. Plant Growth Regul **15:** 193–206

Sharrock RA, Quail PH (1989) Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Dev **3:** 1745–1757

Shinomura T, Nagatani A, Chory J, Furuya M (1994) The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B, and secondarily by phytochrome A. Plant Physiol **104:** 363–371

Sun T-P, Goodman HM, Ausubel FM (1992) Cloning the Arabidopsis *GA1* locus by genomic subtraction. Plant Cell **4:** 119–128

Szekeres M, Németh K, Koncz-Kalman Z, Mathur J, Kauschmann A, Altmann T, Rédei G, Nagy F, Schell J, Koncz C (1996) Brassinosteroids rescue the deficiency of CYP90, a cytochrome P450, controlling cell elongation and de-etiolation in Arabidopsis. Cell **85:** 171–182

Talon M, Koornneef M, Zeevaart JAD (1990a) Accumulation of C_{19}-gibberellins in the gibberellin-insensitive dwarf mutant *gai* of *Arabidopsis thaliana* (L.) Heynh. Planta **182:** 501–505

Talon M, Koornneef M, Zeevaart JAD (1990b) Endogenous gibberellins in *Arabidopsis thaliana* and possible steps blocked in the biosynthetic pathways of the semidwarf *ga4* and *ga5* mutants. Proc Natl Acad Sci USA **87:** 7983–7986

Talon M, Zeevaart JAD, Gage DA (1991) Identification of gibberellins in spinach and effects of light and darkness on their levels. Plant Physiol **97:** 1521–1526

Thompson WF, White MJ (1991) Physiological and molecular studies of light-regulated nuclear genes in higher plants. Annu Rev Plant Physiol Plant Mol Biol **42:** 423–466

Weller JL, Nagatani A, Kendrick RE, Murfet IC, Reid JB (1995) New *lv* mutants of pea are deficient in phytochrome B. Plant Physiol **108:** 525–532

Weller JL, Reid JB (1993) Photoperiodism and photocontrol of stem elongation in two photomorphogenic mutants of *Pisum sativum* L. Planta **189:** 15–23

Weller JL, Ross JJ, Reid JB (1994) Gibberellins and phytochrome regulation of stem elongation in pea. Planta **192:** 489–496

Whitelam GC, Smith H (1991) Retention of phytochrome-mediated shade avoidance responses in phytochrome-deficient mutants of *Arabidopsis*, cucumber and tomato. J Plant Physiol **139:** 119–125

Wilson RN, Heckman JW, Somerville CR (1992) Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol **100:** 403–408

Zeevaart JAD, Gage DA (1993) *ent*-Kaurene biosynthesis is enhanced by long photoperiods in the long-day plants *Spinacia oleracea* L. and *Agrostemma githago* L. Plant Physiol **101:** 25–29

Zeevaart JAD, Talon M (1992) Gibberellin mutants in *Arabidopsis thaliana*. *In* CM Karssen, LC van Loon, D Vreugdenhil, eds, Progress in Plant Growth Regulation. Kluwer Academic, Dordrecht, The Netherlands, pp 34–42