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The phytochrome family: dissection of functional roles and signalling pathways among family members

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There is considerable evidence that individual members of the five-membered phytochrome family of photoreceptors in *Arabidopsis* have differential functional roles in controlling plant photomorphogenesis. Emerging genetic evidence suggests that this differential activity may involve initially separate signalling pathway branches specific to individual family members.

Keywords: phytochromes; sensory photoreceptors; signalling pathways; photomorphogenesis; seedling development; *Arabidopsis* phy mutants

1. INTRODUCTION

The diversity and complexity of photoinduced plant responses attributed to the phytochrome (phy) photoreceptor system has for a long time suggested to physiologists and photobiologists that several phytochromes with differential activities are necessary to rationalize the observed phenomena (Smith & Whitelam 1990; Quail 1991, 1994*b*; Smith 1994; Pratt 1995). The discovery of a five-membered family of phytochromes (designated phyA to phyE) in *Arabidopsis* (Sharrock & Quail 1989; Clack *et al.* 1994), and subsequent studies with mutants defective in one or more individual members of the family, have provided evidence that at least some members of the family do indeed have differential (albeit sometimes partly overlapping) photosensory and/or physiological roles in controlling plant growth and development (Smith & Whitelam 1990; Quail *et al.* 1994, 1995, 1996; Smith 1994; Whitelam & Harberd 1994; Whitelam & Devlin 1997). However, the mechanism(s) by which this differential activity is achieved is unresolved. In a simplistic, formal sense, two contrasting possibilities can be envisioned (figure 1).

1. Each phy could interact with the same, single molecular partner, X, in the process of signal transfer following activation of the photoreceptor (model 1). All downstream signal transduction would then be common to all phytochromes.
2. Each phy could interact with a different molecular partner (X, V, T, R, P in figure 1) in effecting signal transfer (model 2). In this case, one or more signal transduction steps would be specific to each phytochrome, with downstream convergence of the individual pathways leading to any given common response.

In model 1, differential activity among family members could be achieved by qualitative or quantitative differential expression patterns, spatially and/or temporally within the plant or the cell. In model 2, the sequence differences between the phytochromes could permit specific interaction with different cognate reaction partners at the apex of at least initially separate signalling pathways in the absence of differential phy expression patterns. In either model, the biochemical mechanism of signal transfer to the reaction partner could be either the same (e.g. phosphorylation of the partner) or different for each phy family member. Here, I briefly review the principal evidence that individual phytochromes have differential functional roles in mediating light-regulated growth and development, and discuss the accumulating genetic evidence that at least some of the individual phytochromes use initially separate signalling pathway branches.

2. DIFFERENTIAL PHOTOSENSORY AND PHYSIOLOGICAL ROLES AMONG PHY FAMILY MEMBERS

Analysis of *Arabidopsis* mutants carrying mutations in the structural genes for phyA and phyB provided the first compelling evidence that these two phytochromes have contrasting photosensory functions in controlling seedling de-etiolation (figure 2). The data indicate that phyA is exclusively responsible for continuous, monochromatic, far-red light (FRc)-driven de-etiolation through the FR high-irradiance response (FR-HIR), whereas phyB is predominantly responsible for continuous, monochromatic, red light (Rc)-driven de-etiolation through the R-HIR (Quail *et al.* 1995; Whitelam & Devlin 1997). Furthermore, in early seedling development these two phytochromes act antagonistically in response to varying ratios of Rc to FRc light in the environment (Quail *et al.* 1995; Smith *et al.* 1997). Rc-enrichment stimulates phyB activity, while negating phyA FR-HIR activity. Conversely, FRc-enrichment stimulates phyA activity, while

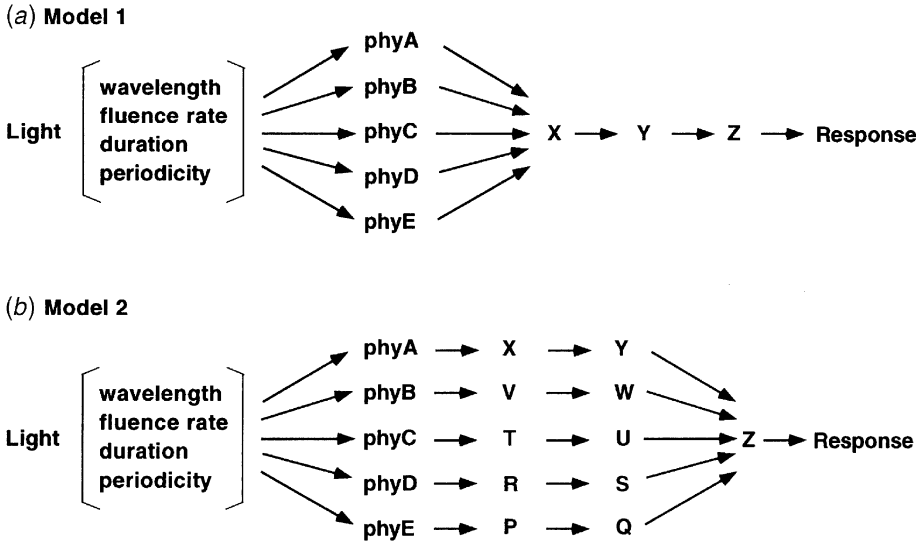

Figure 1. Simplistic alternative models of possible phytochrome (phy) signal transduction mechanisms. Each model indicates that individual members of the phytochrome family (phyA–E) perceive and interpret various informational parameters in impinging light signals (wavelength, fluence rate, duration, periodicity), and transduce these signals downstream to cellular signal transduction components (P–Z), culminating in a monitorable response. (*a*) In model 1, each phy interacts with the same initial component of the transduction chain, X, resulting in immediate convergence of the signalling pathways. (*b*) In model 2, each phy interacts with a different primary transduction component (P, R, T, V, X) leading to initially separate branches of the transduction pathway for each phy and downstream convergence of the pathways leading to common responses (e.g. altered hypocotyl cell elongation).

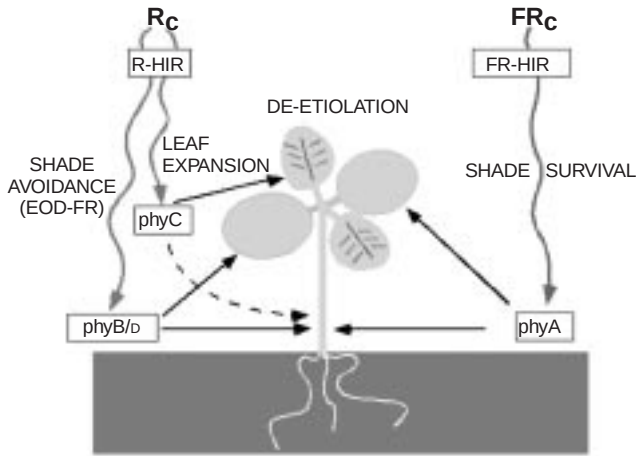

Figure 2. Schematic summary of the differential functional roles of phytochromes A, B, C, and D in controlling *Arabidopsis* seedling photomorphogenesis. phyA functions in FRc perception, inhibiting hypocotyl elongation and stimulating cotyledon expansion via the FR-HIR. This activity enhances young seedling survival on emergence into a vegetatively shaded, FRc-rich environment. phyB, phyC, and phyD mediate responses to Rc via the R-HIR. phyB and phyD inhibit hypocotyl elongation and stimulate cotyledon expansion, with phyB playing the most dominant role. Antagonism of the Rc effect by FRc-enrichment in vegetatively shaded environments leads to the shade avoidance response. End-of-day FR pulses (EOD-FR) have a similar effect by antagonizing the night effects of residual Pfr remaining from the light period. phyC may have a relatively minor effect on inhibition of hypocotyl elongation, but, in contrast to phyA, phyB, and phyD, may have a differential role in stimulating primary leaf expansion.

negating phyB activity. The net outcome of this mutual antagonism is a function of the relative levels of phyA and phyB in the plant. Initially, phyA dominates, providing 'shade survival' capacity to newly emergent seedlings in FRc-enriched, vegetatively shaded environments (Yanovsky *et al.* 1995). However, because of its light-lability, phyA rapidly declines to relatively ineffective levels. phyB is left to dominate in fully de-etiolated plants, where it is the main species responsible for the shade avoidance response (Smith & Whitelam 1997; Whitelam & Devlin 1997). phyD, which is closely related to phyB in sequence (Clack *et al.* 1994), also appears to have similar photosensory and regulatory activity to phyB, but is a minor contributor relative to phyB (Aukerman *et al.* 1997).

The potential role of phyC in seedling de-etiolation has thus far only been inferred from the effects of overexpression in transgenic plants. No monogenic mutants of phyC have yet been reported. Overexpression of phyC in transgenic *Arabidopsis* confers moderately enhanced sensitivity to Rc as regards hypocotyl growth inhibition, but has no detectable effect on sensitivity to FRc (figure 2; Qin *et al.* 1997). These results suggest that phyC has photosensory specificity similar to phyB, but distinct from phyA. On the other hand, the absence of a detectable effect of overexpressed phyC on cotyledon expansion indicates a difference from overexpressed phyB which enhances cotyledon expansion in Rc (Wagner *et al.* 1997). Furthermore, whereas phyA and phyB overexpressors show no enhancement of primary leaf expansion, phyC overexpressors do show enhancement (Qin *et al.* 1997). Overexpression of phyC in transgenic tobacco also enhances leaf expansion, as well as cotyledon expansion, but has no detectable effect on hypocotyl elongation (Halliday *et al.* 1997).

Taken together, these data suggest that phyA, phyB, and phyC each have a differential role in controlling seedling photomorphogenesis, with phyD having a relatively minor role, more or less additive with that of phyB. In addition, clear differences in the roles of phyA and phyB in the control of other stages of development, including flowering (Halliday *et al.* 1994; Johnson *et al.*

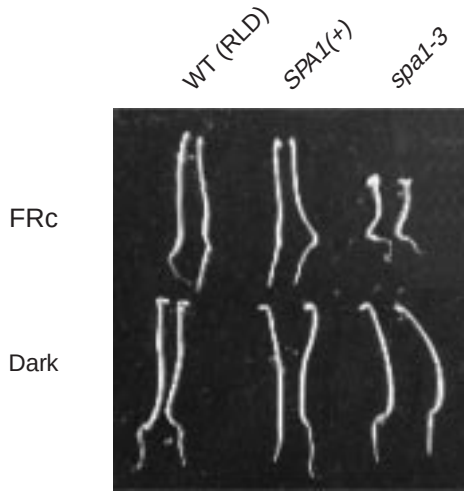

Figure 3. De-etiolation in the *spa1 Arabidopsis* mutant is light-dependent and hypersensitive to FRc. Seedlings were grown in FRc ($1\ \mu mol\ m^{-1}\ s^{-1}$) or darkness for 3 days. Seedling genotypes: WT (RLD), wild-type, RLD ecotype; *SPA1*(+), progeny of a wild-type sibling in a segregating F2 population used for isolation of the homozygous *spa1* mutant; *spa1-3*, homozygous mutant carrying allele number 3 at the *SPA1* locus.

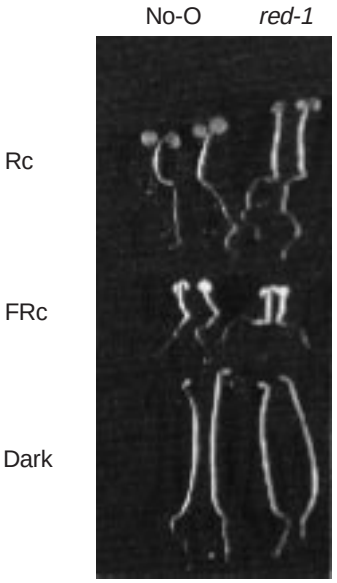

Figure 4. De-etiolation in the *red1 Arabidopsis* mutant is specifically hyposensitive to Rc. Seedlings were grown for three days either in darkness, Rc ($80\ \mu mol\ m^{-2}\ s^{-1}$) or FRc ($12\ \mu mol\ m^{-2}\ s^{-1}$). Seedling genotypes: No-O, wild-type, No-O ecotype; *red1*, homozygous mutant at the *RED1* locus in a wild-type (nontransgenic) No-O background.

1994) and seed germination (Botto *et al*. 1996; Shinomura *et al*. 1996; Poppe & Schäfer 1997) have been documented. The recent isolation of phyE mutants (G. C. Whitelam, personal communication) will significantly expand our understanding of the individual functions of and interactions between the phytochrome family members.

3. GENETICALLY SEPARABLE SIGNALLING-PATHWAY SEGMENTS AMONG PHY FAMILY MEMBERS

Genetic approaches to signalling-intermediate identification in *Arabidopsis* have led to the isolation of a series of mutants, including *hy5*, and the *cop–det–fus* class, defective in components that act very early in the pathway controlling seedling photomorphogenesis (Koornneef *et al*. 1980; Deng 1994; Quail 1994*a*; Chory *et al*. 1996; von Arnim & Deng 1996; Wei & Deng 1996). However, the existing evidence has been interpreted to suggest that these components act at or downstream of the convergence of both phytochrome and blue-light photoreceptor signalling pathways (McNellis & Deng 1995; Chory *et al*. 1996; Wei & Deng 1996). In addition, microinjection and pharmacological studies with the phytochrome-deficient aurea mutant of tomato have provided evidence for the involvement of G-proteins, Ca^{2+}–calmodulin and cGMP in both phyA and phyB activity (Neuhaus *et al*. 1993, 1997; Bowler *et al*. 1994; Millar *et al*. 1994; Kunkel *et al*. 1996; Schäfer *et al*. 1997).

Evidence for the existence of signalling intermediates specific to individual phytochromes was first presented by Whitelam and co-workers (1993). These authors isolated two recessive, *Arabidopsis* non-photoreceptor mutants, *fhy1* and *fhy3*, lacking responsiveness to FRc, but apparently normal in Rc responsiveness. These mutants thus potentially represent components specifically necessary for phyA signalling. More recently, we have used a different genetic strategy to identify phyA-pathway-specific components by screening for extragenic suppressors of a previously identified weak phyA mutant, *phyA-105* (Xu *et al*. 1995; Hoecker *et al*. 1998). From this screen, five recessive alleles at a new mutant locus, designated *spa1*, were identified (see figure 3) and mapped to the bottom of chromosome 2 (Hoecker *et al*. 1998). These mutants exhibit enhanced responsiveness to FRc in both the parental *phyA-105* and wild-type *PHYA* backgrounds, but not in a *phyA* null mutant background. They also have enhanced responsiveness to Rc, but this effect is dependent on wild-type phyA as it is absent in a *phyA* null background. The *spa1* mutant seedlings are like wild-type when grown in darkness (figure 3). The data indicate, therefore, that the *spa1* mutant phenotype is light-dependent, that phyA is necessary for this phenotype, and that the other phytochromes (phyB, C, D, and E) are not sufficient for expression of this phenotype. Together with the recessive nature of the *spa1* mutations, these data suggest that the wild-type SPA1 gene-product normally acts to negatively regulate phyA-specific signalling and that *spa1* mutations specifically amplify the phyA signalling pathway.

Evidence for Rc-specific signalling components has come from two separate studies. In a screen for early flowering *Arabidopsis* mutants, Ahmad & Cashmore (1996) identified two lines, *pef2* and *pef3* that also exhibit reduced seedling responsiveness to Rc, but wild-type responsiveness to FRc. In a screen for extragenic suppressors of a phyB-overexpressor phenotype, we isolated a mutant, designated *red1*, that also has reduced seedling responsiveness to Rc, but not FRc, whether in the transgenic phyB-overexpressor or wild-type phyB background (figure 4) (Wagner *et al*. 1997). Because phyB has been shown to be the main photoreceptor mediating Rc effects on seedling morphogenesis (Quail *et al*. 1995; Smith & Whitelam 1997; Whitelam & Devlin 1997), it is suggested

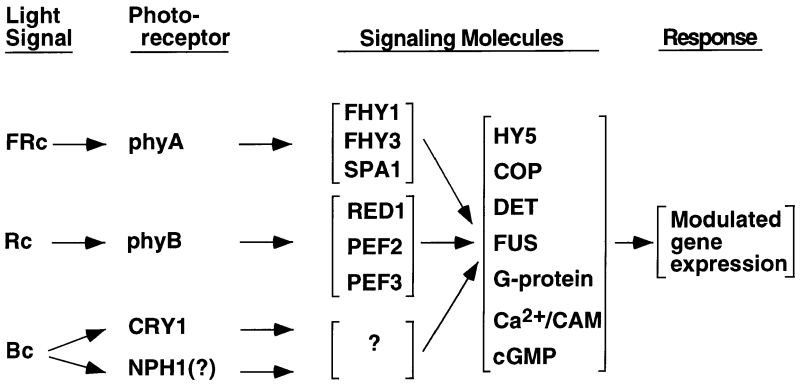

Figure 5. Simplified scheme summarizing postulated signalling pathways for phyA, phyB, and the blue-light (Bc) photoreceptors CRY1 and, potentially, NPH1. Potential signalling components specific to the phyA pathway (FHY1, FHY3, and SPA1), and specific to the phyB pathway (RED1, PEF2, and PEF3), as well as those apparently downstream of the convergence of the phyA and phyB and possibly blue-light pathways (HY5, COP, DET, FUS, G-protein, Ca^{2+}–calmodulin, cGMP) are indicated.

that the *pef2*, *pef3*, and *red1* mutants may represent components necessary for phyB-specific signalling.

Taken together, these genetic data support the notion of initially separate phyA and phyB signalling pathway segments, each involving up to at least three components, presumably upstream of the HY5, COP–DET–FUS and second messenger components considered common to these pathways (figure 5). At face value, then, the data are more consistent with model 2 in figure 1 than with model 1. However, it is not unlikely that the picture will ultimately prove to be much more complex, including the possibility of several reaction partners for each phy, and interaction and cross-talk between pathways. It is hoped that the molecular cloning of the phyA- and phyB-specific loci described here will soon provide significant insight into this question.

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