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Commentary

Higher-plant phytochrome: "I used to date histidine, but now I prefer serine"

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We all know that plants harvest light energy via the process of photosynthesis. Not quite so widely appreciated is that in addition to the chlorophylls and other light-absorbing pigments associated with the photosynthetic apparatus, plants use a separate class of photoreceptors to enable them to sense both the quality and quantity of light in the surrounding environment. In these respects plants can see, in a manner not totally distinct from you and I. In response to this information, they adjust their growth habits accordingly and hence maximize their capacity to use radiant energy.

The primary photoreceptors of higher plants are the phytochromes and cryptochromes—quite distinct from the rhodopsins that animals use for vision. Phytochrome mediates responses to red/far-red light through a tetrapyrolle chromophore (1, 2), whereas the cryptochromes respond to blue/UV-A light through a flavin (3, 4). An important and distinguishing feature of the phytochrome light-sensing system is that it measures, not the absolute amount of red/far-red light, but the ratio of the amount of light (the number of photons) corresponding to these two regions of the spectrum. Phytochrome acts as a light-regulated molecular switch, with the capacity to undergo repeated interconversion between the red light-absorbing phytochrome (Pr) form and the far-red light-absorbing phytochrome (Pfr).

Whereas it has been generally agreed that Pfr is the active form of phytochrome, the actual biochemical nature of this activity has remained unclear and a matter of some controversy. This, in spite of almost a half century of intense research. The work described by Yeh and Lagarias (5) in this issue of the *Proceedings* sheds some light on this question, and in the eyes of many, it will be argued that the basic question concerning the general activity of phytochrome has now been laid to rest.

From early studies, it had been speculated that phytochrome may function as a light-activated enzyme (6); however determining the identity of this enzymatic activity has proven difficult. The first evidence that phytochrome may be a protein kinase came from the laboratory of Lagarias. They showed in a series of publications that phytochrome preparations possessed serine/threonine protein kinase activity (7–9). This activity was stimulated by polycations and strongly inhibited by pyrophosphate. The phytochrome-associated kinase was active with respect to phosphorylation of phytochrome and also was capable of phosphorylating histone H1.

These early indications that phytochrome may be a protein kinase met with considerable skepticism—this response reflecting the fact that phytochrome showed no obvious sequence homology to the known superfamily of serine/threonine/tyrosine kinases (10). Whereas novel protein kinases have now been identified (see ref. 11 and references therein), the obvious question was raised: How does one distinguish the possibility that phytochrome itself may be a kinase, from a contaminant that copurifies with phytochrome? Indeed, two groups independently reported that protein kinase activity could be removed from phytochrome by extensive purification (12, 13). The Lagarias laboratory countered by noting that the properties of this separated kinase were not the same as they had characterized for their phytochrome preparations (14). During these investigations, Lagarias was careful not to claim that they had proven that phytochrome was a protein kinase—at a minimum they had demonstrated that there was a tightly associated protein kinase activity of unusual properties.

This question took on new life when it was shown that the phytochrome C-terminal domain showed considerable sequence-relatedness to bacterial sensory kinases (15, 16). However the significance of this observation was not immediately clear because the bacterial sensor proteins are histidine kinases, whereas the activity associated with phytochrome was that of a serine/threonine kinase. Furthermore, the histidine residue that is commonly conserved in the bacterial kinases is not generally found in phytochromes, and site-directed mutation of another histidine, as well as other residues shared between phytochrome and the bacterial sensor proteins, appeared not to affect phytochrome activity (17, 18).

The next chapter in this story came with the sequencing of genes from two cyanobacteria. The *rcaE* gene from *Fremyella diplosiphon* was shown by Kehoe and Grossman (19) to have some sequence relatedness to both bacterial histidine kinases and to higher plant phytochromes. Furthermore, additional phytochrome-like genes were identified in the genome of *Synechocystis* (20, 21). Expression of a *Synechocystis* phytochrome-like gene in *Escherichia coli* was demonstrated by Hughes *et al.* (20) to provide a readily soluble protein molecule that would bind phycocyanobilin, a tetrapyrolle similar to the chromophore bound by higher-plant phytochromes. Significantly, this reconstituted protein was photochromic: its absorption properties were differentially altered by prior irradiation with red or far-red light. Furthermore, this cyanobacterial phytochrome molecule was shown by Yeh *et al.* (21) to undergo autophosphorylation, generating an acid-labile phosphate residue suggestive of histidine kinase activity. The Pr form of this phytochrome (Cph1) was more active in autophosphorylation than was the Pfr form—this observation being in contrast to the normal assumption that it is the Pfr form of higher plant phytochrome that is the active species. Yeh *et al.* (21) identified an adjacent *Synechocystis* gene encoding a response regulator, Rcp1. This response regulator was phosphorylated by the Pr form of Cph1. This phosphotransfer was lacking in both a mutant of Cph1 lacking a conserved histidine residue and a mutant of Rcp1 lacking a conserved aspartate. In these respects—autophosphorylation on a histidine residue followed by phosphotransfer to an aspartate—these cyanobacterial proteins are acting as a typical two-component regulatory system, with the phytochrome moiety acting as a light-regulated sensory histidine kinase (18, 22, 23).

Abbreviations: Pr, red light-absorbing phytochrome; Pfr, far-red light-absorbing phytochrome.

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The latest step in this continuing saga has come again from the laboratory of Lagarias (5). In this publication, the authors have used a yeast expression system designed to obtain highly purified samples of oat phyA (24). This apoprotein was converted to holoprotein by addition of chromophore, which autocatalytically undergoes covalent attachment. The availability of highly purified phytochrome, expressed not from plants but from yeast, allowed one to ask the obvious question: Does this phytochrome, purified from an alternate source, possess protein kinase activity? As convincingly demonstrated by Yeh and Lagarias, the answer to this question is clearly yes.

In these latest studies, the authors first demonstrated that phytochromes expressed from genes from both oat and the green alga *Mesotaenium caldariorum*, displayed kinase activity on expression from either of the yeasts *Saccharomyces cerevisiae* or *Pichia pastoris*. Both of these phytochrome preparations underwent autophosphorylation. The Pfr forms were more active with respect to autophosphorylation than were the Pr forms—contrasting with the finding for the cyanobacterial phytochrome. Both the higher plant phytochrome and the algal phytochrome phosphorylated histone H1—in this case the activity was the same for both Pr and Pfr. Furthermore, histone H1 stimulated the autophosphorylation of phytochrome, particularly the Pr form. Also, as in the case of the earlier studies with phytochrome purified from oat, the protein kinase activity was strongly inhibited by pyrophosphate.

The authors then addressed the following question. They argued that if the phosphorylation by phytochrome represented a contaminating kinase, then such a reaction might be expected to show bimolecular kinetics—conversely, intramolecular phosphorylation by phytochrome should result in a linear increase in reaction rate in response to increasing concentration of protein. Indeed, the latter was the result obtained, ostensibly strongly supporting the idea that phytochrome itself is the source of the kinase activity. I say ostensibly, in the sense that the only alternative model that we are entertaining here is that there is an associated kinase, so tightly bound that it cannot be readily separated from native phytochrome—as noted by the authors, presumably such a kinase activity also would show kinetics of an intramolecular reaction.

As noted, plant phytochrome shows sequence relatedness to bacterial histidine kinases, and cyanobacterial phytochrome has the enzymatic properties of such a kinase. Indeed, higher plant phytochrome contains not one, but two regions within its C terminus that show sequence relatedness to the signal-transducing domain of the cyanobacterial phytochrome Cph1 (5, 25). Because both of these regions show a similar degree of sequence relatedness to Cph1 as they do to one another, it would appear that these regions of phytochrome diverged at a very early stage in the evolution of the higher plant molecule.

In their final experiment, the authors asked if phytochrome would phosphorylate Rcp1, the response regulator substrate of the cyanobacterial phytochrome. The answer to this question was an unequivocal yes. Both Pr and Pfr oat phytochrome phosphorylated Rcp1. This phosphorylation was not on aspartate residues—the normal substrate for transphosphorylation by sensory histidine kinases—but once again higher plant phytochrome was seen to act as serine/threonine protein kinase.

So what do we conclude from these elegant series of experiments. The first question we need to ask is the following: Has it now been convincingly demonstrated that higher plant phytochrome really is a protein kinase—was Lagarias right all along with his persistent claims that phytochrome has the properties of a protein kinase? Well, what is the alternative explanation for the reported observations? That there is a tightly associated kinase activity that copurifies with phytochrome isolated from oat, from green algae, and two species of yeast. This tightly associated hypothetical protein kinase would need to have similar properties, apparently irrespective of the source—these properties include phosphorylation of phytochrome in a light- and chromophore-dependent manner and inhibition by pyrophosphate. This hypothetical contaminating kinase is apparently undetectable on SDS/PAGE. These are all possibilities; however, using the law of parsimony, it seems clear that for the time being a more attractive hypothesis is the one emanating from the Lagarias laboratory.

So what needs to be done? For my money, the "final proof" (if there is such a thing) would be to characterize the phytochrome-binding site for ATP and demonstrate that there is an appropriate correlation between mutations affecting ATP binding, phytochrome phosphorylation, and biological activity. Here it is noted that present indications are that this presumptive ATP-binding site may be unrelated to that used by the bacterial histidine kinases (9, 17). Concerning a related matter, it could be argued that higher plant phytochrome retains histidine kinase activity, the phosphate being rapidly transferred onto serine (22). If this turns out to be not the case, then we will be left with the following question: What then is functionally conserved between these two classes of molecules, which show clear sequence relatedness but apparently relatively little functional conservation? Whatever the eventual outcome is in reference to these questions, it is abundantly clear that there are some fascinating structural and mechanistic details to be sorted out in order for us to fully appreciate the contortions that phytochrome has undergone in the course of its evolutionary history.

Another question that needs addressing is to characterize biological substrates for the phytochrome kinase activity. In my laboratory, we have demonstrated that cryptochrome is a substrate for phosphorylation by phytochrome (26). Whereas, as always, it is difficult to prove the biological relevance of this observation, we know from other studies that for many cryptochrome responses there is a strong requirement for phytochrome (27, 28). Furthermore we demonstrated, both in yeast and *in vitro*, that phytochrome interacted with cryptochrome, an observation consistent with the notion that the observed kinase activity is indeed an inherent property of phytochrome A (26).

In the future it will be interesting to determine if partners for phytochrome that are being isolated by the yeast two-hybrid assay are substrates for phosphorylation by phytochrome. One class of substrate may be the higher plant homologs of the bacterial histidine kinase response regulators. Such regulators have been described for *Arabidopsis* (29), and other two-component regulators have been described for higher plants (30). Eventually, to accommodate the kinase hypothesis into the current model that argues that Pfr is the active species, it will be necessary to find a phytochrome substrate that is more readily activated by Pfr than by Pr.

In conclusion, a reasonable bet is that the current paper will be seen to represent a milestone in phytochrome research. And rumor has it that if you listen carefully while flying over Davis, you can hear a whispered, "see—I told you so."

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