Plant biochemistry

Phytochrome feedback

from G.C. Whitelam

PHYTOCHROME is the photoreceptor which regulates almost all aspects of plant development including germination, seedling development, flowering and adaptation to the light environment. The molecular mechanisms underlying its action have yet to be elucidated but several examples of phytochrome-regulated gene expression have been recognized[1-3]. A particularly intriguing example was described recently: phytochrome exerts autoregulatory control over its own translatable mRNA level. The finding was one of a number of significant new insights into the nature of the phytochrome molecule to be reported at the European Symposium on Photomorphogenesis in Plants*.

Phytochrome is a soluble protein existing in two forms — a red-light-absorbing form (Pr, λ_{max} = 666 nm) and a far-red-light-absorbing form (Pfr, λ_{max} = 730 nm) — which are converted into one another by light of suitable frequency. Photoconversion of Pr to Pfr induces a diverse array of morphogenic responses, and induction is halted when Pfr is converted back to Pr. Pfr is thus often considered to be the active form and Pr the inactive form of phytochrome.

It has been held that phytochrome is synthesized *de novo* as Pr at a constant rate and accumulates in dark-grown plant tissue. The rapid decline in phytochrome concentration seen on photoconversion of Pr to Pfr has been believed to be the result of the very much greater rate of Pfr degradation relative to Pr. The low steady-state concentration of phytochrome in continuous light, characteristically 1–3 per cent of the initial dark-grown tissue level, has thus been accounted for solely in terms of the differential turnover rates of Pr and Pfr: a slower rate of Pr degradation and a more rapid rate of Pfr degradation against a background of constant Pr synthesis.

New work reported from Peter Quail's group in Madison, Wisconsin now clearly shows that there is another level of control. In a rabbit reticulocyte lysate *in vitro* translation system, the translatable phytochrome mRNA accounts for only about 0.005 per cent of the poly(A) RNA from dark-grown oat shoots. As little as five seconds' exposure to red light induces a marked and rapid reduction in the level after about 15 min and a 95 per cent reduction of phytochrome mRNA after only 2 h. Control experiments show that this represents a selective decrease in translatable phytochrome mRNA. Partial reversal of the red-light-induced decline by subsequent far-red light indicates that phytochrome itself is the photoreceptor. Only partial reversibility is possible since the 1 per cent Pfr level established by far-red light alone is sufficient to induce a significant response. As Pfr becomes depleted in extended periods of darkness the feedback control appears to be reversed since translatable phytochrome mRNA re-accumulates. The rapidity of the regulation, with a lag of only 15 min, makes this one of the most promising systems for the elucidation of the transduction chain between receptor and gene expression.

An obvious consequence of the demonstration of the autoregulatory control mechanism is that current concepts regarding the control of phytochrome levels *in vivo* will have to be revised. Indeed, introduction of the second level of control has already created something of a problem. It is known that the rate of phytochrome degradation is increased by up to 100-fold in the light and we now know that its rate of synthesis decreases by about 20-fold in these conditions. This being the case, then the levels of phytochrome present in continuous light should be an order of magnitude lower than observed.

Of the possible explanations put forward to account for the discrepancy, one which has created most interest, as well as obtaining some experimental support, is the long-established notion that there may be two distinct populations of phytochrome. The major population, with a high Pfr destruction rate, would be rapidly depleted in the light, leaving a minor more stable population to predominate. Recent experimental evidence on the kinetics of Pfr degradation in *Amaranthus* supports this suggestion[4].

One view is that the two populations represent distinct gene products with different immunological properties as well as distinct turnover rates. Support for this notion was presented by Brian Thomas (Glasshouse Crops Research Institute, Littlehampton) who described the development of an enzyme-linked immunosorbent assay for phytochrome using antibodies raised against phytochrome from dark-grown oats. The results of immunotitration experiments revealed that the avidity of the antigen–antibody reaction for phytochrome from dark-grown oat shoots was much higher than that for phytochrome from light-grown tissue. Independently, Quail's group has also reported these immunological differences based on the results of immunoprecipitation experiments. The inability of antibodies to phytochrome from dark-grown tissue to precipitate effectively phytochrome from light-grown tissue explains the failure to detect a phytochrome translation product among the total *in vitro* translation products encoded by poly(A) RNA from light-grown tissue[5,6].

The existence of two different phytochromes seems a real possibility then, although more trivial explanations such as post-homogenization modifications do have to be considered. New techniques may soon provide a firm conclusion. Quail described the production of a cDNA probe for the mRNA for dark-grown oat phytochrome, which should prove an invaluable tool in elucidating further the molecular biology of phytochrome; it will be interesting to see whether there is indeed a second phytochrome gene. Rick Vierstra (University of Wisconsin, Madison) and Clark Lagarius (University of California, Davis) both described new purification protocols for production of proteolytically undenatured phytochrome from dark-grown tissues. However, no one has so far succeeded in the daunting task of purifying phytochrome from green tissue. A number of groups are currently producing monoclonal antibodies to phytochrome. M-M. Cordonnier (University of Geneva) described work carried out jointly with L. Pratt (University of Athens, Georgia) on the characterization of monoclonal antibodies to oat and pea phytochromes. Interest is currently focused on the development of clones which will be specific for Pr and Pfr and on the characterization of clones exhibiting extensive cross-reactivity in the expectation that cognate antigenic sites represent highly conserved regions of the phytochrome molecule and so may be involved in its biological activity. □

*The symposium was held in Frostvallen, Sweden on 19–23 July 1983.

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PROF. BROWN GOODE, the Commissioner of the United States to the International Fisheries Exhibition, has just received a telegram from Prof. Baird, the United States Commissioner of Fish and Fisheries, to the effect that Mr. Ryder, the embryologist of the Fish Commission, has finally solved the problem of the culture of oysters from artifically impregnated eggs, and that on the 4th inst., at the Government station at Stockton, Maryland, there were many millions of young oysters three-quarters of an inch in diameter which had been hatched from eggs artificially impregnated forty-six days before. It may be added that oysters were artificially impregnated in America by Dr. Brooks, of Baltimore, in 1879, but the difficulty hitherto met with in hatching them has been to prevent the young oysters from escaping and being lost immediately after they are hatched, since the spat passes through the meshes of most finely-woven fabrics, such as flannel.
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