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Phytochrome and the regulation of the expression of its genes

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In attempting to understand the mechanism of phytochrome action we are studying structural properties of the photoreceptor molecule and the autoregulation of expression of phytochrome genes. Run-off transcription assays in isolated nuclei from *Avena* indicate that phytochrome decreases the transcription of its own genes threefold in less than 15 min from Pfr formation. The extent of this decrease is insufficient to account for the observed 10- to 50-fold decrease in mature phytochrome mRNA levels, suggesting that enhanced degradation may also play a significant role in determining the level of this mRNA. Structural analysis of native phytochrome from *Avena* indicates that the molecule is an elongated dimer of 124 kDa monomers, each consisting of a globular, 74 kDa, NH_2-terminal domain bearing the single chromophore at Cys-321, and a more open COOH-terminal domain that bears the dimerization site. Controlled proteolysis and binding of monoclonal antibodies to mapped epitopes has identified two regions, one in the 6–10 kDa NH_2-terminal segment and the other *ca.* 70 kDa from the NH_2-terminus, that undergo photoconversion-induced conformational changes and are therefore candidates for involvement in the molecule's regulatory function. Comparison of the full-length amino acid sequences of *Avena* and *Cucurbita* phytochromes, derived from nucleotide sequence analysis, indicates overall homology of 65%. The most highly conserved regions are those immediately surrounding the chromophore attachment site, where 29 residues are invariant, and a hydrophobic region between residues 150 and 300, postulated to form a cavity containing the chromophore. In contrast, a strikingly lower level of homology exists at the COOH-terminus of the polypeptide between residues 800 and 1128, indicating a possible lack of involvement of this region in phytochrome function.

Introduction

The proposal of Mohr (1966) that phytochrome controls plant development by differential gene regulation at the transcriptional level has received direct experimental support in recent years (Mösinger *et al.* 1985; Silverthorne & Tobin, 1984; Tobin & Silverthorne 1985). We are attempting to understand the molecular mechanism by which that regulation occurs. Our approach is to study genes whose expression is rapidly altered in response to Pfr formation and to define structural properties of the phytochrome molecule potentially related to its regulatory function. In this approach we have focused considerable attention on the gene for phytochrome itself, which, in *Avena*, is subject to a rapid, autoregulatory decrease in expression upon Pfr formation (Colbert *et al.* 1983, 1985).

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Transcriptional regulation of phytochrome gene expression

The capacity for synthesis of phytochrome protein in etiolated oat (*Avena sativa* L.) seedlings is decreased by light owing to a dramatic decline in the amount of phytochrome mRNA per microgram of poly(A)$^+$ RNA (Colbert *et al.* 1983, 1985; Gottmann & Schäfer 1983; Quail *et al.* 1986). The decline results in an amount of phytochrome mRNA that is half of the dark level by 1 h after irradiation and is one tenth to one fiftieth of the dark level by 5 h after irradiation. This response to light is mediated by phytochrome itself (Colbert *et al.* 1983, 1985; Otto *et al.* 1983) and is not a result of deadenylation of phytochrome mRNA (Colbert *et al.* 1985). There are at least three possible explanations for this rapid decline: (*a*) synthesis of mature phytochrome mRNA is decreased 10- to 50-fold, resulting in its rapid diminution by a prevailing high rate of degradation; (*b*) degradation of phytochrome mRNA is specifically increased 10- to 50-fold without a change in rate of synthesis; (*c*) both the rates of synthesis and degradation of phytochrome mRNA are changed.

To begin to distinguish between these possibilities we have isolated oat nuclei and performed *in vitro* run-off transcription assays essentially as described by Beach *et al.* (1985). Incorporation of [^{32}P]UTP into trichloracetic acid-precipitable material by the isolated nuclei is approximately linear for 20 min and then continues at a lower rate for at least 60 min (Colbert 1985). An average of 10.5 fmol of [^{32}P]UMP is incorporated per microgram DNA per minute over the 30 min assay period (Colbert 1985). Incorporation is reduced 70–80% by actinomycin D (10 μg ml^{-1}), is reduced 30–40% by α-amanitin (10 μg ml^{-1}) and is abolished in the absence of unlabelled ribonucleoside triphosphates. RNA products up to 4–5 kilobases (kb) are produced in the presence of placental ribonuclease inhibitor, which we included routinely in these assays. Based on these parameters, the isolated oat nuclei used in our experiments are similar to other isolated plant nuclei used in *in vitro* run-off transcription assays (Beach *et al.* 1985; Gallagher & Ellis 1982; Mössinger *et al.* 1985; Silverthorne & Tobin 1984).

To ensure that hybridization signals obtained in subsequent dot-blot assays would be specific for the desired transcripts we performed a Southern blot analysis with the hybridization probes (figure 1). *In vitro* labelled RNA transcripts produced in nuclei isolated from dark-grown plants were hybridized with the various probes used. Specific hybridization was observed with an oat phytochrome copy DNA (cDNA) clone (pAP-3) (Hershey *et al.* 1984), a corn light-harvesting chlorophyll *a*/*b* binding protein cDNA clone (pAB1084) (A. H. Christensen, unpublished), and a wheat rRNA genomic clone (pTA71) (Gerlach & Bedbrook 1979), with no detectable hybridization to the pBR322 control (figure 1).

Transcription of the phytochrome gene(s) is down-regulated by light (figure 2*b*). The decline in transcription rate is induced by either a red light pulse or continuous white light and results in a level *ca.* one third of the dark level by 2.5 h after irradiation. Based on the effects of far-red and red plus far-red light treatments, this light-regulated transcription of the phytochrome gene(s) appears to be a phytochrome-mediated process (figure 2*b*). In the same isolated oat nuclei, transcription of rRNA genes is unaffected by light (figure 2*a*) whereas, as expected (Gallagher & Ellis 1982; Mössinger *et al.* 1985; Silverthorne & Tobin 1984), transcription of the light-harvesting chlorophyll *a*/*b* binding protein gene(s) is stimulated by light in a phytochrome-mediated fashion (figure 2*c*).

Time-course experiments indicate that the light-induced decline in transcription of the phytochrome genes occurs rapidly, reaching a level *ca.* one third of the dark level by 15 min

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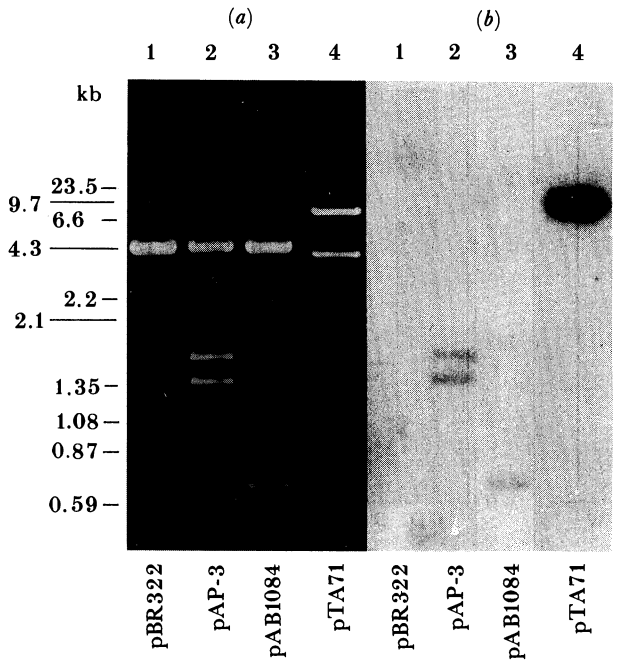

FIGURE 1. Specific hybridization of labelled transcripts to cloned DNAs. Plasmids were isolated by the alkaline lysis method as described by Maniatis *et al.* (1982) and banded twice in CsCl–ethidium bromide gradients. The plasmids pBR322, pAP-3 and pAB1084 (250 ng each) were digested with *Pst*I. pTA71 (250 ng) was digested with *Eco*R1. The resulting fragments were resolved on a 1% agarose gel and stained with ethidium bromide (*a*). The fragments were then transferred to Gene Screen Plus (*b*) by the alkaline blot procedure (Chomczynski & Qasba 1984), and hybridized with labelled RNA transcripts isolated from nuclei prepared from 4-day-old etiolated oats (Colbert 1985). Hybridization was done in 1.5 ml of 33% deionized formamide, 0.55 M NaCl, 40 mM sodium phosphate (pH 6.8), 0.04% sodium dodecyl sulphate (SDS), 5X Denhardt's solution (Denhardt 1966), 4 mM EDTA, denatured salmon sperm DNA (100 μg ml^{-1}), poly(A) (100 μg ml^{-1}), with 10^7 c.p.m. of labelled RNA at 42 °C for 40 h. The filter was washed twice for 15 min in 0.3 M NaCl, 0.03 M sodium citrate (pH 7.0), 0.5% SDS, 40 mM sodium phosphate (pH 6.8), 5 mM EDTA, 2 mM sodium pyrophosphate at room temperature and then for 1 h in 15 mM NaCl, 1.5 mM sodium citrate (pH 7.0), 0.5% SDS at 62 °C with continuous agitation. Autoradiography was performed at −70 °C for 48 h (pBR322, PAP-3, pAB1084) or 4 h (pTA71). *Hin*dIII digested λ and *Hae*III digested ϕX174 DNA served as molecular mass markers.

after irradiation (figure 3). We observe a significant decrease in phytochrome-gene transcription even at our shortest time point, approximately 2 min after irradiation. This is one of the most rapid modulations of the rate of transcription of a plant gene yet observed. Similarly rapid light-regulated modulation of the transcription rates of the small subunit of ribulosebisphosphate carboxylase genes and light-harvesting chlorophyll *a*/*b* binding protein genes have been reported (Gallagher *et al.* 1985), as have rapid auxin-induced changes in the transcription of as yet unidentified sequences (Hagen & Guilfoyle 1985). Transcription of the phytochrome genes remains constant at the new level for at least 10 h from irradiation (figure 3). This lower level does not represent the limit of detection of the hybridization assays because the inclusion of α-amanitin with light-treated nuclei during *in vitro* transcription results in a phytochrome hybridization signal that is reduced still further by a factor of 10 (Colbert 1985). Transcription of the rRNA genes is unaffected by light treatment over the 10 h time course (data not shown).

The quantitative discrepancy between the decline in mature phytochrome mRNA abundance (10- to 50-fold) and the decrease in rate of transcription of the phytochrome gene (threefold) over the same time-course (figure 3) indicates that post-transcriptional events may be involved in the regulation of phytochrome gene expression. Similar evidence has been obtained for

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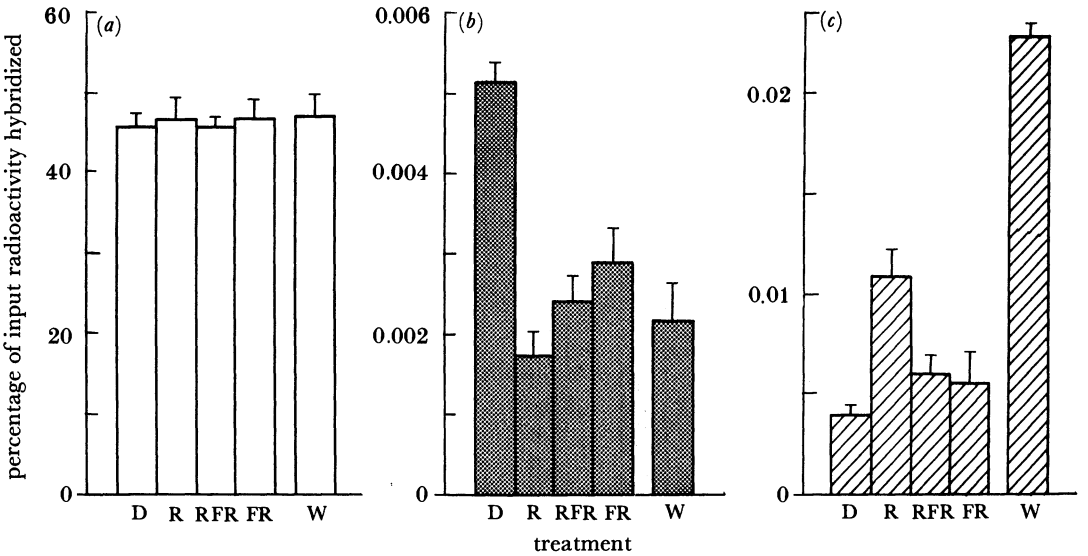

FIGURE 2. Light-mediated changes in specific run-off transcript levels. Four-day-old etiolated oats were given saturating pulses of red light (R), far-red light (FR) or red plus far-red light (RFR) and returned to darkness for 2.5 h. Control seedlings were left in the dark (D). In separate experiments etiolated oat seedlings were transferred to continuous white light for 2.5 h (W). Nuclei were prepared and *in vitro*-labelled transcripts were isolated (Colbert 1985). Nitrocellulose filter discs bearing (*a*) pTA71 (rRNA) were hybridized with 1.5, 7.5 or 15×10^3 c.p.m. of labelled RNA, and filters bearing (*b*) pAP-3 (phytochrome) or (*c*) pAB1084 (LHCP) were hybridized with 2, 4 or 6×10^6 c.p.m. of labelled RNA. A filter with immobilized pBR322 was included in each hybridization reaction. Hybridization and washing conditions were as described in the legend to figure 1 except that the volume of the hybridization buffer was 100 µl. The bound radioactivity was measured by liquid scintillation spectrometry. Individual filters were counted for 5–10 min. Background hybridization to the pBR322 filters was subtracted from other values. Specific hybridization was linear with input radioactivity. Data points are the means of at least three experiments ± standard error.

post-transcriptional regulation of several other plant genes including those for α-amylase (Nolan *et al.* 1985), vicilin (Beach *et al.* 1985), protochlorophyllide reductase (Mössinger *et al.* 1985), seed-protein genes (Walling *et al.* 1986) and auxin-regulated genes (Kroner & Key 1985).

Differential stability of mRNAs has emerged as an important post-transcriptional mechanism by which the amount of a given mRNA species present in the cytoplasm can be controlled by the cell. Some well-characterized examples include the mRNAs for vitellogenin (Brock & Shapiro 1983), casein (Guyette *et al.* 1979), β-tubulin (Pittenger & Cleveland 1985; Schedl *et al.* 1984), c-myc (Dony *et al.* 1985), and a number of unidentified mRNAs in *Dictyostelium* (Mangiarotti *et al.* 1985), *Drosophila* (Winkles & Grainger 1985) and sea urchin (Cabrera *et al.* 1984). Consequently we have analysed our data further from a theoretical perspective to explore whether a change in the stability of phytochrome mRNA would be consistent with our experimental results. In this analysis we have assumed that the synthesis of phytochrome mRNA is a zero-order process and that the degradation of phytochrome mRNA is a first-order process (Brock & Shapiro 1983; Chung *et al.* 1981; Kafatos 1972; Schimke 1975). The kinetics of phytochrome mRNA metabolism can then be described by the equation

$$\mathrm{d}m/\mathrm{d}t = R_s - R_d m,$$

where m is the amount of phytochrome mRNA at time t, R_s is the rate constant of synthesis of phytochrome mRNA, and R_d is the rate constant of degradation of phytochrome mRNA.

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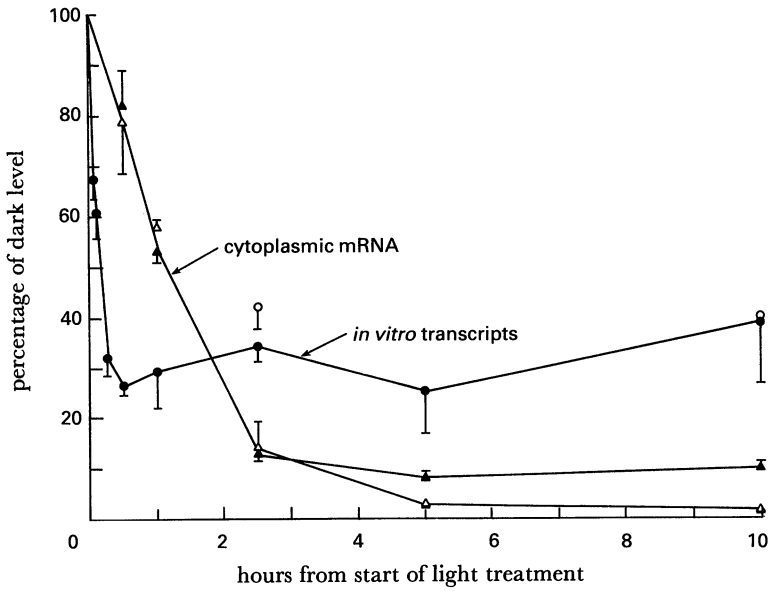

FIGURE 3. Time-course of change in transcription rate of phytochrome genes and of mature phytochrome mRNA levels in response to light treatment. Four-day-old dark grown oat seedlings were given either a saturating pulse of red light and returned to the dark (solid circles) or transferred to continuous white light (open circles) at time zero. Nuclei were isolated from plants harvested at various times after the light treatment and *in vitro* run-off transcription assays were performed (Colbert 1985). The *in vitro* transcripts were isolated and hybridized to filter-bound pAP-3. Hybridization and washing conditions were as described in the legend to figure 1, except that the volume of the hybridization buffer was 100 µl. Input of labelled transcripts and measurement of hybridized RNA were as described in the legend to figure 2. Data points are from two independent experiments except: 2 and 5 min points ($N = 4$), 15 min point ($N = 3$), 10 h continuous white light ($N = 1$). Mean hybridization for dark controls in this set of experiments was 0.0055% of the input radioactivity. Data are expressed as the means ± standard error relative to phytochrome transcription in dark-grown control seedlings. The 2.5 h data points are replotted from the experiments presented figure 2. The mean abundance of mature, hybridizable phytochrome mRNA in the cellular poly(A)$^+$ fraction after either a red-light pulse (closed triangles) or transfer to continuous white light (open triangles) is replotted from Colbert *et al.* (1985).

With this equation we have calculated the predicted time-course of the Pfr-induced change in phytochrome mRNA levels (table 1; figure 4) using (1) the measured steady-state level of 1 ng µg^{-1} poly(A)$^+$ RNA at time zero in etiolated tissue (Colbert *et al.* 1985), and (2) two different values for R_s (0.015 and 0.045 ng phytochrome mRNA per microgram poly(A)$^+$ RNA per hour) at time zero before irradiation. The value of 0.015 ng phytochrome mRNA per microgram poly(A)$^+$ RNA per hour is that measured for long-term reaccumulation in tissue returned to the dark after irradiation (Colbert *et al.* 1985). Use of this value corresponds to the scenario (designated A) that the observed threefold decrease in transcription induced by Pfr (figures 2 and 3) is transient and returns subsequently in the dark to the initial level before irradiation. The value of 0.045 ng phytochrome mRNA per microgram poly(A)$^+$ RNA per hour corresponds to the scenario (designated B) that the measured reaccumulation rate of 0.015 does not represent a return to the initial rate, but rather a prolongation of the threefold lower rate induced by initial Pfr formation.

In neither case is a threefold decrease in R_s alone sufficient to account for the rapid decline in phytochrome mRNA levels (table 1; figure 4). Whereas the apparent half-life measured experimentally for phytochrome mRNA is *ca.* 1 h after Pfr formation (Colbert *et al.* 1985), a

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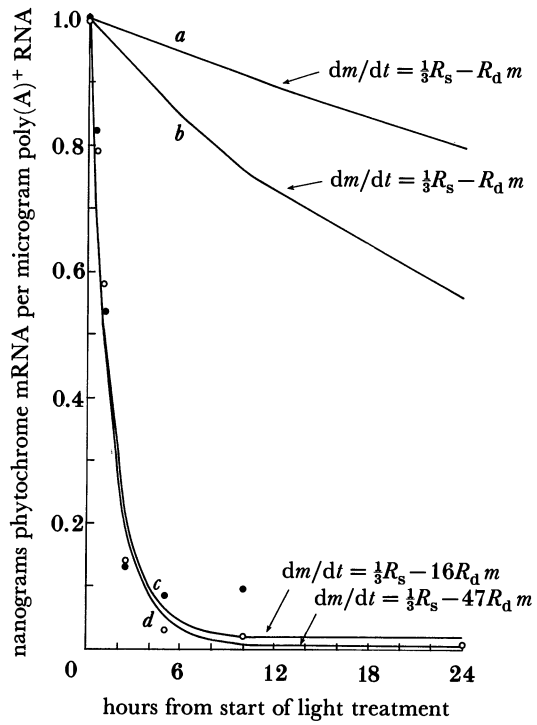

FIGURE 4. Theoretical curves predicting the abundance of phytochrome mRNA after a light treatment. The abundance of phytochrome mRNA was predicted (solid lines) assuming: (curves *a* and *d*) that R_s is 0.015 ng phytochrome mRNA per microgram poly(A)$^+$ RNA per hour, or (curves *b* and *c*) that R_s is 0.045 ng phytochrome mRNA per microgram poly(A)$^+$ RNA per hour. The experimentally determined change in abundance of phytochrome mRNA measured after either a red pulse (closed circles) or transfer to continuous white light (open circles) is replotted from Colbert *et al.* (1985). Curves *c* and *d* were generated to match the experimental data points, requiring a 16-fold increase in R_d (curve *c*) or a 47-fold increase in R_d (curve *d*) in addition to a threefold decrease in R_s.

threefold decrease in R_s alone results in half-lives of 90 and 30 h for scenarios A and B respectively. To account for the observed 1 h half-life it is necessary to invoke increases in R_d of 47-fold and 16-fold respectively for scenarios A and B, in addition to a decrease in R_s of threefold (table 1; fig. 4).

If the indicated assumptions are correct, this analysis is consistent with the conclusion that a Pfr-induced increase in the rate of phytochrome mRNA degradation, in addition to a decrease in transcription of the phytochrome gene, plays a significant role in regulating the amount of this mRNA in the cell. However, direct verification of this apparent differential stability of phytochrome mRNA awaits further experimentation. For example, although in general there is good evidence that run-off transcription in isolated nuclei accurately reflects relative transcription rates *in vivo* (Darnell 1982; Derman *et al.* 1981), one study of globin mRNA synthesis has presented evidence that, in some cases, changes in transcription rates measured by run-off transcription in isolated nuclei were less than the changes detected by pulse-labelling nuclear RNA in living cells (Ganguly & Skoultchi 1985). Moreover, the data we have obtained so far do not exclude the possibility of regulation at other levels including processing and/or transport of phytochrome mRNA.

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Table 1. Theoretical analysis of phytochrome mRNA levels in response to Pfr formation

	scenario A	scenario B
steady-state phytochrome mRNA level (m) at time zero before irradiation[1]	1 ng μg^{-1} poly(A)$^+$ RNA	1 ng μg^{-1} poly(A)$^+$ RNA
R_s for phytochrome mRNA measured in irradiated plants returned to prolonged darkness[2]	0.015 ng μg^{-1} poly(A)$^+$ RNA h^{-1}	0.015 ng μg^{-1} poly(A)$^+$ RNA h^{-1}
Assumed R_s for phytochrome mRNA at steady state before irradiation[3]	0.015 ng μg^{-1} poly(A)$^+$ RNA h^{-1}	0.045 ng μg^{-1} poly(A)$^+$ RNA h^{-1}
R_d at steady state before irradiation[4]	0.015 ng μg^{-1} poly(A)$^+$ h^{-1}	0.045 ng μg^{-1} poly(A)$^+$ h^{-1}
R_s in short term after irradiation[5]	0.005 ng μg^{-1} poly(A)$^+$ h^{-1}	0.015 ng μg^{-1} poly(A)$^+$ h^{-1}
time for phytochrome mRNA to decrease to 50% assuming only a decrease in R_s of threefold (no change in R_d)	90 h	30 h
increase in R_d required to account for observed rate of decline in phytochrome mRNA[6]	47-fold	16-fold

[1] From Colbert *et al.* (1985).
[2] Value for R_s is the rate of reaccumulation measured by Colbert *et al.* (1985) in irradiated plants returned to prolonged darkness.
[3] Values derived from the assumption that irradiation induces a rapid, threefold decrease in R_s that is either transient with R_s returning in prolonged darkness to the level before irradiation (scenario A), or irreversible for the duration of the experimental period resulting in a sustained threefold decrease in R_s (scenario B).
[4] $R_s = Rdm$ at steady state; $m = 1$ ng μg^{-1} poly(A)$^+$ RNA.
[5] Values represent threefold decrease in R_s in response to Pfr formation as indicated by data in figures 2 and 3.
[6] Assuming a threefold decrease in R_s as well.

The phytochrome molecule

Our perception of the phytochrome molecule has undergone rapid revision in recent times (see Lagarias (1985) for a review) as the result of a combination of studies involving nucleotide sequence analysis (Hershey *et al.* 1985; Sharrock *et al.* 1986), peptide mapping (Daniels & Quail 1984; Jones *et al.* 1985; Lagarias & Mercurio 1985; Vierstra *et al.* 1984), probing with monoclonal antibodies (McAb) (Cordonnier *et al.* 1984, 1985; Daniels & Quail 1984), spectral analysis (Kelly & Lagarias 1985; Vierstra & Quail 1982*b*, 1983*a*, *b*), the use of chemical probes (Hahn *et al.* 1984*b*; Song 1985) and quaternary structure analysis (Jones & Quail 1986; Lagarias & Mercurio 1985). Current data for *Avena* indicate that the molecule is a slightly elongated dimer of 124 kDa subunits with each monomer of 1128 amino acids having a single chromophore attached at Cys-321 in a globular, 74 kDa NH_2-terminal domain (Daniels & Quail 1984; Hershey *et al.* 1985; Jones & Quail 1986; Lagarias & Mercurio 1985; Vierstra *et al.* 1984). The overall shape of the dissociated monomer may also be elongated, with the COOH-terminal 55 kDa domain having a relatively open structure (Jones & Quail 1986; Lagarias & Mercurio 1985).

Additional studies have begun to define submolecular regions of phytochrome involved in photoconversion-induced conformational changes, in protein–chromophore interactions and in dimerization. A 4–10 kDa region near the NH_2-terminus undergoes photoconversion-induced

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conformational changes as indicated by differential proteolysis of, and differential McAb binding to, the Pr and Pfr forms (see Daniels & Quail (1984), Jones & Quail (1986), Jones *et al.* (1985), Lagarias & Mercurio (1985) and Vierstra & Quail (1982*a*, *b*) for the former, and Cordonnier *et al.* (1985) and Quail *et al.* (1986) for the latter). Other regions at 74, 83–84 and 100 kDa from the NH_2-terminus also differ in accessibility to proteases as Pr and Pfr (Jones *et al.* 1985; Lagarias & Mercurio 1985; Vierstra *et al.* 1984). These conformational changes are conserved in phytochrome from a variety of plant species (Kerscher & Nowitzki 1982; Vierstra *et al.* 1984). Gross indications of undefined conformational changes have also come from studies with circular dichroism (Litts *et al.* 1983; R. D. Vierstra, H. K. Sarkar, P. S. Song & P. H. Quail, unpublished data), ^1H–^3H exchange (Hahn *et al.* 1984*a*) and size-exclusion chromatography (Jones & Quail 1986; Lagarias & Mercurio 1985).

The 55 kDa COOH-terminal domain can be cleaved from phytochrome without affecting its spectral properties, indicating that this domain does not interact with the chromophore (Jones *et al.* 1985). In contrast, the 6–10 kDa NH_2-terminal segment of the polypeptide is involved directly or indirectly in protein interaction with the Pfr but not with the Pr form of the chromophore. This conclusion is based on the changes in several major spectral and photochemical properties that result from proteolytic removal (Eilfeld & Rüdiger 1984; Kelly & Lagarias 1985; Litts *et al.* 1985; Quail *et al.* 1983; Vierstra & Quail 1982*b*, 1983*a*, *b*), and on the spectral perturbation of the Pfr spectrum and enhanced dark reversion that are induced by McAbs that bind to epitopes in that segment (Cordonnier *et al.* 1985; Quail *et al.* 1986). Proteolytic cleavage of the 6–10 kDa segment causes a fivefold increase in the rate of TNM-induced bleaching of Pfr but not of Pr (Hahn *et al.* 1984*b*). Because ozone, a small, penetrating molecule, reacts equally with Pr and Pfr, indicating equal intrinsic chemical reactivity of the two forms of the chromophore (Thümmler *et al.* 1985), it has been suggested that the 6–10 kDa segment shields the tetrapyrrole in a hydrophobic pocket from the external, hydrophilic environment (Song 1985). Binding studies with the hydrophobic probe 8-anilinonaphthalene-1-sulphonate have been interpreted similarly (Eilfeld & Rüdiger 1984).

One approach to defining structural features important to the function of a regulatory molecule is to identify sequences that are conserved between evolutionarily divergent species. The limited immunochemical cross-reactivity observed between phytochrome from monocot and dicot plants (Cordonnier & Pratt 1982; Cordonnier *et al.* 1984; Pratt 1982; Vierstra & Quail 1985) indicates that this approach might prove fruitful for the photoreceptor. To this end we have cloned and sequenced a *Cucurbita* phytochrome cDNA containing the entire coding sequence and have compared it with the *Avena* sequence (Sharrock *et al.* 1986). Overall the level of conservation is 64% at the nucleotide level and 65% at the amino acid level (table 2). However, the distribution of homology is non-uniform along the sequences as indicated by dot matrix comparisons of the nucleotide and amino acid sequences (figure 5).

Table 2. Nucleotide (coding region) and amino acid sequence homologies between oat and zucchini phytochromes

sequence region	homology (%) amino acid	nucleotide
full length	65	64
amino acids 1–800	71	67
amino acids 801–1128	49	59

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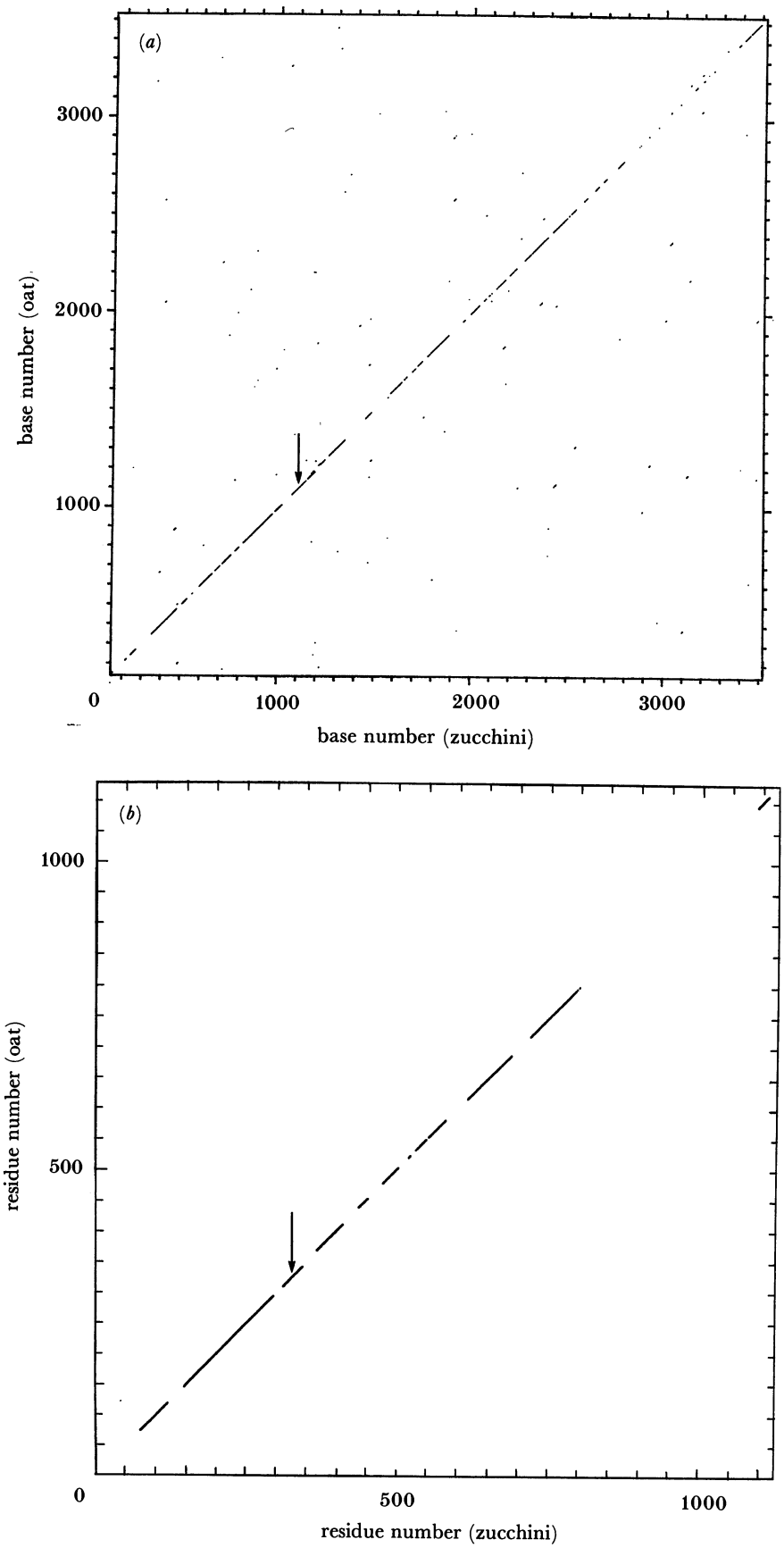

FIGURE 5. Dot-matrix comparison of oat and zucchini phytochrome nucleotide (*a*) and amino acid (*b*) sequences. The oat sequence is the type 3 sequence determined by Hershey *et al.* (1985) and the zucchini sequence is from Sharrock *et al.* (1986). Only the coding regions of the two nucleotide sequences are displayed. The arrows indicate the location of the chromophore attachment site.

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The most striking feature is the extensive region of reduced homology in the COOH-terminal domain relative to the rest of the molecule (figure 5). The amino acid conservation is only 49% in the COOH-terminal one third of the polypeptide, whereas it is 71% in the remainder (table 2). This observation decreases the probability that the bulk of the COOH-terminal one third of the molecule is directly involved in the molecular action of the photoreceptor. Moreover, since there is evidence that the dimerization site(s) lies within 40 kDa of the COOH-terminus (i.e. between residues 765 and 1128), the region between residues 765 and 800 becomes a candidate for this site, given the high level of conservation relative to the remainder of this 40 kDa region.

Localized regions of high homology are distributed throughout the rest of the NH_2-terminal 800 amino acid residues, interspersed with regions of lower homology (figure 5). The most highly conserved region is that immediately surrounding the chromophore attachment site (arrow) where 29 consecutive residues are invariant. The most extensive region of relatively high homology is between amino acids 150 and 300, a hydrophobic region postulated to be involved in forming the cavity thought to house the chromophore away from the surface of the phytochrome molecule (Hershey *et al.* 1985; Sharrock *et al.* 1986; Song 1985). A high degree of conservation is expected of those regions of the polypeptide that interact directly with the chromophore given the almost identical spectral properties of phytochrome from oats and zucchini (Vierstra *et al.* 1984; Vierstra & Quail 1985). Surprisingly the NH_2-terminal 70 amino acids exhibit relatively low homology overall (figure 5). Strong conservation might have been expected in this region given its involvement in fidelity of protein–chromophore interaction (Jones *et al.* 1986; Vierstra & Quail 1982*b*, 1983*a*, *b*; Vierstra *et al.* 1984), in photoconversion-induced conformational changes (Cordonnier *et al.* 1985; Litts *et al.* 1985; Jones *et al.* 1985; Quail *et al.* 1986; Vierstra & Quail 1982*a*, *b*; Vierstra *et al.* 1984) and the presence of a conserved epitope for a type 1 monoclonal antibody (Daniels & Quail 1984). Examination of the hydropathy profiles for the two sequences, however, reveals substantial conservation of the hydropathic properties in this region as a result of predominantly conservative amino acid substitutions (Sharrock *et al.* 1986).

Thus steady progress is being made towards assembling an image of the phytochrome molecule that may ultimately aid in resolving its mechanism of action. Sequence analysis of phytochrome from additional evolutionarily diverse plants should provide further refinement of this image.

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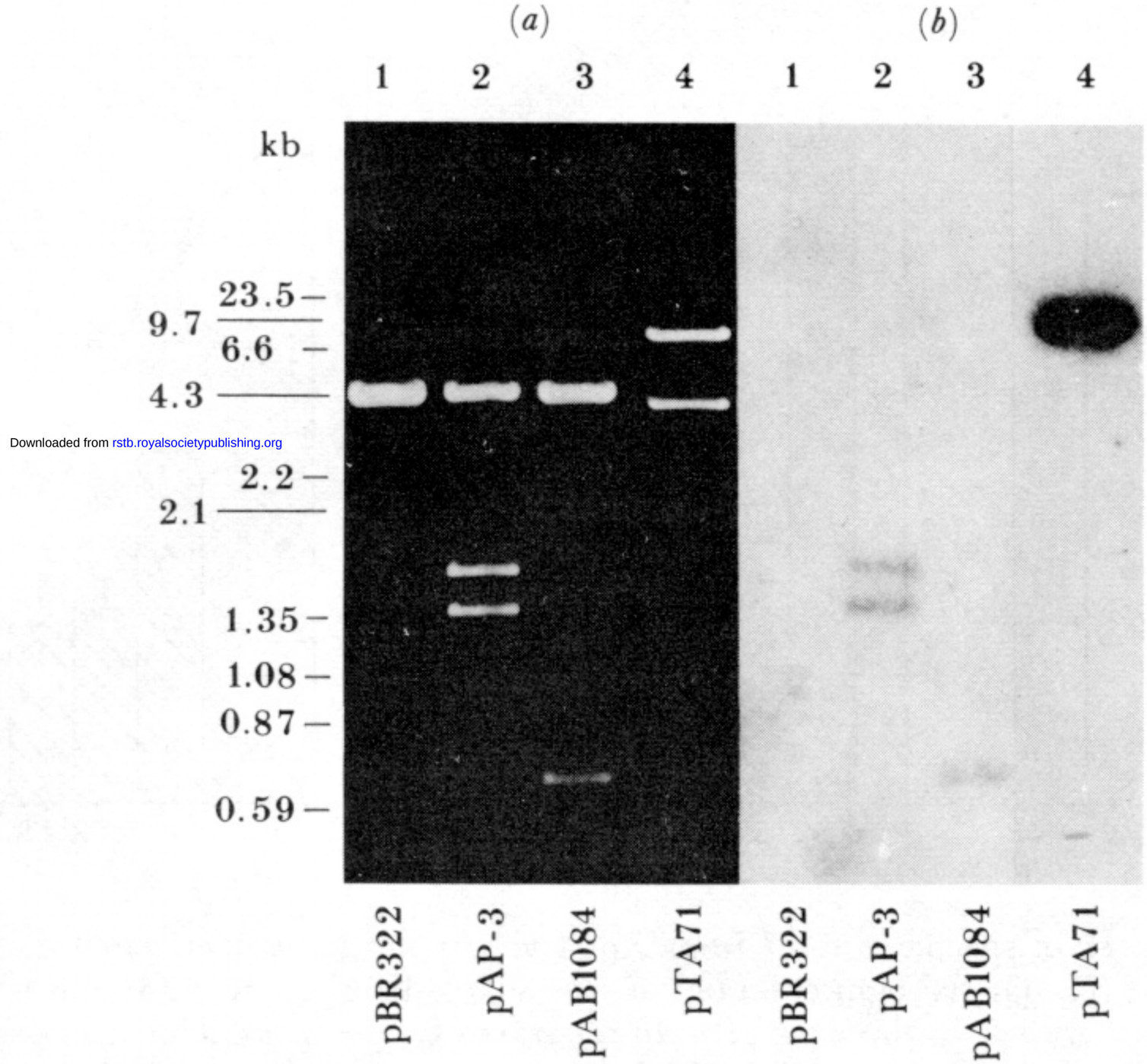

FIGURE 1. Specific hybridization of labelled transcripts to cloned DNAs. Plasmids were isolated by the alkaline lysis method as described by Maniatis *et al.* (1982) and banded twice in CsCl–ethidium bromide gradients. The plasmids pBR322, pAP-3 and pAB1084 (250 ng each) were digested with *Pst*I. pTA71 (250 ng) was digested with *Eco*R1. The resulting fragments were resolved on a 1% agarose gel and stained with ethidium bromide (*a*). The fragments were then transferred to Gene Screen Plus (*b*) by the alkaline blot procedure (Chomczynski & Qasba 1984), and hybridized with labelled RNA transcripts isolated from nuclei prepared from 4-day-old etiolated oats (Colbert 1985). Hybridization was done in 1.5 ml of 33% deionized formamide, 0.55 M NaCl, 40 mM sodium phosphate (pH 6.8), 0.04% sodium dodecyl sulphate (SDS), 5X Denhardt's solution (Denhardt 1966), 4 mM EDTA, denatured salmon sperm DNA (100 μg ml^{-1}), poly(A) (100 μg ml^{-1}), with 10^7 c.p.m. of labelled RNA at 42 °C for 40 h. The filter was washed twice for 15 min in 0.3 M NaCl, 0.03 M sodium citrate (pH 7.0), 0.5% SDS, 40 mM sodium phosphate (pH 6.8), 5 mM EDTA, 2 mM sodium pyrophosphate at room temperature and then for 1 h in 15 mM NaCl, 1.5 mM sodium citrate (pH 7.0), 0.5% SDS at 62 °C with continuous agitation. Autoradiography was performed at −70 °C for 48 h (pBR322, PAP-3, pAB1084) or 4 h (pTA71). *Hin*dIII digested λ and *Hae*III digested ϕX174 DNA served as molecular mass markers.