Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2332–2336, April 1984
Biochemistry

Molecular cloning of cDNA for *Avena* phytochrome

(regulatory photoreceptor/low-abundance mRNA/light–dark difference screening/hybridization-selected translation/gene expression)

HOWARD P. HERSHEY*, JAMES T. COLBERT*, JAMES L. LISSEMORE*, RICHARD F. BARKER†, AND PETER H. QUAIL*

*Department of Botany, University of Wisconsin, Madison, WI 53706; and †Agrigenetics Advanced Research Laboratory, Madison, WI 53716

Communicated by Folke Skoog, January 9, 1984

ABSTRACT **We have isolated several cDNA clones for phytochrome, a plant regulatory photoreceptor. A cDNA library was constructed by using etiolated *Avena* poly(A)$^+$ RNA enriched for phytochrome mRNA by size fractionation. Replicate arrays of colonies were differentially screened with cDNA probes made from poly(A)$^+$ RNA that had been either enriched in or depleted of phytochrome mRNA. Of the colonies hybridizing preferentially with the enriched probe, several contained plasmids that specifically selected phytochrome mRNA when assayed by hybridization-selection and translation. The largest such plasmid, pAP-2, was used to isolate clones from an *Avena* genomic library. One of these genomic clones was then used to screen a second cDNA library in an attempt to identify full-length phytochrome clones. The largest of the plasmids thus obtained, pAP-3, contains a 3.4-kilobase-pair (kbp) insert, verified to contain phytochrome sequences by hybridization-selection and translation. Sequence analysis of pAP-2 and pAP-3 revealed that the two clones are identical in sequence through a 2.4-kbp region in which they overlap. However, the pAP-2 insert contains, in addition, 1.5 kbp of sequence of unknown origin, the apparent result of a recombination event. Blots of poly(A)$^+$ RNA hybridized with ^{32}P-labeled pAP-2 or pAP-3 show a single mRNA band at 4.2 kilobases. Blot analysis of RNA from dark-grown and from red-irradiated tissue demonstrates that a previously reported light-induced decrease in translatable phytochrome mRNA results from a decrease in physical abundance of this mRNA.**

Phytochrome is the best characterized of the regulatory photoreceptors that control plant development in response to light (1). The molecule is a chromoprotein consisting of a linear tetrapyrrole chromophore covalently linked to a polypeptide of molecular mass in the range of 120–127 kilodaltons (kDa), depending on plant species (2, 3). The photoreceptor has two forms that are reversibly interconvertible by light: the Pr form that absorbs maximally in the red (λ_{max} = 666 nm) region of the spectrum and the Pfr form that absorbs maximally in the far-red (λ_{max} = 730 nm) region. Photoconversion of Pr to Pfr *in vivo* induces a large array of morphogenic responses, whereas reconversion of Pfr to Pr cancels the induction of those responses. It is this property of repeated photointerconvertibility that allows phytochrome to function as a regulatory molecule, with Pr considered to be the inactive form and Pfr the active form.

Despite considerable research effort, the molecular mechanism by which Pfr induces the various developmental changes observed remains obscure. The prevailing expectation that these changes result from phytochrome-regulated gene expression has been verified recently by direct experimental evidence (4–10). The photoreceptor has been shown to control the expression of a number of genes, including those encoding the small subunit of ribulosebisphosphate carboxylase (6, 7), chlorophyll a/b binding protein (5–7), protochlorophyllide reductase (4), and several unidentified mRNA species (10). In the first two cases there is evidence that the change in expression is controlled at the transcriptional level (7, 8). Nevertheless, neither the primary mechanism of phytochrome action (the first molecular change elicited by Pfr in the cell) nor the subsequent steps in any putative signal chain leading to altered transcription are understood.

To approach this problem we have initiated a study aimed both at providing detailed structural information on the photoreceptor molecule itself and at determining the basis for the observed changes in gene expression at the molecular level. In this effort we are exploiting a unique feature of the phytochrome system. We have determined recently that phytochrome controls not only the expression of other genes but also that of its own gene in negative feedback fashion (11). In dark-grown tissue, translatable phytochrome mRNA constitutes $\approx 5 \times 10^{-3}$% of the total poly(A)$^+$ RNA. Red-light-induced conversion of Pr to Pfr triggers a rapid decline in this translatable mRNA, detectable within 15–30 min and resulting in the loss of >90% of the translatable activity within 2 hr. This dramatic reduction in phytochrome mRNA activity is prevented by immediate reconversion of the Pfr to Pr with subsequent far-red light. In addition, return of light-grown tissue to the dark results in reaccumulation of translatable phytochrome mRNA (12), confirming that the feedback control is reversible upon Pfr withdrawal. Because of the rapidity of this regulation, the phytochrome gene itself offers an attractive model system for the study of the mechanism of phytochrome-regulated gene expression.

Both the primary structure of the photoreceptor and its regulation of gene expression can be investigated simultaneously by using recombinant DNA technology. cDNA clones for phytochrome can be used to determine the amino acid sequence of the polypeptide, and as hybridization probes both for quantitating cytoplasmic and nuclear phytochrome mRNA sequences and for isolating phytochrome genomic clones for structural and organizational studies. To this end we report here the successful isolation of several cDNA clones containing up to 3.4 kilobase pairs (kbp) of phytochrome sequence.

MATERIALS AND METHODS

Growth of Plants. *Avena sativa* Linnaeus cv. Garry (oat) seedlings were grown in moist vermiculite at 25°C for 4 days in the dark. Some seedlings were given a saturating dose of red light and returned to the dark for an additional 3 hr before harvest. Shoot tissue from all treatments was harvested under green safelight, frozen immediately in liquid nitrogen, and used to isolate RNA either directly or after a period of storage at −80°C.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: kDa, kilodalton(s); Pr and Pfr, red-absorbing and far-red-absorbing forms of phytochrome, respectively; kb, kilobases; kbp, kilobase pairs.

Isolation of RNA. Small quantities of RNA were isolated by a modified guanidine thiocyanate procedure (11). Milligram quantities for sucrose gradient fractionation were isolated by the procedure of Silflow *et al.* (13) as modified by Spiker *et al.* (14). Poly(A)$^+$ RNA for cDNA synthesis was separated according to size by rate zonal centrifugation through linear sucrose gradients. Phytochrome mRNA-containing fractions were identified by *in vitro* translation and immunoprecipitation (11, 15).

Construction of cDNA Clones. Two libraries were constructed, using different procedures. In the first, DNA complementary to the poly(A)$^+$ RNA in phytochrome mRNA-enriched fractions was synthesized with reverse transcriptase as described by Murray *et al.* (16) except that the final $MgCl_2$ and KCl concentrations were 7.5 mM and 140 mM, respectively. RNA was hydrolyzed by addition of NaOH to 400 mM, followed by 5 hr of incubation at room temperature. The mixture was neutralized with HCl, extracted with phenol/chloroform/octanol (25:24:1, vol/vol), and applied to a Sephadex G-100 column. Nucleic acids eluting in the void volume were precipitated with ethanol. Reaction conditions for second-strand synthesis were as described by Wickens *et al.* (17) except that 20 units of the large fragment of DNA polymerase I were used per μg of cDNA. After 18 hr of incubation at 14°C, the reaction was stopped by addition of EDTA (as Na_3EDTA) to 50 mM and the mixture was extracted with phenol/chloroform/octanol (25:24:1) and subjected to gel filtration chromatography on Sephadex G-100. The cDNA was precipitated twice with ethanol, treated with 150 units of S1 nuclease per μg of cDNA for 30 min (18), extracted with phenol, and subjected to gel filtration chromatography. The material eluting in the void volume was sized on alkaline agarose gels (19) and oligo(dC) tails averaging 12–14 residues in length were added by incubation of DNA at 100 pmol/ml with a 5:1 molar ratio of terminal deoxynucleotidyltransferase for 6 min at 30°C (20). The reaction was terminated by addition of EDTA to 50 mM, and the tailed cDNA was precipitated with ethanolic perchlorate (21) and redissolved in 10 mM Tris·HCl (pH 7.6)/1 mM EDTA. The tailed cDNA was annealed with *Pst* I-cut oligo(dG)-tailed pBR322 in a 1:1 molar ratio in 10 mM Tris·HCl, pH 7.5/100 mM NaCl/1 mM EDTA and used directly to transform *Escherichia coli* RR1.

For the second library, double-stranded cDNA was synthesized as described by Land *et al.* (22) as modified by Murray *et al.* (16). Conditions for oligo(dC)-tailing of single- and double-stranded cDNAs and annealing of oligo(dC)-tailed cDNA with oligo(dG)-tailed pBR322 were as described above. Annealed DNA was used directly to transform *E. coli* HB101.

Preparation of cDNA Probes for Difference Screening. One probe was prepared by reverse transcription of etiolated-tissue poly(A)$^+$ RNA enriched for translatable phytochrome mRNA by sucrose gradient centrifugation (15). A second probe was prepared in the same manner from poly(A)$^+$ RNA in a corresponding fraction from a parallel gradient in which translatable phytochrome mRNA had been depleted by red-irradiation of tissue 3 hr prior to harvest. Conditions for synthesis of the probes were identical to those used for first-strand cDNA synthesis described above except that α-^{32}P-labeled dCTP with a specific radioactivity of 3000 Ci/mmol (1 Ci = 37 GBq) was used as the only source of that dNTP. RNA was hydrolyzed with alkali, and high molecular weight cDNA was isolated by gel filtration chromatography.

Difference Screening of the cDNA Library in *E. coli* RR1. After transformation (23) and plating, individual colonies were picked and arrayed on Millipore HATF filters on fresh L-agar plates containing tetracycline at 15 μg/ml. Two replicas of each master filter were made and prepared for hybridization (24). Each replica filter was hybridized to 5×10^6 cpm of probe, one to the enriched and one to the depleted probe, in a mixture containing 0.9 M NaCl/0.09 M trisodium citrate (6× NaCl/Cit), 5× Denhardt's solution (25), 10 mM EDTA, 0.5% $NaDodSO_4$, sonicated denatured calf thymus DNA at 100 μg/ml, and poly(rA) at 20 μg/ml for 48 hr at 64°C. The filters were washed twice at room temperature for 15 min in 2× NaCl/Cit/0.5% $NaDodSO_4$ and twice for 1 hr in 0.1× NaCl/Cit/0.5% $NaDodSO_4$ at 64°C and were autoradiographed for 4 days at −70°C with a single DuPont Lightning Plus intensifying screen.

Hybridization-Selection. Colonies showing a stronger hybridization signal with the enriched than with the depleted probe were further screened by hybridization-selection and translation, using a modification of the method of Parnes *et al.* (26). Individual positive colonies were grown overnight in 4 ml of L broth containing tetracycline at 15 μg/ml. Pools of the clones were made by inoculating 75 μl of each of four overnight cultures into 75 ml of L broth plus antibiotics and growing these at 37°C until the OD_{600} of the cultures reached 0.6. Chloramphenicol was then added to 175 μg/ml, and incubation at 37°C was continued for 16 hr. Cleared lysates were prepared from these cultures by the alkaline $NaDodSO_4$ lysis method (27). The lysates from pairs of cultures were combined, made 4.75 M in CsCl and 600 μg/ml in ethidium bromide, and centrifuged for 15 hr at 194,000 × g_{max} in a Beckman VTi 65 rotor to isolate plasmid DNA. Denaturation of plasmid DNA for hybridization-selection was performed as described (26), and the DNA was bound to nitrocellulose by filtration through 13-mm Millipore HAWP filters. The filters were extensively washed in 6× NaCl/Cit and baked for 2 hr at 80°C under reduced pressure. Discs (0.5 cm) were punched from the filters, and loosely bound DNA was removed by boiling in water for 1 min. The discs were then washed twice more with water at room temperature, and groups of up to eight discs were hybridized together to 250 μg of *Avena* poly(A)$^+$ RNA at a concentration of 500 μg/ml as described (26). Hybridization was performed for 4 hr at 50°C. The discs were then washed and RNA was eluted from each individual disc. Conditions for the translation of hybridization-selected mRNA as well as for the immunoprecipitation, electrophoresis, and fluorography of translation products were as described (11). Pools containing phytochrome clones were identified by the ability of hybridization-selected mRNA to direct the synthesis of an immunoprecipitable translation product that comigrated with an *in vitro* synthesized phytochrome standard. Clones from each positive pool were then grown separately, and cDNA from each was used for hybridization-selection to identify individual phytochrome clones.

RNA Blot Analysis. Poly(A)$^+$ RNA was electrophoresed in 1.5% agarose gels containing 3% (wt/vol) formaldehyde (28) and transferred to nitrocellulose (29). Filters were incubated in 50% (vol/vol) deionized formamide/6× NaCl/Cit/5× Denhardt's solution/1% $NaDodSO_4$/40 mM sodium phosphate, pH 6.8/5 mM EDTA/100 μg of denatured calf thymus DNA per ml/20 μg of poly(rA) per ml for 8 hr. Blots were hybridized to nick-translated cDNA probes in 50% formamide/5× NaCl/Cit/1× Denhardt's solution/40 mM sodium phosphate, pH 6.8/5 mM EDTA/10% dextran sulfate. After 14 hr, the blots were washed twice for 15 min in 2× NaCl/Cit/0.5% $NaDodSO_4$/40 mM sodium phosphate, pH 6.8/5 mM EDTA/2 mM sodium pyrophosphate and then twice for 1 hr in 0.1× NaCl/Cit/0.5% $NaDodSO_4$ at 64°C. Autoradiography was performed at −70°C with a single intensifying screen. Nick-translations of plasmids to $>5 \times 10^8$ cpm/μg of DNA were performed essentially as described by Rigby *et al.* (30).

DNA Sequence Analysis. Sequences were determined by the method of Maxam and Gilbert (31) with modifications described by Barker *et al.* (32). Computer analyses of the

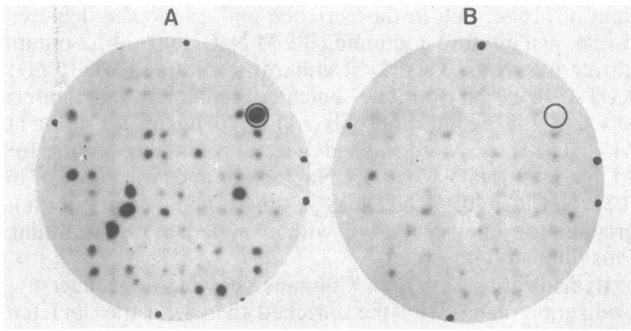

FIG. 1. Isolation of cDNA clones representing red-light regulated mRNAs from etiolated *Avena*. Double-stranded oligo(dC)-tailed cDNA was prepared from poly(A)$^+$ RNA enriched for phytochrome mRNA, annealed with *Pst* I-cut oligo(dG)-tailed pBR322, and used directly to transform *E. coli* RR1. About 1400 tetracycline-resistant ampicillin-sensitive colonies were arrayed and grown on a series of Millipore filters. Two replica filters were produced from each master. One replica filter was hybridized with a cDNA probe made from etiolated-tissue poly(A)$^+$ RNA enriched for phytochrome mRNA by sucrose gradient fractionation (*A*). The second replica was hybridized with a cDNA probe made from a corresponding poly(A)$^+$ RNA gradient fraction in which phytochrome mRNA had been depleted by red irradiation of tissue 3 hr prior to harvest (*B*). Colonies displaying stronger hybridization with the enriched probe were deemed potential phytochrome clones and were tested further by hybridization-selection and translation. The circled colony proved to be a phytochrome cDNA clone designated pAP-2.

DNA sequences were performed by using programs made available by O. Smithies and F. Blattner (University of Wisconsin, Madison).

RESULTS

Difference Screening of cDNA Clones. The first cDNA library was prepared in *E. coli* RR1 with poly(A)$^+$ RNA enriched 120-fold for translatable phytochrome mRNA. From this library approximately 1400 tetracycline-resistant ampicillin-sensitive colonies were arrayed on nitrocellulose filters and replica plated. One replica of each filter was probed with the ^{32}P-labeled cDNA probe enriched for phytochrome sequences, and a second replica was probed with the ^{32}P-labeled probe depleted in phytochrome sequences by irradiation of tissue 3 hr prior to harvest. Of these colonies, 175 showed stronger hybridization signals with the enriched probe than with the depleted probe. Fig. 1 shows one representative pair of replica filters. The circled colony has been shown to be a phytochrome cDNA clone, designated pAP-2.

Hybridization-Selection of Phytochrome mRNA with Cloned cDNA. The difference screening procedure identifies cDNA clones representing a family of genes with mRNA levels that decline within 3 hr of red-light irradiation. A modification of the batch hybridization-selection and translation procedure of Parnes *et al.* (26) was used to rapidly screen the large number of these positive clones for those containing phytochrome sequences. Twelve pools of eight cDNA clones each were analyzed for their ability to hybrid-select phytochrome mRNA specifically. Of these pools, five showed specific selection of phytochrome mRNA on the basis of *in vitro* translation and immunoprecipitation of the phytochrome apoprotein. All clones from the two pools showing strongest selection of phytochrome mRNA were tested individually to identify which ones contained phytochrome cDNA sequences. Each pool yielded a single plasmid complementary to phytochrome mRNA. These plasmids were designated pAP-1 and pAP-2 in order of their confirmation as containing phytochrome sequences.

Fig. 2 shows the data used to identify pAP-2 as a phytochrome clone. The mRNA selected by pAP-2 codes for a 124-kDa protein (Fig. 2, lane B), which is immunoprecipitated from the translation mixture by anti-phytochrome immunoglobulins (Fig. 2, lane E) but not by nonimmune immunoglobulins (Fig. 2, lane F). Translation of the mRNA selected by pL99, a nonphytochrome plasmid containing an insert similar in size to that of pAP-2, yields a 100-kDa translation product (Fig. 2, lane C) not recognized by anti-phytochrome immunoglobulins (Fig. 2, lane G).

Characterization of Phytochrome cDNA Clones. pAP-2 has an insert size of 4.1 kbp, whereas that of pAP-1 is 220 base pairs (data not shown). Initial RNA blot analysis using ^{32}P-labeled pAP-2 showed that the plasmid identifies a single band 4.2 kilobases (kb) in length (Fig. 3*A*). This result indicated that the pAP-2 insert was nearly full-length relative to the size of the mature phytochrome mRNA. The cDNA, however, was known to lack 5′-terminal mRNA sequences, since S1 digestion of hairpin loops was used in construction of the first library. Therefore, a second library was constructed with a procedure designed to maximize the yield of full-length cDNA clones. Screening of this second library was performed with one of several phytochrome genomic clones isolated from an *Avena* genomic library by using pAP-2 as a probe. Details of the isolation and characterization of the genomic clones will be presented elsewhere.

During screening of this second cDNA library, sequence analysis of pAP-2 revealed that the insert contains a structural anomaly. A restriction map with salient features of the sequence is shown in Fig. 4. The insert starts with an

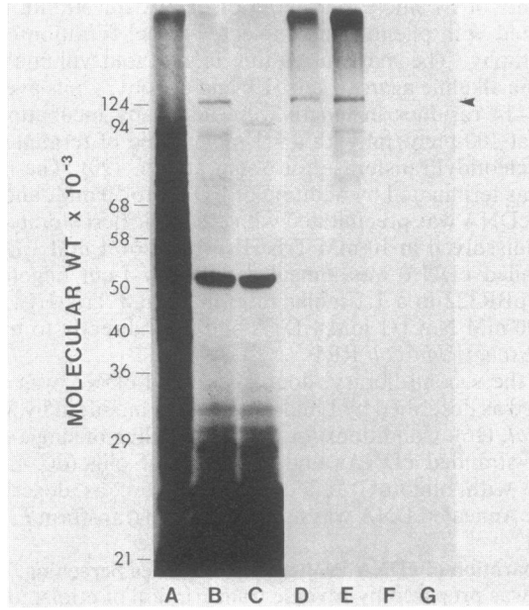

FIG. 2. Identification of cDNA clone pAP-2 containing phytochrome sequences by hybridization-selection and translation. Poly (A)$^+$ RNA from etiolated *Avena* was hybridized with filter-bound pAP-2 or with pL99, a plasmid containing a cDNA insert similar in size to that in pAP-2. The filters were washed extensively and bound mRNAs were eluted by boiling. Total poly(A)$^+$ RNA and hybridization-selected mRNAs were translated in the rabbit reticulocyte cell-free protein-synthesizing system. Aliquots of each reaction mixture were withdrawn for analysis of total translation products and the remainder of each was precipitated with either antiserum against phytochrome or nonimmune serum. Total translation products (lanes A–C), anti-phytochrome immunoprecipitates (lanes D, E, and G) and nonimmune control immunoprecipitates (lane F) were separated by NaDodSO$_4$/polyacrylamide gel electrophoresis and fluorographed. Lanes A and D, total poly(A)$^+$ RNA; lanes B, E, and F, mRNA selected by pAP-2; lanes C and G, mRNA selected by pL99. Arrow to the right indicates the position of the phytochrome apoprotein. The prominent band at ≈52 kDa in lanes B and C is an endogenous product of the reticulocyte lysate system.

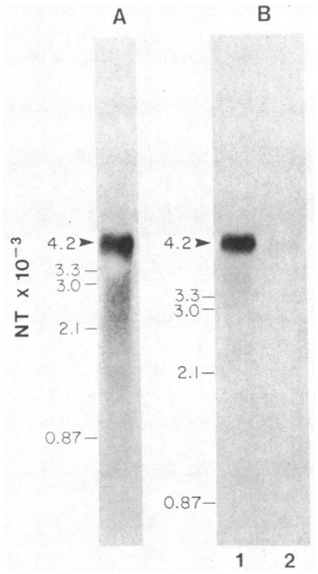

FIG. 3. RNA blot analysis of size and red-light-regulated change in abundance of phytochrome mRNA. Poly(A)$^+$ RNA was isolated from etiolated tissue (*A* and lane 1 of *B*) and from tissue irradiated with red light 3 hr before harvest (lane 2 of *B*). Fifteen micrograms of total poly(A)$^+$ RNA from each preparation was denatured, electrophoresed in a 1.5% agarose/formaldehyde gel, transferred to nitrocellulose, and hybridized with ^{32}P-labeled pAP-2 (blot *A*) or with pAP-3 (blot *B*). Brome mosaic virus RNAs were used as molecular weight markers. Arrow indicates position of the phytochrome mRNA.

oligo(dC) tail, followed by a single potential open reading frame 2.15 kb in length. This reading frame ends in a TAG termination codon followed by a 3′ untranslated region of 456 bases terminating with a poly(dA) tract 33 nucleotides long and an oligo(dC) tail 9 nucleotides long. These features are all characteristic of cloned cDNA representing a eukaryotic mRNA. However, following the central dC-tailed region is 1.51 kbp of additional sequence of undetermined origin that ends in the *Pst* I site of pBR322. These unexpected data suggest that some form of recombination event may have occurred in the *recA*$^+$ *E. coli* RR1 strain used for the first library, giving rise to a plasmid containing an insert composed of a phytochrome cDNA construct linked to an anomalous sequence. Sequence analysis of the 1.51-kbp tract shows that it does not originate from pBR322. RNA blots probed with pAP-2 identify only a single mRNA band (Fig. 3), indicating that the 1.51-kbp segment is likewise not a second cDNA sequence linked to phytochrome as a result of a homologous recombination between two plasmids present in the same *recA*$^+$ bacterium. Finally, this sequence is apparently not derived from *E. coli* chromosomal DNA, since no background hybridization to filters was observed when nitrocellulose replicas of a genomic library were probed with pAP-2.

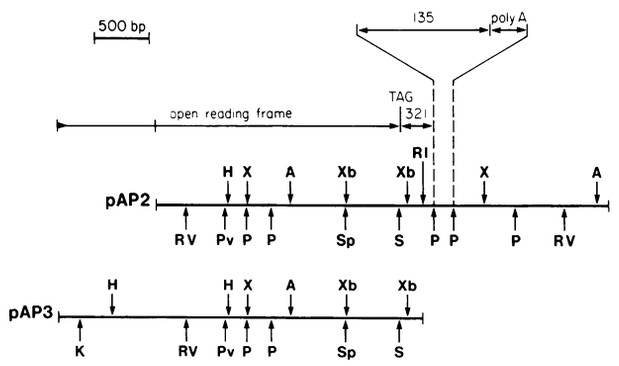

FIG. 4. Restriction map and sequence information for cDNA inserts in recombinant plasmids pAP-2 and pAP-3. The positions of sites for *Ava* I (A), *Eco*RI (RI), *Eco*RV (RV), *Hin*dIII (H), *Kpn* I (K), *Pst* I (P), *Pvu* II (Pv), *Xba* I (Xb), *Xmn* I (X), *Sac* I (S), and *Sph* I (Sp) are shown. The two clones share identical sequences in the region of overlap, which includes an open reading frame, a TAG termination codon, and a 3′ untranslated sequence. The number of base pairs between TAG and the poly(dA) tract of pAP-2 is indicated.

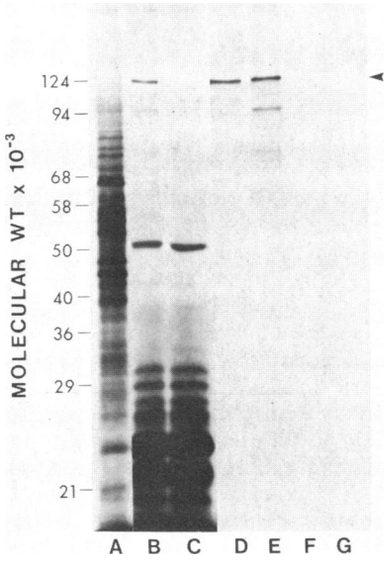

FIG. 5. Identification of phytochrome cDNA clone pAP-3 by hybridization-selection and translation. The conditions for the identification of pAP-3 as a phytochrome clone are the same as those in Fig. 2. Lanes A–C, total translation products; lanes D, E, and G, anti-phytochrome immunoprecipitates; lane F, nonimmune control immunoprecipitate; lanes A and D, total poly(A)$^+$ RNA; lanes B, E, F, mRNA selected by pAP-3; lanes C and G, mRNA selected by pL99.

As a result of these findings, the largest cDNA clone isolated from the second cDNA library prepared in *E. coli* HB101 (a *recA*$^-$ strain), was characterized. Hybridization-selection and translation analysis indicates that this plasmid, designated pAP-3, does indeed contain phytochrome sequences as expected (Fig. 5). Restriction enzyme analysis shows that the pAP-3 insert has >2.4 kbp of map homology with pAP-2 but also contains 0.83 kbp of additional sequence not found in pAP-2 (Fig. 4). Sequence analysis of pAP-3 shows that its sequence is identical to pAP-2 in the region of map homology, indicating that the event that produced the mixed sequence found in pAP-2 did not affect the sequence of the phytochrome portion of the insert. The analysis also shows that the additional 0.83 kbp of sequence in pAP-3 is a continuation of the 2.15 kb of open reading frame common to pAP-2 and pAP-3.

Photoregulation of Phytochrome mRNA Levels. Hybridization of ^{32}P-labeled pAP-3 to a blot of oat poly(A)$^+$ RNA also identifies a single band 4.2 kb in length (Fig. 3*B*). A comparison of RNA from etiolated tissue (Fig. 3*B*, lane 1) and from tissue irradiated with red light 3 hr prior to harvest (Fig. 3*B*, lane 2) demonstrates that the previously observed red-light-induced decline in translatable phytochrome mRNA (11, 12) results from loss of phytochrome mRNA sequences rather than from some form of translational control. Densitometric scanning of the autoradiogram indicates that the mRNA level of the irradiated tissue is reduced to ≈10% of that in etiolated tissue, consistent with the *in vitro* translation data (11). While the success of the colony screening procedure in selecting phytochrome clones demonstrates that the earlier observed red-light-induced loss of phytochrome mRNA activity must result from loss of the mRNA, the blot analysis clearly shows the magnitude of this decline over 3 hr.

DISCUSSION

The cloning of phytochrome cDNA reported here is an essential step in the approach we are taking to investigating the molecular mechanism of phytochrome action and its control of gene expression. Three major facets of this approach are

facilitated by the availability of these clones: (*i*) amino acid sequence determination, (*ii*) phytochrome mRNA quantitation, and (*iii*) phytochrome gene isolation and characterization.

The present cDNA sequences represent 3.4 kb of the *Avena* phytochrome mRNA. These sequences thus make up >80% of the mature phytochrome mRNA, which is estimated to be 4.2 kb from RNA blot analysis. The complete nucleotide sequence of the message will be published as a unit once available from the analysis of additional cDNA clones. Determination of the amino acid sequence of the phytochrome polypeptide may permit identification of regions of the protein related to its mechanism of action. The low degree of immunological crossreactivity between phytochrome from monocotyledons and dicotyledons (33) suggests that only limited regions of the molecule from the two plant groups share amino acid sequence homology and that these regions may therefore represent conserved sequences related to function. The primary sequence also has the potential to provide information on the secondary and tertiary structure of the molecule (34).

Experiments designed to elucidate the molecular basis of phytochrome-regulated gene expression must distinguish between those changes induced directly by the phytochrome signal and those that arise secondarily in response to signal cascading at the genome level (35). Clearly, those changes detected most rapidly after Pfr formation have the highest probability of representing direct phytochrome action. The rapidity of the Pfr-induced reduction in translatable phytochrome mRNA levels (11) has established the phytochrome gene itself as a potential target for this direct action. In exploring this possibility it is necessary first to identify the level at which the regulation occurs. The present data demonstrate that the decrease in translatable phytochrome mRNA represents a decrease in physical abundance rather than some modification reducing its translatability. No evidence is yet available to determine whether this regulation occurs at the transcriptional or posttranscriptional level.

The low abundance of the phytochrome mRNA [$\approx 5 \times 10^{-3}$% of total poly(A)$^+$ RNA in etiolated tissue] makes it one of the least abundant eukaryotic mRNAs cloned to date (36–39). Since low-abundance mRNAs represent the largest class of expressed genes, information on the regulation of the phytochrome gene may be more applicable to the majority of higher plant genes than that obtained for the relatively high-abundance species thus far investigated (4–10, 40–42).

We thank Richard D. Vierstra for antiserum against native 124-kDa *Avena* phytochrome, J. Tokuhisa for *Staphylococcus aureus* cells, and Elizabeth A. Craig and Michael Slater for helpful advice. We extend special thanks to Michael G. Murray for assistance in the initial stages of this work and for helpful scientific discussions and advice. This work was supported by National Science Foundation Grant PCM 8003792, and U.S. Department of Agriculture, Science and Education Administration Grant 59-2551-1-1-744-0. J.T.C. was supported as a predoctoral trainee by National Institutes of Health Grant 5T32GM07215. J.L.L. is the recipient of a National Science Foundation Graduate Fellowship.

1. Smith, H. (1975) *Phytochrome and Photomorphogenesis* (McGraw-Hill, London).
2. Pratt, L. H. (1982) *Annu. Rev. Plant Physiol.* **33,** 557–582.
3. Vierstra, R. D., Cordonnier, M.-M., Pratt, L. H. & Quail, P. H. (1984) *Planta*, in press.
4. Apel, K. (1981) *Eur. J. Biochem.* **120,** 89–93.
5. Gollmer, I. & Apel, K. (1983) *Eur. J. Biochem.* **133,** 309–313.
6. Stiekema, W. J., Wimpee, C. F., Silverthorne, J. & Tobin, E. M. (1983) *Plant Physiol.* **72,** 717–724.
7. Silverthorne, J. & Tobin, E. M. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1112–1116.
8. Gallagher, T. F. & Ellis, R. J. (1982) *EMBO J.* **1,** 1493–1498.
9. Schroder, J., Kreuzaler, F., Schafer, E. & Hahlbrock, K. (1978) *J. Biol. Chem.* **254,** 57–65.
10. Thompson, W. F., Everett, M., Polans, N. O., Jorgensen, R. A. & Palmer, J. D. (1983) *Planta* **158,** 487–500.
11. Colbert, J. T., Hershey, H. P. & Quail, P. H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2248–2252.
12. Gottmann, K. & Schafer, E. (1983) *Planta* **157,** 392–400.
13. Silflow, C. D., Hammett, J. R. & Key, J. L. (1979) *Biochemistry* **18,** 2725–2731.
14. Spiker, S., Murray, M. G. & Thompson, W. F. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 815–819.
15. Quail, P. H., Colbert, J. T., Hershey, H. P. & Vierstra, R. D. (1983) *Phil. Trans. R. Soc. London B* **303,** 387–402.
16. Murray, M. G., Hoffman, L. M. & Jarvis, N. P. (1983) *Plant Mol. Biol.* **2,** 75–84.
17. Wickens, M. P., Buell, G. N. & Schimke, R. T. (1978) *J. Biol. Chem.* **253,** 2483–2495.
18. Maquat, L. E., Kinniburgh, A. J., Rachmilenvitz, E. A. & Ross, J. (1981) *Cell* **27,** 543–553.
19. McDonnel, M. W., Simon, M. N. & Studier, F. W. (1977) *J. Mol. Biol.* **110,** 119–146.
20. Deng, G. & Wu, R. (1981) *Nucleic Acids Res.* **9,** 4173–4188.
21. Bolton, G. W. & Quail, P. H. (1982) *Planta* **155,** 212–217.
22. Land, H., Grez, M., Hauser, H., Lindenmaier, W. & Schütz, G. (1981) *Nucleic Acids Res.* **9,** 2251–2266.
23. Mandel, M. & Higa, A. (1970) *J. Mol. Biol.* **53,** 154–162.
24. Grunstein, M. & Hogness, D. S. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3961–3965.
25. Denhardt, R. T. (1966) *Biochem. Biophys. Res. Comm.* **23,** 641–646.
26. Parnes, J. R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appela, E. & Seidman, J. G. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2253–2257.
27. Birnboim, H. C. & Doly, J. (1979) *Nucleic Acids Res.* **7,** 1513–1523.
28. Hoffman, L. M., Fritsch, M. K. & Gorski, J. (1981) *J. Biol. Chem.* **256,** 2597–2600.
29. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
30. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
31. Maxam, A. M. & Gilbert, W. (1980) in *Methods in Enzymology*, ed. Grosman, L. & Moldave, K., Vol. 65, pp. 499–560.
32. Barker, R. F., Idler, K. B., Thompson, D. V. & Kemp, J. D. (1983) *Plant Mol. Biol.* **2,** 335–350.
33. Cordonnier, M.-M. & Pratt, L. H. (1982) *Plant Physiol.* **70,** 912–916.
34. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 251–276.
35. Britten, R. J. & Davidson, E. H. (1969) *Science* **169,** 349–356.
36. Yoo, C. J., Powell, T. & Agarwal, K. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1049–1053.
37. Korman, A. J., Knudsen, P. J., Kaufman, J. F. & Strominger, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 1844–1848.
38. Kraus, J. R. & Rosenberg, L. E. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4015–4019.
39. Fischer, R. L. & Goldberg, R. B. (1982) *Cell* **29,** 651–660.
40. Sun, S. M., Slightom, J. L. & Hall, T. C. (1981) *Nature (London)* **289,** 37–41.
41. Marks, M. D. & Larkins, B. A. (1982) *J. Biol. Chem.* **257,** 9976–9983.
42. Meinke, D. W., Chen, J. & Beachy, R. N. (1981) *Planta* **153,** 130–139.