Autoregulatory control of translatable phytochrome mRNA levels

(regulatory photoreceptor/dose-response curves/rapid regulation/low-abundance mRNA/gene expression)

JAMES T. COLBERT, HOWARD P. HERSHEY, AND PETER H. QUAIL

Botany Department, University of Wisconsin, Madison, Wisconsin 53706

Communicated by Folke Skoog, January 17, 1983

ABSTRACT Translatable phytochrome mRNA represents $\approx 5 \times 10^{-3}\%$ of the total poly(A)-RNA present in etiolated *Avena* seedlings, as determined by incorporation of radioactivity into the immunoprecipitable apoprotein in a cell-free translation system. Irradiation of such seedlings with 5-s red light induces a decline in this mRNA that is detectable within 15–30 min, shows a 50% reduction within 50–60 min, and results in a $>95\%$ reduction within 2 hr. The effect of the red light pulse is reversed by an immediately subsequent far-red pulse to the level of the far-red-light control, indicating that phytochrome exerts autoregulatory control over its own translatable mRNA level. This result necessitates revision of existing concepts of how phytochrome concentrations are modulated *in vivo*. Red-light dose-response curves show that the response is sensitive to very low light levels. Conversion of $<1\%$ of the total cellular phytochrome to the biologically active far-red-absorbing form is sufficient to induce $\approx 60\%$ of the maximal response, and 20% far-red-absorbing form saturates the response. The observed change in translatable phytochrome mRNA level is one of the most rapid phytochrome-induced alterations in any cellular mRNA yet recorded. Thus, autoregulation of phytochrome mRNA provides an attractive opportunity to examine the early sequence of events in phytochrome control of gene expression.

Phytochrome is a regulatory photoreceptor with a central role in plant development (1, 2). The molecule consists of a linear tetrapyrrole chromophore covalently linked to a polypeptide (3, 4) with a monomeric molecular mass of 124 kilodaltons (kDa) in *Avena* (5) and ranging from 120 to 127 kDa in other species (unpublished data). The photoreceptor has two photointerconvertible forms: a red-absorbing form (Pr), which absorbs maximally in the red ($\lambda_{\max} = 666$ nm), and a far-red-absorbing form (Pfr), which absorbs maximally in the far-red ($\lambda_{\max} = 730$ nm) region of the spectrum. Photoconversion of Pr to Pfr induces a diverse array of morphogenic responses, whereas reconversion of Pfr to Pr cancels the induction of the responses. Thus, Pfr is considered to be the active form and Pr the inactive form of the photoreceptor. The molecular mechanism by which Pfr induces these responses has been the subject of much research but is yet to be elucidated (6, 7).

Phytochrome is synthesized *de novo* as Pr (8) and accumulates up to 0.5% of the soluble protein in dark-grown *Avena* shoots (ref. 9; unpublished data). Photoconversion of Pr to Pfr *in vivo* results in a rapid decline in phytochrome concentration because Pfr has a much greater rate of degradation than Pr (7, 8, 10, 11). Three hours after the onset of irradiation the concentration of phytochrome is decreased by $\approx 80\%$ in *Avena* (11, 12), eventually reaching a new steady state in continuous light at 1–3% of the initial etiolated-tissue concentration (10, 13). Return of the tissue to the dark results in reaccumulation of the photoreceptor as Pr (8, 14).

Previously this modulation of phytochrome concentration has been accounted for strictly at the protein level in terms of the disparate turnover rates of Pr and Pfr (8, 11). Implied in this view is a constant rate of *de novo* synthesis of the photoreceptor. However, the recent observation that no translatable phytochrome mRNA is detectable in fully green tissue grown in continuous white light (15, 16) has led us to consider the possibility of a second level of control. Here we examine the time course of the change in translatable phytochrome mRNA after irradiation of etiolated seedlings and identify the photoreceptor mediating this response. Some of these data have been reported (17).

MATERIALS AND METHODS

Plants. Seedlings of *Avena sativa* L. cv. Garry (Olds Seed, Madison, WI) were grown uncovered in moist vermiculite for 4 days in the dark at 25°C. Irradiations and harvesting of seedlings were performed under green safelight (18). Irradiations were given with a Kodak Ektagraphic slide projector fitted with a red (660 nm, Baird Atomic) interference filter or a far-red (>720 nm, Corning type CS7-69) cut-off filter. Unless otherwise noted, red irradiations were for 5 s (total dose, 137 nanoeinsteins $\cdot\text{cm}^{-2}$) and far-red irradiations, for 6 s (total dose, 6,080 nanoeinsteins $\cdot\text{cm}^{-2}$). After irradiation, seedlings were returned to the dark at 25°C for the periods indicated. Tissue for RNA isolation was frozen in liquid nitrogen and stored at -70°C until extraction. Tissue for phytochrome extraction was added to ice-cold medium and homogenized immediately.

Phytochrome Measurements. All phytochrome determinations were performed with a dual-wavelength spectrophotometer designed and constructed by L. H. Pratt (University of Georgia). For the measurement of *in vivo* destruction, tissue was extracted and phytochrome was determined in the crude homogenate with CaCO_3 as a scattering agent, as described (19). The percentage of phytochrome photoconverted to Pfr by varying doses of red light was measured *in vivo* at 0°C in the top 0.5 cm of etiolated coleoptiles with enclosed leaves (11). The photoequilibrium value of 86% Pfr in red light, recently determined for 124-kDa *Avena* phytochrome (unpublished data), was used in all calculations.

RNA Isolation. Extraction of total cellular RNA was performed according to Maniatis *et al.* (20) with several modifications. The 4 M guanidine thiocyanate reagent was buffered with 50 mM Tris $\cdot\text{HCl}$ (pH 7.6) and contained 2% (wt/vol) lauroyl sarcosine, 1% (vol/vol) 2-mercaptoethanol, and 10 mM vanadyl ribonucleoside complex. Stock 200 mM vanadyl ribonucleoside complex was prepared according to Berger and Birkenmeier (21). Frozen tissue was powdered in a mortar and then was added to the guanidine thiocyanate reagent at a ratio of 4–5 ml of reagent per gram of fresh weight of tissue. Homoge-

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.

nization was completed with an Ultra Turrax blender (Tekmar, Cincinnati, OH). After an initial centrifugation at $3,000 \times g$, the homogenate was made 2.4 M in CsCl and centrifuged at $30,000 \times g$ for 10 min. The resulting supernatant was layered over a 2-ml pad of 5.7 M CsCl in 0.1 M EDTA (pH 7.5) and centrifuged in a Beckman SW 41 Ti rotor at $209,000 \times g_{\max}$ for 18 hr at 7°C. The RNA pellet was then treated according to Maniatis *et al.* (20), except that storage was in high-salt buffer (10 mM Tris-HCl, pH 7.5/200 mM NaCl/1 mM EDTA) made 0.5% (wt/vol) in NaDodSO₄. Typically, 0.5 mg of RNA was obtained per gram of fresh weight of tissue. Poly(A)-RNA was isolated from total cellular RNA by two passages over oligo(dT)-cellulose (22). The yield of poly(A)-RNA was between 0.5% and 1.5% of the total RNA.

In Vitro Translation. Translations were performed with micrococcal nuclease-treated rabbit reticulocyte lysate (23). Nuclease treatment (30 μ g of nuclease per ml of lysate) was carried out at 20°C for 10 min. Final concentrations of all components of the lysate were: 0.71 mM Mg(OAc)₂; 100 mM KOAc; the 20 common L amino acids, excluding L-methionine, 0.04 mM each; 0.032 mM hemin; 0.2 mM GTP; 1 mM ATP; 0.76 mM

dithiothreitol; 11 mM creatine phosphate; creatine phosphokinase at 100 μ g/ml; and tRNA at 30 μ g/ml. [³⁵S]Methionine (>600 Ci/mmol; 1 Ci = 3.7×10^{10} Bq; Amersham) was added to a final concentration of 2–2.5 mCi/ml. Poly(A)-RNA was added to 15 μ g/ml and the translation mixtures were incubated for 1 hr at 30°C. To determine incorporation into total translation products duplicate 1.5- μ l aliquots were taken from each tube and were added to 2 ml of 0.5 M NaOH at 0°C. These samples were then incubated at 37°C for 20 min before 4 ml of 15% (wt/vol) trichloroacetic acid containing 20 mM unlabeled methionine was added. After an additional 45-min incubation at 0°C the trichloroacetic acid-precipitable material was collected on Whatman GF/C filters and the radioactivity was counted. Incorporation of [³⁵S]methionine into trichloroacetic acid-precipitable products was shown to respond linearly to poly(A)-RNA concentration up to 15 μ g/ml.

Immunoprecipitations. *In vitro*-synthesized phytochrome apoprotein was immunoprecipitated by using the solid-phase *Staphylococcus aureus* procedure (24, 25). The boiled translation mixtures were diluted with buffer [50 mM Tris-HCl, pH 7.5/150 mM NaCl/1 mM EDTA/2% (vol/vol) Triton X-100]

FIG. 1. The effect of red irradiation on the levels of phytochrome and phytochrome mRNA in etiolated *Avena* shoots. (Left) Time course of the decrease in translatable phytochrome mRNA in darkness after a 5-s, saturating red irradiation. At the times indicated poly(A)-RNA was isolated and translated and the phytochrome apoprotein (arrow) was immunoprecipitated. The immunoprecipitates were subjected to NaDodSO₄/polyacrylamide gel electrophoresis and were fluorographed. Molecular mass markers are given in kDa. Lane A, nonimmune control (N), unirradiated tissue. Lanes B–I, antiphytochrome IgG immunoprecipitates. Lane B, unirradiated tissue (U). Lanes C–I, 5 s of red light followed by incubation in the dark for the times indicated: lane C, 15 min; lane D, 30 min; lane E, 45 min; lane F, 60 min; lane G, 75 min; lane H, 90 min; and lane I, 120 min. The minor band at ≈ 58 kDa is thought to be a contaminant recognized by nonphytochrome antibodies in the antiphytochrome IgG preparations because this band is not observed in immunoprecipitates from phytochrome mRNA-containing fractions after size fractionation of poly(A)-RNA on sucrose gradients (15). (Right) Time course of the change in cellular phytochrome and translatable phytochrome mRNA in etiolated shoots in darkness after a saturating red (5 s) irradiation. At the times indicated, either poly(A)-RNA was isolated and translated or phytochrome was extracted and measured. The *in vitro* synthesized phytochrome apoprotein was immunoprecipitated, subjected to NaDodSO₄/polyacrylamide gel electrophoresis, and quantitated by scanning fluorographs of these gels (○—○). Data points are from four different experiments, including that in Left, and are expressed as a percentage of the initial level in the unirradiated control in each case. Phytochrome [$\Delta(\Delta A) \times 10^{-3}$] was measured spectrophotometrically in crude extracts with CaCO₃ as a scattering agent. Ptot = total amount of spectrally detectable phytochrome (●—●); Pr (▲—▲) and Pfr (■—■) = amounts of the two spectral forms that comprise Ptot. The levels of Pr and Pfr at time zero are those immediately after the 5-s red irradiation. Data points are the means of two different experiments in which each time point was duplicated.

and were centrifuged twice in a Microfuge at room temperature. Antiphytochrome IgG (2 μ g) or nonimmune IgG (2.2 μ g) was added to the supernatant and allowed to incubate for 45 min at room temperature before 12 μ l of a 10% (wt/vol) *S. aureus* suspension was added. After an additional 15 min, the *S. aureus* cells were pelleted through a 40% (wt/vol) sucrose pad and were further treated as described by Bolton and Quail (18).

NaDodSO₄/Polyacrylamide Gel Electrophoresis. Samples were electrophoresed on 10% polyacrylamide gels according to Laemmli (26) and fluorography was performed according to Laskey (27). The molecular mass markers were the same as reported (5). When total translation products were compared, equal amounts of trichloroacetic acid-precipitable radioactivity were applied to all lanes. Immunoprecipitates were applied to the gels in proportion to the [³⁵S]methionine incorporated into total translation products per microgram of poly(A)-RNA in the individual translation mixtures from which they were derived. This procedure corrected for the minor variations in total incorporation per microgram of poly(A)-RNA between samples, thus ensuring that all immunoprecipitates within an experiment were derived from equal amounts of total translation products.

Band intensity was quantitated by scanning fluorographs at 540 nm with a Gilford spectrophotometer. Fluorograph exposure times were such that maximal band intensity did not exceed $A_{540} = 1.5$ to ensure linearity of film response to the amount of radioactivity present (27). The proportion of the total translation products represented by the 124-kDa phytochrome apoprotein was estimated from the fluorographs as follows. The percentage of the total radioactivity in a gel lane actually pres-

ent in the 124-kDa band of an immunoprecipitate was determined by scanning the full length of the lane. This percentage was then used to estimate how much of the radioactivity measured directly in the immunoprecipitate by scintillation counting could be attributed to the phytochrome band itself (as opposed to other minor bands and to the top and bottom of the gel). This final value was then expressed as a percentage of the total trichloroacetic acid-precipitable radioactivity.

RESULTS

From the proportion of the total trichloroacetic acid-precipitable translation products that are immunoprecipitable as the 124-kDa phytochrome apoprotein, it was calculated that translatable phytochrome mRNA is only about 0.005% of the total poly(A)-RNA extractable from etiolated *Avena* shoots (Fig. 1 Left, lane B). Five seconds of red light caused the concentration of this translatable phytochrome mRNA to decline rapidly to yet lower levels (Fig. 1). After an apparent lag of about 15 min, a 50% decrease occurred within 50–60 min, with a >95% decrease occurring within 2 hr (Fig. 1 Right). This decline most likely represents a selective decrease in translatable phytochrome mRNA per cell because over the 3-hr experimental period no significant effect of irradiation on the following parameters was observed: (i) the amount of poly(A)-RNA per gram of fresh weight of tissue (unpublished data); (ii) the amount of radioactivity incorporated into trichloroacetic acid-precipitable total translation products per microgram of poly(A)-RNA (unpublished data); and (iii) the qualitative pattern of total translation products (Fig. 2, lanes A–D).

By comparison, onset of the decrease in amount of spectrally detectable phytochrome extracted from the tissue exhibited a lag of ≈ 60 min and required ≈ 150 min to decrease to 50% of the initial level. This pattern has been observed previously and the lag has been shown to be a function of both the developmental stage of the tissue (28) and the effects of ethylene when seedlings are grown in closed containers (29). The lag observed here is probably due to the developmental stage of the tissue rather than to ethylene, because the seedlings were grown in open containers.

Fig. 2 shows that phytochrome is the photoreceptor for the red light-induced decrease of its own translatable mRNA. The red light-induced decrease is reversed to the level of the far-red control by subsequent far-red irradiation. From the intensities of the 124-kDa bands in Fig. 2 it was calculated that the levels of translatable phytochrome mRNA in the red-, red plus far-red-, and far-red-irradiated tissues are 5%, 34%, and 37%, respectively, of the dark control value. The failure of the far-red irradiation to reverse the red light-induced effect to the level of the dark control, and the effect of far-red irradiation alone in causing a reduction in phytochrome mRNA concentration below the dark control level, is not an uncommon pattern in phytochrome-mediated responses (1, 2). This pattern suggests that the <1% Pfr established at photoequilibrium by far-red light (1, 2, 11) is already sufficient to induce a significant response.

This possibility was tested directly by constructing a red-light dose-response curve (Fig. 3). Pfr levels from <1% to 86% were established by increasing doses of red light. After a subsequent 2 hr of darkness, the tissue was harvested and the amount of translatable phytochrome mRNA was assayed. Substantial reductions in translatable phytochrome mRNA were induced by levels of Pfr well below the detection limit of the spectrophotometer. Approximately 1% Pfr was sufficient to decrease the translatable mRNA level by $\approx 60\%$, with the response saturating at $\approx 20\%$ Pfr.

FIG. 2. Autoregulatory control of translatable phytochrome mRNA levels. Etiolated seedlings were irradiated as indicated and returned to the dark for 3 hr before poly(A)-RNA was isolated and translated. Total translation products (lanes A–D) and immunoprecipitates of the phytochrome apoprotein from each translation (arrow, lanes E–H) were separated by NaDodSO₄/polyacrylamide gel electrophoresis and were fluorographed. Molecular mass markers are given in kDa. Lanes A and E, unirradiated control (U); lanes B and F, 5-s red light (R); lanes C and G, 5-s red plus 6-s far-red light (R/F); lanes D and H, 6-s far-red light (F).

DISCUSSION

The observation that phytochrome exerts autoregulatory control over its own translatable mRNA levels necessitates that present concepts of the control of phytochrome levels *in vivo* be revised. In the past, the well-documented, light-controlled modulation of phytochrome concentration *in vivo* (Fig. 1 *Right*) has been explained by the following generalized scheme (8, 11):

in which 0k_s = zero-order rate constant of synthesis; $^1k_d^{\text{Pr}}$ and $^1k_d^{\text{Pfr}}$ = first-order rate constants of degradation of Pr and Pfr, respectively, with $^1k_d^{\text{Pr}} \ll ^1k_d^{\text{Pfr}}$; and Pr' and Pfr' = degradation products of Pr and Pfr, respectively. In this scheme phytochrome levels are considered to be modulated strictly at the protein level by the disparate rate constants of degradation for Pr and Pfr against a background of constant Pr synthesis determined by an unchanging 0k_s . Clearly implied in this scheme is a constant level of translatable phytochrome mRNA supporting a constant rate of *de novo* synthesis of the chromoprotein. The data for spectrally measurable phytochrome in Fig. 1 *Right* illustrate the basis for this interpretation. The decline in total phytochrome over 3 hr in the dark after a red pulse results almost entirely from the destruction of Pfr. The level of Pr remains essentially constant throughout this period, consistent with the slow turnover rate estimated for this form of the molecule (8). The steady-state level of phytochrome established in continuous white light at 1–3% of that initially present in etiolated tissue (10, 13) is also consistent with the notion that 0k_s is constant and that $^1k_d^{\text{Pfr}}$ is 50–100 times greater than $^1k_d^{\text{Pr}}$ (8).

The data presented here indicate that the above scheme should be modified as follows:

Conversion to Pfr not only enhances degradation of the chromoprotein but also decreases the rate of synthesis due to a concomitant reduction in the level of translatable phytochrome mRNA. The degradation process might be considered "passive" in the sense that the enhanced rate appears to result simply from exposure of Pfr to the cellular environment, whereas the decreased mRNA synthesis appears to involve active regulation by Pfr. The feedback inhibition of translatable phytochrome mRNA production appears to be reversible upon depletion of Pfr in extended darkness. Gottmann and Schäfer (16) have reported the reappearance of detectable levels of translatable phytochrome mRNA in green tissue returned to the dark for 10–24 hr.

In this revised scheme, the short-term modulations in levels of the photoreceptor that accompany dark/light transitions are still dominated by the differential turnover rates of Pr and Pfr as previously visualized. The constant level of Pr over the present experimental period (3 hr) indicates that the large reduction in rate of synthesis presumed to result from the decline in translatable mRNA (Fig. 1 *Right*) has no significant short-term effect on total phytochrome levels. However, in the long term, new steady-state levels of phytochrome established under continuous irradiation are expected to involve a balance between the prevailing rate of Pfr destruction and the presumably much slower rate of synthesis caused by the Pfr-induced reduction in

FIG. 3. Translatable phytochrome mRNA and spectrally measurable Pfr each as a function of red light dose. For translatable mRNA determinations etiolated seedlings were irradiated as indicated and returned to the dark for 2 hr before isolation and translation of poly(A)-RNA. The *in vitro* synthesized phytochrome apoprotein was immunoprecipitated, subjected to NaDodSO₄/polyacrylamide gel electrophoresis, and was quantitated as in Fig. 1 (○—○). The data are from two separate experiments and are expressed as a percentage of the unirradiated control in each case. The % Pfr formed by the red light irradiations was determined by *in vivo* spectrophotometry of etiolated shoots at 0°C (●—●). (Inset) Translatable phytochrome mRNA as a function of Pfr level. Data from the main body of the figure were replotted to show the relationship between the level of translatable phytochrome mRNA and the % Pfr produced by various doses of red light (Δ—Δ).

translatable mRNA. Why then are the new steady-state levels of the photoreceptor in continuous light observed to be as high as 1–3% of the initial, etiolated tissue concentration (10, 13)? If the rate of destruction of the chromoprotein increases 50- to 100-fold and the rate of synthesis declines >20-fold upon Pfr formation, the level of phytochrome in continuous light should be more than an order of magnitude lower than observed.

In the absence of conclusive experimental evidence, several alternative explanations of this discrepancy are possible. These include the following: (i) It might be expected from Fig. 3 that, as the absolute Pfr level drops below a few percent of that initially present, the feedback inhibition will be partially relieved and the translatable phytochrome mRNA level will begin to rise again, thereby increasing the rate of phytochrome synthesis once more. For example, when total phytochrome drops to 1% of the etiolated tissue level [Pfr, 0.6% in continuous white light (2)], the translatable phytochrome mRNA level corresponds to 30–40% of the level in the etiolated tissue (Fig. 3). Thus, the steady-state level of translatable phytochrome mRNA may be higher in continuous light than predicted from the decline observed after 5-s red light (Fig. 1). (ii) There may be a time-dependent decrease in the rate constant for Pfr destruction that compensates for the decline in rate of synthesis under prolonged irradiation. (iii) There may be two populations of phytochrome: one major, with a high Pfr destruction rate, and one minor, with a low Pfr destruction rate. The major population would be rapidly depleted upon transfer of etiolated seedlings to light, leaving the more stable, minor population to predominate under prolonged irradiations. A recent study of the kinetics of Pfr destruction in *Amaranthus* is consistent with this latter notion (30). The two populations theoretically could represent physically separate but otherwise identical pools of molecules within the same cell or in separate cells or they could represent two distinct gene products with intrinsically different turnover rates and immunological properties. The failure to detect an immunoprecipitable phytochrome translation product among the total cell-free translation products encoded by green tissue poly(A)-RNA (15, 16) when spectrally measurable phytochrome is present in the tissue (13, 31) may be indicative of such a different phytochrome gene product in green tissue.

Study of the Pfr-induced change in translatable phytochrome mRNA levels may contribute to the broader question of phytochrome-regulated gene expression. Phytochrome has been shown previously to control the expression of mRNAs for ribulose 1,5-bisphosphatase carboxylase (32), chlorophyll a/b binding protein (32, 33), protochlorophyllide reductase (34), and several unidentified mRNAs (35). Ironically, however, phytochrome mRNA, together with protochlorophyllide reductase mRNA (34), exhibits the most rapid change in a phytochrome-regulated mRNA level thus far recorded (15 min lag) and represents one of the most rapid changes in any plant cellular mRNA (36–38). The rapidity of this autoregulation should help facilitate investigations of the sequence of events between light-signal perception and altered gene expression by defining a relatively short period over which the relevant events need be sought. In addition, because phytochrome mRNA is a low-abundance species, it may be considered more representative of the majority of structural genes expressed in eukaryotic cells than the relatively high-abundance mRNAs that have been most intensively studied in plants to date (32–34, 39–41). Thus, information gained from the phytochrome system may contribute to the general question of the regulation of gene expression.

We thank L. H. Pratt for initial assistance in producing antiphytochrome IgG and W. R. Briggs, in whose laboratory the antiphytochrome IgG was produced. R. D. Vierstra provided valuable comments on the manuscript. This work was supported in part by National Science Foundation Grants PCM 8003921, PCM 7723584, and PCM 7514161 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.

- Mohr, H. (1972) *Lectures in Photomorphogenesis* (Springer, Berlin).
- Smith, H. (1975) *Phytochrome and Photomorphogenesis* (McGraw-Hill, London).
- Fry, K. T. & Mumford, F. E. (1971) *Biochem. Biophys. Res. Commun.* **45**, 1466–1473.
- Lagarias, J. C. & Rapoport, H. (1980) *J. Am. Chem. Soc.* **102**, 4821–4828.
- Vierstra, R. D. & Quail, P. H. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5272–5276.
- Pratt, L. H. (1982) *Annu. Rev. Plant Physiol.* **33**, 557–582.
- Pratt, L. H. (1979) *Photochem. Photobiol. Rev.* **4**, 59–124.
- Quail, P. H., Schäfer, E. & Marmé, D. (1973) *Plant Physiol.* **52**, 128–131.
- Bolton, G. W. (1979) Dissertation (Univ. of Minnesota, St. Paul, MN).
- Hunt, R. E. & Pratt, L. H. (1979) *Plant Physiol.* **64**, 327–331.
- Schäfer, E., Lassig, T.-U. & Schopfer, P. (1975) *Photochem. Photobiol.* **22**, 193–202.
- Pratt, L. H., Kidd, G. H. & Coleman, R. A. (1974) *Biochim. Biophys. Acta* **365**, 93–107.
- Jabben, M. & Deitzer, G. F. (1978) *Planta* **143**, 309–313.
- Hunt, R. E. & Pratt, L. H. (1980) *Plant Cell Environ.* **3**, 91–95.
- Quail, P. H., Bolton, G. W., Hershey, H. P. & Vierstra, R. D. (1982) in *Current Topics in Plant Biochemistry-Physiology*, ed. Randall, D. (Univ. of Missouri, Columbia, MO).
- Gottmann, K. & Schäfer, E. (1982) *Photochem. Photobiol.* **35**, 521–525.
- Hershey, H. P., Colbert, J. T., Murray, M. G. & Quail, P. H. (1982) in *Structure and Function of Plant Genomes*, ed. Ciferri, O. (NATO Conference, Porto Portese, Italy), pp. 65 (abstr.).
- Bolton, G. W. & Quail, P. H. (1982) *Planta* **155**, 212–217.
- Quail, P. H. & Briggs, W. R. (1978) *Plant Physiol.* **62**, 773–778.
- Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning—A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 196.
- Berger, S. L. & Birkenmeier, C. S. (1979) *Biochemistry* **18**, 5143–5149.
- Bantle, J. A., Maxwell, I. H. & Hahn, W. E. (1976) *Anal. Biochem.* **72**, 413–427.
- Pelham, R. B. & Jackson, R. J. (1976) *Eur. J. Biochem.* **67**, 247–256.
- Kessler, S. W. (1975) *J. Immunol.* **115**, 1617–1624.
- Ivarie, R. D. & Jones, P. P. (1979) *Anal. Biochem.* **97**, 24–35.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
- Laskey, R. A. (1980) *Methods Enzymol.* **65**, 363–371.
- Kidd, G. H. & Pratt, L. H. (1973) *Plant Physiol.* **52**, 309–311.
- Stone, H. J. & Pratt, L. H. (1978) *Plant Physiol.* **62**, 922–923.
- Brockman, J. & Schäfer, E. (1982) *Photochem. Photobiol.* **35**, 555–558.
- Bolton, G. W. & Quail, P. H. (1981) *Plant Physiol.* **67**, S104.
- Tobin, E. M. (1981) *Plant Mol. Biol.* **1**, 35–51.
- Apel, K. (1979) *Eur. J. Biochem.* **97**, 183–188.
- Apel, K. (1981) *Eur. J. Biochem.* **120**, 89–93.
- Everett, M. S., Polans, N., Jorgensen, R. A., Palmer, J. D. & Thompson, W. F. (1982) in *Structure and Function of Plant Genomes*, ed. Ciferri, O. (NATO Conference, Porto Portese, Italy), p. 64 (abstr.).
- Theologis, A. & Ray, P. M. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 418–421.
- Walker, J. C. & Key, J. L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 7185–7189.
- Zurfluh, L. L. & Guilfoyle, T. J. (1982) *Plant Physiol.* **69**, 332–337.
- Hall, T. C., Sun, S. M., Buchbinder, B. U., Pyne, J. W., Bliss, F. A. & Kemp, J. D. (1980) in *Genome Organization and Expression in Plants*, ed. Leaver, C. J. (Plenum, New York), pp. 259–273.
- Meinke, D. W., Chen, J. & Beachy, R. N. (1981) *Planta* **153**, 130–139.
- Larkins, B. A., Pedersen, K., Hurkman, W. J., Handa, A. K., Mason, A. C., Tsai, C. Y. & Hermodson, M. A. (1980) in *Genome Organization and Expression in Plants*, ed. Leaver, C. J. (Plenum, New York), pp. 203–218.