A potential role for RNA turnover in the light regulation of plant gene expression: ribulose-1,5-bisphosphate carboxylase small subunit in soybean

B.W.Shirley+ and R.B.Meagher*
Department of Genetics, University of Georgia, Athens, Georgia 30602, USA

Received October 26, 1989; Revised and Accepted April 20, 1990

ABSTRACT

Post-transcriptional regulation of the genes encoding the small subunit (rbcS) of ribulose-1,5-bisphosphate carboxylase was examined in soybean seedlings. Substantial discrepancies were detected between relative *in vitro* transcription rates and steady-state RNA levels in light- and dark-grown seedling leaves, indicating that rbcS RNA may be degraded more rapidly in light than in darkness. Additional data imply that the turnover mechanism is rapidly induced by light, maintained for some time in darkness, and that it may be negatively controlled by far-red light. The proposed RNA turnover system does not affect all RNAs equally since a soybean actin gene showed equivalent *in vitro* transcription rates and RNA levels in light and darkness. Soybean rbcS genes may be subject to a novel mode of control in which light-induced expression is accompanied by an increased rate of RNA degradation. Models for the specific regulation of rbcS RNA stability in response to light are presented.

INTRODUCTION

Increasing evidence points to the fundamental role of post-transcriptional processes such as RNA turnover in the control of gene expression. Complex mechanisms for the regulation of histone and tubulin mRNA degradation have recently been described (1, 2). In these cases nucleases associated with the translation machinery may control RNA degradation (3). However, the specific post-transcriptional mechanisms that control RNA levels have not been identified in most systems.

The nuclear-encoded small subunit (rbcS) of the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase (Rubisco) has been a model system for the study of light-regulated gene expression [reviewed by Tobin and Silverthorne (4) and Watson (5)]. The steady-state levels of rbcS mRNA have been shown to be higher in light than in darkness in many plants, including amaranth (6), *Lemna* (7), maize (8, 9), mung bean (10), pea (10, 11, 12), petunia (13, 14), radish (15), rye (16), soybean (17, 18), and tobacco (19) and in the green alga, *Chlamydomonas* (20). Far-red light has been found to reverse white or red light induction of rbcS RNA levels in *Lemna* (21), maize (9), mung bean (10), pea (10) and rye (16), indicating that the chromoprotein, phytochrome, is involved in light regulation of rbcS expression in these plants.

Changes in rbcS RNA levels in response to light are regulated, at least in part, at the level of transcription. This has been demonstrated in *Lemna* (22), pea (23), petunia (24), rye (16) and soybean (25) by examining the effects of light on rates of transcription *in vitro* in isolated nuclei. In addition, the 5′ flanking sequences of the genes from pea (26, 27), soybean (24, 28, 29) and tobacco (19) have been shown to function as light-inducible promoters in transgenic plant tissue.

Translational and post-translational regulation of SSU expression has also been described in some organisms. In volvox (30) and amaranth (31) pulse-labeling experiments have shown that synthesis of SSU proteins changes rapidly in response to light in the absence of changes in rbcS RNA levels. These data indicate that light regulates the translation of rbcS RNA. Furthermore, pulse-chase labeling of proteins in a LSU-deficient mutant of *Chlamydomonas* demonstrated that, in the absence of LSU protein, SSU protein is degraded (32). Thus in wild type *Chlamydomonas* accumulation of SSU protein may also be regulated post-translationally.

In this study the *in vitro* transcription rates and steady-state RNA levels of the soybean rbcS genes were compared in light-grown and etiolated (dark-grown) seedlings. Changes in rbcS expression in response to white light, darkness and far-red light were also examined. The results of these experiments indicate that rbcS RNA is degraded more rapidly in light than in darkness. These experiments also indicate that light regulates the degradation of rbcS RNA independent of the turnover of most mRNAs. A potential link between transcription rates and the levels of SSU and LSU polypeptides was examined in greening soybean leaves.

* To whom correspondence should be addressed

+ Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA 02114, USA

MATERIALS AND METHODS

Growth and light conditions for soybean seedlings

Seeds of *Glycine max* (var. Wayne or Pella '86) were soaked in water for 1h and then grown in vermiculite at 28°C in darkness or in a 12 h (6AM/6PM) light/dark cycle. The growth conditions were changed from the continuous light of lower intensity used previously (25) in order to more closely duplicate the conditions found in nature. In experiments examining rbcS RNA levels in light-grown seedlings or in etiolated seedlings shifted to the light, rbcS RNA accumulation was the same whether a 12 h light/dark cycle or a lower level of continuous light was used (data not shown). Primary leaves from eight day old light-grown or etiolated seedlings or from seedlings that were eight days old at the start of light treatment were used in these experiments. Far-red light or dark treatment of light-grown seedlings and light treatment of etiolated seedlings were always started in the middle of the light cycle (noon). Far-red light treatments were performed as described in Berry-Lowe and Meagher (25). Leaves were harvested directly into liquid nitrogen and stored at −70°C.

Probes

rbcS gene expression was measured using the genomic clone, pSRS0.8 (17), which contains the 3′ EcoRI fragment of the soybean rbcS gene, SRS1, including the 2nd intron, 3rd exon and 300 nt of 3′ untranslated sequences. Actin gene expression was measured using pSAc6 (33), a genomic clone containing the entire transcribed region of the soybean actin gene, SAc6, beginning 20 nt upstream of the cap site and extending approximately 200 nt past the termination codon (L. Pearson and R.B. Meagher, unpublished data). In run-on transcription assays, levels of 18S rRNA transcripts were determined using the clone, pSR1.2B3 (34), containing a 1071 nt BamHI-EcoRI fragment internal to the soybean 18S rRNA gene. A synthetic oligonucleotide probe, complimentary to nt 1764−1803 of the soybean 18S rRNA gene, was used to determine 18S rRNA levels on RNA dot blots (18, 34).

For transcription assays the plasmid clones were applied to nitrocellulose filters and hybridized to [α-^{32}P]UTP labeled run-on transcripts as described in Berry-Lowe and Meagher (25). Northern blots and RNA dot blots were hybridized with plasmid inserts purified on DE-81 paper (35) and labeled with [α-^{32}P]dATP by the random primer method (36). Hybridizations were in a solution containing 3× SSC and 50% formamide (25); filters were washed 4× 10 min in 0.2× SSC/0.2% SDS. Filters containing DNA clones were hybridized to run-on transcripts and washed at 56°C. Northern blots and RNA dot blots were hybridized and washed at 56°C for the rbcS probe and at 42°C for the actin probe. The oligonucleotide probe for 18S rRNA was 5′-end labeled using polynucleotide kinase and [γ-^{32}P]ATP (37). Filters were hybridized in 2× SSC, 0.2% SDS, 0.25% dry milk (38) at 60°C. The filters were washed in 2× SSC, 0.2% SDS, 2× 10 min at 25°C and 1× 1 min at 60°C. Under these conditions, the rRNA probe was specific for the 18S rRNA gene on genomic Southern blots.

Studies on several rbcS gene families including that in soybean, demonstrate that rbcS coding sequences from all gene family members are homogenized by gene conversion and thus have significant sequence homology within a species (39). Under the hybridization and wash conditions used in these experiments the rbcS probe binds to a single band on Northern blots (Figure 1A). On Southern blots at 51°C this probe hybridizes strongly to the previously identified SRS1 and SRS4 genes and one additional family member (17, 18), and weakly to three other members of the rbcS gene family (data not shown). Genomic and cDNA sequences demonstrate that SRS1 and SRS4 are 95% homologous in sequence, account for greater than 80% of all rbcS transcripts in soybean leaves and exhibit parallel patterns of expression in response to light (40). Thus the rbcS DNA probe used in these experiments measures the combined transcription rates and RNA levels for the soybean rbcS gene family.

The actin probe hybridizes strongly to the soybean gene, SAc6, and weakly to the other kappa actin gene, SAc1, but does not hybridize to the other four known soybean actin genes on genomic Southern blots at 52°C (33). SAc6 is one of the most highly expressed of the soybean actin genes. The probe hybridizes to a single band on Northern blots at 42°C (data not shown) and at 56°C (33). Therefore run-on transcripts hybridized to DNA dot blots at 56°C are assumed to reflect transcription of only the SAc6 and SAc1 genes. Although RNA dot blots were hybridized to the SAc6 probe at 42°C, these experiments still measured actin RNA primarily from SAc6 and SAc1, members of the kappa class of soybean actin genes.

Northern blots and RNA dot blots

Total RNA was extracted from soybean leaves and roots according to the method of Hall *et al.* (41). For Northern blots, RNA was electrophoresed in 1.5% agarose formaldehyde gels (42) and electrophoretically transferred (43) to Biotrans filters (I.C.N.). RNA dot blots were prepared by direct application of RNA to Biotrans filters which results in higher retention of RNA than does suction blotting (33).

Quantification

As in previous studies, dilution series of RNA or DNA on dot blots were used instead of Northern or Southern blots to more accurately quantify relative *in vitro* transcription rates (25) and RNA levels (18, 33, 40, 64). Measurements of RNA levels were made by hybridizing dilution series of RNA immobilized on filters with excess amounts of DNA probes. In transcription assays sufficient DNA was applied to filters so that the hybridization reaction was not limited by these probes. This was previously confirmed for the rRNA standard, which is estimated to comprise 50% of total *in vitro* transcription (25). However, the amount of DNA in each dot also affects the efficiency with which it competes for the RNA in solution, the limiting component in these hybridization reactions. Thus the two-fold dilution series of filter-bound DNA provide a linear range of concentrations which can interact with various levels of labeled input RNA (25).

Based on previous work (25, 33) the following criteria were established for using dot blots in these types of experiments: 1. Positive and negative controls are included in order to determine the specificity of hybridization and wash conditions in individual experiments. 2. A two fold dilution series of equivalent amounts of RNA or DNA (based on A_{260} measurements) is examined and only dots of similar hybridization intensity on autoradiograms are compared. The amount of dilution giving dots of similar intensity defines the relative levels of a specific RNA in different samples. The dilution series used in these assays covers a 132 fold range. Whenever possible, three or more dots of similar intensity are compared between lanes to control for small errors in applying samples and the nonlinear response of X-ray film. This method of quantification was validated by comparisons of densitometric scanning of autoradiograms and radioactive counting of numerous blots (25). 3. Differences in individual

transcription assay experiments are normalized to transcription of ribosomal RNA or to the transcription rates of another appropriate control RNA such as that encoding actin. 4. Filters are not reused. Duplicate RNA dot blots are hybridizedwith the rRNA probe in order to confirm that all RNA samples are loaded equivalently. 5. RNA samples are always spotted and dried on the filters rather than applied by suction blotting in order to increase efficiency and linearity of binding for all samples (33).

These methods were used to determine only relative levels of expression among samples in the work presented herein. All experiments quantifying *in vitro* transcription rates and RNA levels were performed a minimum of three times and when appropriate one representative experiment is presented.

Western blots

Crude protein extracts were made by freezing tissue in liquid nitrogen and grinding with a mortar and pestle. Tissue (120 mg) was extracted with Laemmli SDS sample buffer (0.5 ml; 44) at 100°C for 15 min and the debris removed by centrifugation two times at 11,000×g. Protein concentrations were normalized based on Coomassie staining of an SDS-PAGE gel. Approximately equal amounts of protein were separated by SDS-PAGE using 12.5% polyacrylamide gels and then electrophoretically transferred (45) to Immobilon filters (Millipore). Duplicate filters were stained with Coomassie blue or incubated with antibodies against denatured *Chlamydomonas* SSU or LSU polypeptides (46). Immunoblots were incubated with peroxidase-conjugated goat anti-rabbit IgG antibodies (1:10,000 dilution; Calbiochem) and the antigen-antibody complexes were visualized with the substrate, 4-chloro-1-naphthol (Sigma).

RESULTS

rbcS RNA may be more stable in etiolated seedlings than in light-grown seedlings

The *in vitro* transcription rates measured for the rbcS genes are approximately 32-fold higher in leaves from light-grown soybean seedlings than in leaves from seedlings grown in darkness relative to actin and ribosomal RNA. A minimum of a 32-fold difference has been observed in numerous repetitions of this experiment. This confirmation of the results of Berry-Lowe and Meagher (25) is summarized in Figure 1C.

In order to determine whether this transcription rate difference is reflected at the steady-state RNA level, the amount of rbcS RNA in light-grown and etiolated soybean leaves was compared. Dot blots containing two fold dilution series of total RNA samples were hybridized to DNA probes for rbcS, actin and 18S ribosomal RNA (Figure 1B). The rbcS probe gave a similar hybridization signal for the 1st through 5th dots of RNA from dark-grown leaves and for the 4th through 8th dots of RNA from light-grown leaves. Therefore leaves from light-grown seedlings contained eight fold more rbcS RNA per μg of total RNA than did leaves from etiolated seedlings. This eight fold difference in rbcS steady-state RNA levels was the maximum observed in several independent experiments. One experiment showed only a four fold difference in rbcS steady-state RNA levels. The steady-state RNA levels for the actin gene were the same in RNA samples from light- and dark-grown leaves (Figure 1B). Thus actin RNA levels were not controlled by light relative to the total RNA population. Hybridization of an identical filter with a probe for soybean 18S rRNA, which together with 26S rRNA

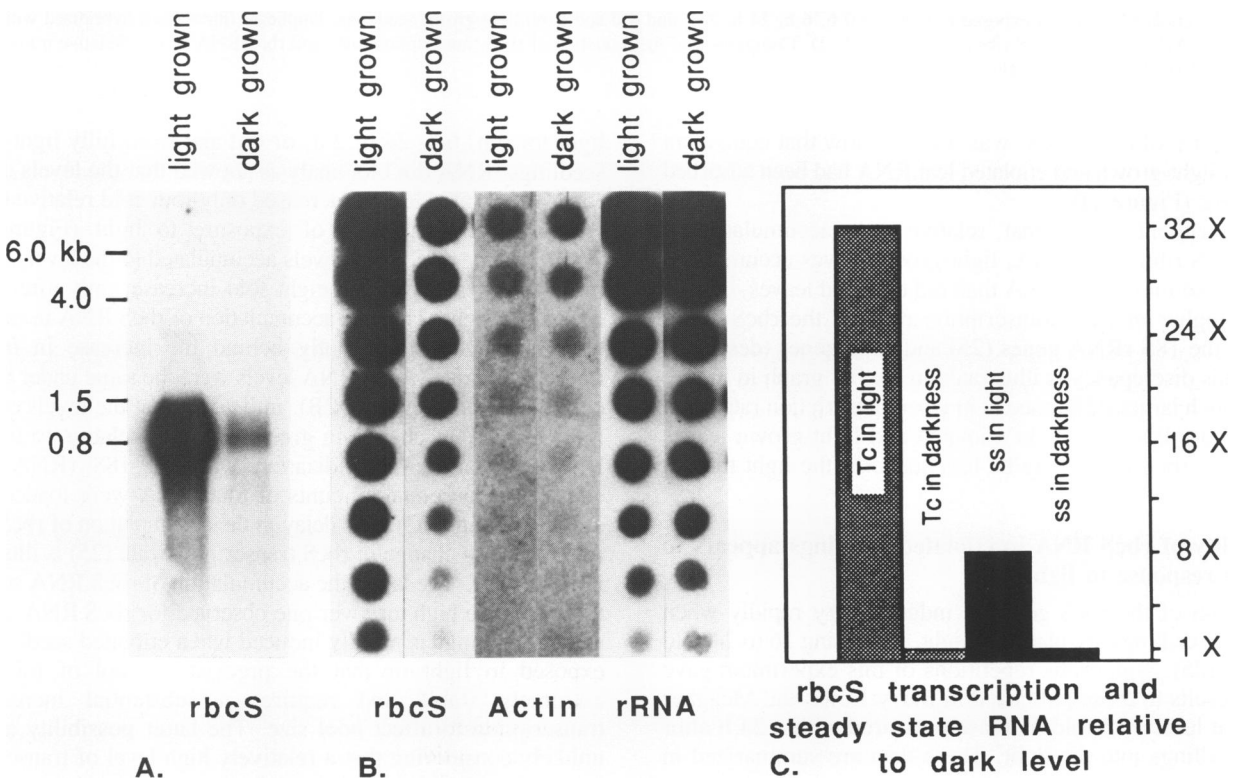

Figure 1. *Comparison of rbcS transcription and steady-state RNA levels in leaves of light-grown and etiolated seedlings.* A. Northern blot of total RNA (20 μg per lane) hybridized with pSRS0.8. B. RNA dot blots containing total RNA applied in two fold dilution series (10 μg in the first dot) hybridized with DNA probes for rbcS, actin and 18S ribosomal RNA. C. Bar diagram comparing rbcS steady-state RNA levels (ss) and transcription rates (tc) in light-grown and dark-grown soybean leaves.

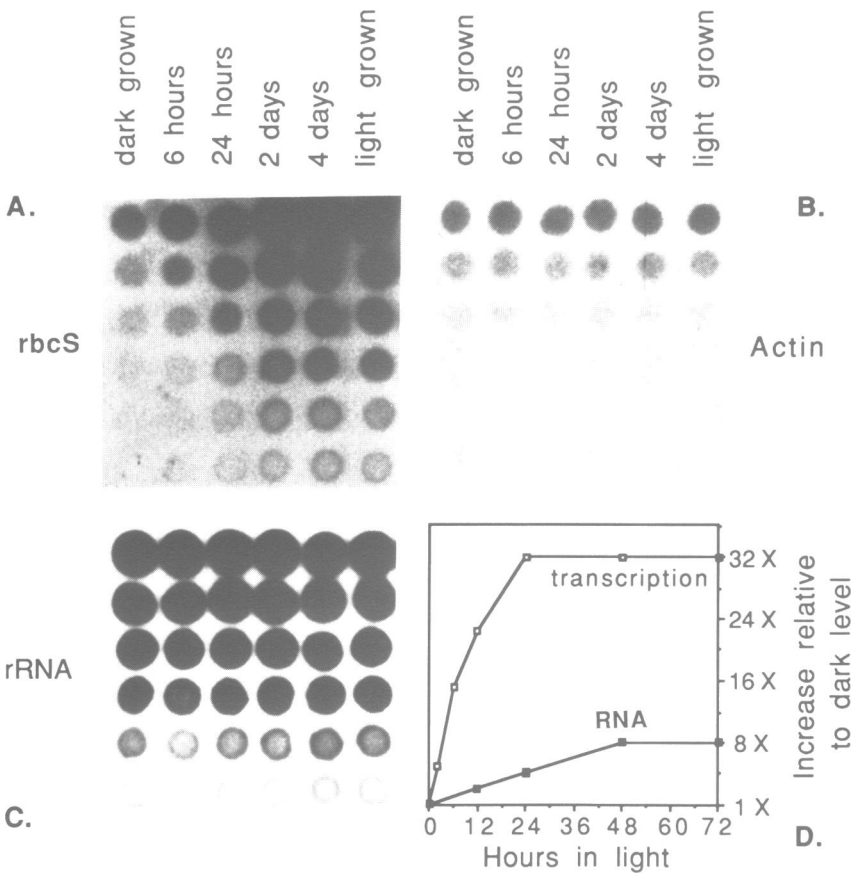

Figure 2. *Time course of accumulation of rbcS RNA in etiolated seedlings exposed to light.* Dot blots contained two fold dilution series (10 μg in the first dot) of total RNA from etiolated seedlings exposed to light for 0 h, 6 h, 24 h, 2 d, and 4 d and from light-grown seedlings. Duplicate filters were hybridized with DNA probes for rbcS (A), actin (B) or 18S ribosomal (C) RNA. D. Comparison of the induction of rbcS transcription rates and rbcS RNA levels. Relative transcription rates are from Berry-Lowe and Meagher (25).

comprises 90% of total RNA, was used to show that equivalent amounts of light-grown and etiolated leaf RNA had been adsorbed to the filters (Figure 1B).

This experiment showed that, relative to the accumulation of actin and 18S ribosomal RNA, light-grown leaves accumulated only eight-fold more rbcS RNA than did etiolated leaves, despite a 32-fold higher *in vitro* transcription rate for the rbcS genes relative to the 18S rRNA genes (25) and actin genes (described below). This discrepancy is illustrated in the bar graph in Figure 1C. The much larger difference in *in vitro* transcription rates than in steady state RNA levels in etiolated and light-grown leaves suggests that rbcS RNA may be less stable in the light than in darkness.

The stability of rbcS RNA in etiolated seedlings appears to change in response to light

Transcription of the rbcS genes is induced very rapidly when dark-grown seedlings are placed in light, increasing 16 to 24-fold within 6 h (25). Numerous repetitions of this experiment gave identical results to those published in Berry-Lowe and Meagher (25) with at least a 32-fold increase measured within 24 h after shifting seedlings into the light. These data are summarized in Figure 2D.

A change in rbcS RNA stability in response to light should influence the rate at which rbcS RNA accumulates in these plants. To examine the effect of light induction on rbcS RNA levels, total RNA was isolated from dark-grown seedlings placed in the light for 0 h, 6 h, 24 h, 2 d, or 4 d and from fully light-grown seedlings. RNA dot blot analysis showed that the levels of rbcS RNA in etiolated leaves increased only four fold relative to total RNA in the first 24 h of exposure to light (Figure 2A). Furthermore, rbcS RNA levels accumulated to the levels present in light-grown leaves, an eight fold increase, only after 2 to 4 days in the light. Thus the accumulation of rbcS RNA in response to light lagged significantly behind the increase in *in vitro* transcription rate. Actin RNA levels were the same under all light conditions tested (Figure 2B), indicating that the levels of some mRNAs did not change in greening leaves relative to the total RNA population. Hybridization with the 18S rRNA probe confirmed that equal amounts of total RNA were loaded in all samples (Figure 2C). The delay in the accumulation of rbcS RNA relative to the change in rbcS transcription rate (25) is illustrated in Figure 2D. The lag in the accumulation of rbcS RNA suggests that either the high turnover rate observed for rbcS RNA in light-grown seedlings is rapidly induced when etiolated seedlings are exposed to light or that the preexisting pool of mRNA is extremely stable and requires a substantial increase in transcription to affect pool size. The latter possibility appears unlikely considering that a relatively high level of transcription (approximately 0.04% of total) was measured in etiolated seedlings (25). It is important to note that the transcription rate and amount of rbcS RNA reach a new steady state level and this allows the RNA turnover rate after light induction to be calculated relative to the turnover rate in darkness (see Discussion).

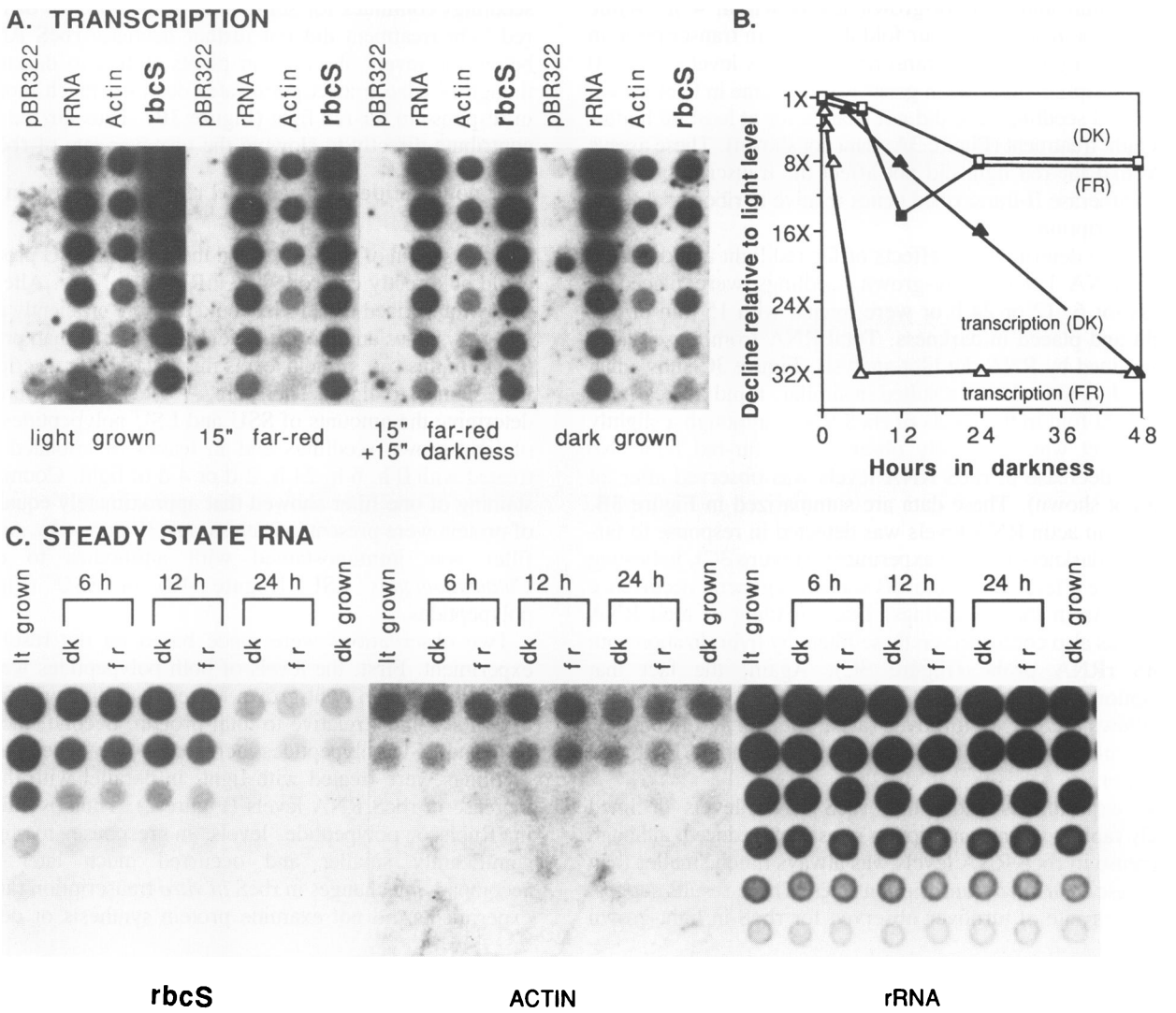

Figure 3. *Time course of decrease in rbcS transcription and RNA levels in light-grown seedlings placed in darkness or treated with far-red light.* A. Run-on transcription assays. DNA dot blots containing pSR1.2B3 (0.25 μg in the first dot), pSB1, pSAc6 and pBR322 (each 2.5 μg in the first dot) were hybridized with run-on transcripts from leaves of light-grown, dark-grown or far-red light treated soybean seedlings. B. Comparison of the rates of decrease in rbcS transcription and rbcS RNA levels. C. RNA levels. RNA dot blots contained two fold dilution series of total RNA (10 μg in the first dot) from light-grown seedlings exposed to darkness or treated with 15 min of far-red light and placed in darkness for 0 h, 6 h, 12 h and 24 h and from etiolated seedlings. Filters were hybridized with DNA probes for rbcS, actin and 18S ribosomal RNA.

rbcS RNA stability appears to change in light-grown seedlings in response to darkness and far-red light

A change in rbcS RNA stability should also influence the rate at which rbcS RNA levels change when rbcS transcription is reduced. Thus rbcS transcription rates and RNA levels were compared in light-grown seedlings treated with darkness and with far-red light.

Berry-Lowe and Meagher (25) showed that placing light-grown seedlings in darkness results in a gradual decline in rbcS transcription, with a 16-fold change occurring in the first 24 h (summarized in Figure 3B). This study also showed that rbcS transcription is reduced at least 16-fold when light-grown seedlings are treated with far-red light and placed in darkness for only 2 h. Similar changes in *in vitro* transcription rates in response to darkness and far-red light have also been observed in transgenic petunia plants using a soybean rbcS promotor fused to a reporter gene (24).

To determine the actual rate at which far-red light reduces rbcS transcription, light-grown seedlings were treated with 15 min of far-red light and shifted to darkness for 0 min, 15 min, 45 min, 1.75 h, 5.75 h, 24 h and 48 h. The levels of rbcS and actin gene transcription in the leaves of these seedlings and in leaves from light- and dark-grown seedlings were compared in nuclear run-on assays. Run-on transcripts labeled with [α-^{32}P]UTP were hybridized to dot blots of two fold dilution series of control (pBR322), 18S ribosomal, actin and rbcS DNA. Hybridization to 18S ribosomal DNA was used to normalize between filters so that levels of rbcS and actin gene transcription were measured relative to the level of rRNA transcription in each sample (Materials and Methods; 25). Figure 3A shows representative filters for the earliest portion of the response to far-red light. The results for all time points are graphed in Figure 3B and are representative of seveal repetitions of these experiments. Far-red light treatments reduced rbcS gene transcription four fold

within 30 min and to dark-grown levels within 4 h. While darkness alone results in a four fold decrease in transcription in 24 h, far-red light reduced transcription to this level within 30 min. Transcription of the actin genes was the same in light-grown and etiolated seedlings and did not change for at least 48 h after far-red light treatment (Figure 3A; data not shown). These assays indicate that far-red light did not affect the transcription of all RNA polymerase II-transcribed genes relative to ribosomal RNA gene transcription.

In order to determine the effects of far-red light and darkness on rbcS RNA levels, light-grown seedlings were placed in darkness for 6, 12 or 24 h or were treated with 15 min of far-red light and placed in darkness. Total RNA from these plants was examined by RNA dot blot analysis. Figure 3C shows that far-red light and darkness resulted in similar, rapid decreases of four to eight fold in the levels of rbcS RNA, although a slightly greater effect was repeatedly observed for far-red light. No additional decrease in rbcS RNA levels was observed after 24 h (data not shown). These data are summarized in Figure 3B. No change in actin RNA levels was detected in response to far-red light or darkness in these experiments (Figure 3C), indicating again that the effect on RNA levels was not a general occurrence for all RNAs in these seedlings. Equal loading of total RNA samples was also confirmed on these filters by hybridization with the 18S rRNA probe (Figure 3C). Again, the fact that transcription rates and amount of RNA reach new steady state levels allows rbcS RNA turnover rates, after far red treatment, to be calculated relative to the turnover rate in the light (see Discussion).

These experiments show that rbcS RNA levels declined relatively rapidly when transcription rates were reduced, although the decrease in rbcS RNA levels was always much smaller than the decrease in *in vitro* transcription rates. These results suggest that the high rate of turnover observed for rbcS in light-grown seedlings continues for some time in the absence of light. Far-red light treatment did not further decrease rbcS RNA levels below the levels observed in plants shifted to darkness, even though *in vitro* transcription rates declined much more rapidly in response to far-red light (Figure 3B). Thus far-red light may contribute directly to slowing the turnover rate of rbcS RNA.

SSU polypeptide levels parallel rbcS RNA levels in response to light

The translation of rbcS RNA and the resulting SSU protein levels could be directly controlled by mRNA pool size. Alternatively, newly transcribed rbcS RNA could be more efficiently assembled into polysomes and more efficiently translated than pre-existing RNA. In this case protein levels might parallel transcription rates in response to light. Therefore, Western blots were used to determine the amounts of SSU and LSU polypeptides in leaves of light-grown seedlings and in leaves of etiolated seedlings treated with 0 h, 6 h, 24 h, 2 d or 4 d of light. Coomassie blue staining of one filter showed that approximately equal amounts of protein were present in each sample (Figure 4A). An identical filter was immunostained with antibodies to denatured *Chlamydomonas* LSU (Figure 4B) or SSU (Figure 4C) polypeptides.

Two observations were made based on the results of this experiment. First, the levels of both polypeptides were five to ten fold higher in light-grown leaves than in leaves from dark-grown seedlings relative to total protein. Second, the levels of SSU and LSU polypeptides increased slowly when dark-grown seedlings were treated with light, in parallel with the gradual increase in rbcS RNA levels (Figure 2). Thus overall changes in Rubisco polypeptide levels in response to light were significantly smaller and occurred much later than the accompanying changes in rbcS *in vitro* transcription rates. These experiments did not examine protein synthesis or degradation

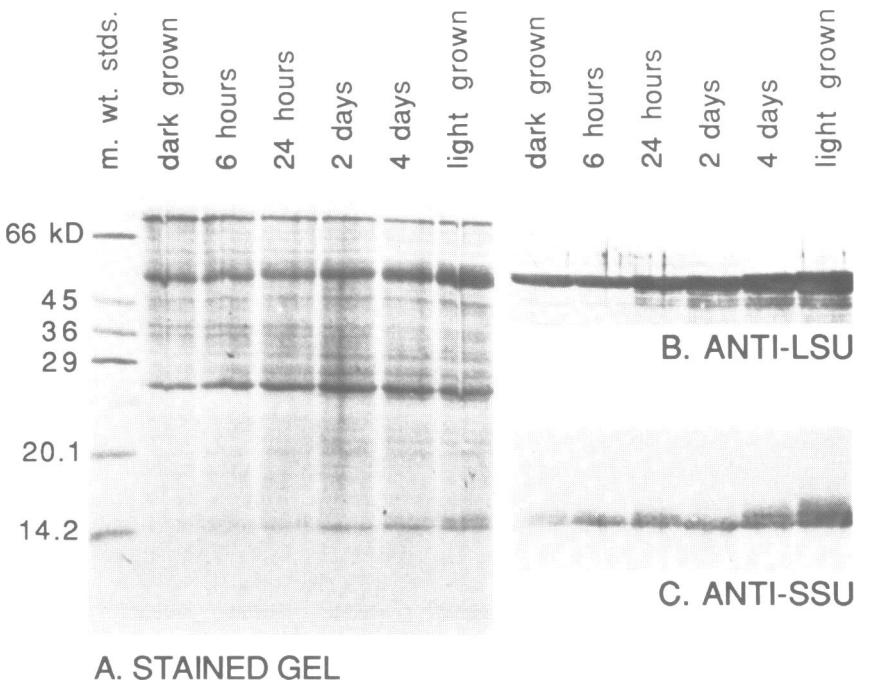

Figure 4. *SSU and LSU polypeptide levels in leaves of etiolated seedlings exposed to light and in leaves and roots of light-grown seedlings.* Crude protein extracts from leaves of etiolated seedlings exposed to light for 0 h, 6 h, 24 h, 2 d and 4 d and from leaves of light-grown seedlings were separated by SDS-PAGE and electrophoretically transferred to Immobilon filters. A. Coomassie-blue stained filter. B. Filter immunostained with antibodies against LSU. C. Filter immunostained with antibodies against SSU.

rates, thus it cannot be concluded that rbcS RNA levels directly control SSU protein levels. However, the five to ten fold difference in SSU protein levels relative to total protein in light- and dark-grown soybean leaves (Figure 4) is in good agreement with the eight fold higher level of rbcS mRNA relative to total RNA in these tissues (Figure 1).

DISCUSSION

RNA turnover may control rbcS RNA levels in response to light

The light regulated expression of genes encoding the small subunit of ribulose-1,5-bisphosphate carboxylase has been studied in detail in a wide variety of photosynthetic organisms. Although transcription exerts substantial control over rbcS gene expression, it is evident that post-transcriptional events such as increased translation in the light and degradation of excess SSU protein in chloroplasts affects the extent to which these genes are ultimately expressed (6, 30, 31, 32). The studies described herein suggest that differential RNA turnover may also control the expression of the rbcS genes in soybean seedlings by reducing RNA levels in the light.

The data presented in Figures 1 and 3 show a discrepancy between rbcS *in vitro* transcription rates and the corresponding steady-state RNA levels in etiolated and light-grown soybean leaves. The higher rates of *in vitro* transcription measured in the light did not result in correspondingly higher steady-state RNA levels. Assuming that run-on transcription assays accurately reflect the *in vivo* situation, these observations indicate that soybean rbcS RNA is less stable in light-grown seedlings than in etiolated seedlings. Thus an increase in RNA degradation may modulate the accumulation of rbcS RNA in light-grown seedlings. In addition, a substantial lag in the accumulation of rbcS RNA was observed relative to a rapid increase in *in vitro* transcription rates when etiolated plants were shifted into the light. In contrast, both the *in vitro* transcription rates and RNA levels measured for rbcS dropped rapidly to etiolated levels when plants were treated with far-red light or placed in darkness. These observations suggest that a high rate of turnover is rapidly induced when etiolated plants are shifted to the light and that it is maintained for some time when light-grown plants are shifted to darkness. It therefore appears that light may control not only transcription of the rbcS genes, but also the rate at which rbcS RNA is degraded in soybean seedlings.

It was previously shown that in *Lemna* there is a rapid decline in translatable rbcS RNA when light-grown plants are transferred to darkness (47). This effect was found to be inhibited to some degree by the hormone, kinetin. It was suggested that kinetin may slow the turnover of rbcS RNA in these plants. In addition, Gallagher and Ellis (23) showed that rbcS RNA in pea is stable in darkness. Both of these results are consistent with the hypothesis for light-induced turnover of rbcS RNA in soybean.

An estimate can be made of the difference in rbcS RNA half-lives in leaves of light- and dark-grown soybean seedlings. rbcS *in vitro* transcription rates and RNA levels reached a maximum 24 h after etiolated plants were shifted to the light and a minumum 48 h after light-grown seedlings were shifted to darkness. Thus it can be assumed that the rates of rbcS RNA synthesis and degradation were at equilibrium in etiolated and light-grown soybean leaves at time zero and after the 24 and 48 h treatments in each of the three experiments presented (Figures 1, 2 and 3). At equilibrium RNA levels are constant, so that if $dC/dt = k_s - k_dC$ (where C is the RNA concentration, k_s is the synthesis rate, k_d is the degradation rate), then for each situation $k_s = k_dC$. From a minimum 32-fold difference in *in vitro* synthesis rates, k_s in the light (k_{sL}) is 32 times greater than k_s in darkness (k_{sD}). Therefore, $k_{sL} = 32\ k_{sD}$. From a maximum eight fold difference in RNA levels, C in the light (C_L) is eight times greater than C in darkness (C_D). Therefore, $C_L = 8\ C_D$. It can thus be estimated by substitution that the turnover rate constant (k_d) is at least four fold higher in the light than in darkness ($k_{dL} = 4k_{dD}$). Assuming that run-on transcription rates accurately reflect the *in vivo* rate of rbcS RNA synthesis, it is estimated that rbcS RNA half-lives are at least four times longer in etiolated leaves than in light-grown leaves (since $T_{1/2} = \ln 2/k_d$).

Run-on transcription assays in isolated nuclei as a measure of *in vivo* transcription rates

Discrepancies in RNA levels and transcription rates measured by run-on assays in isolated nuclei have provided preliminary evidence for changes in RNA stability in response to external stimuli or changes in growth conditions in a variety of systems. Some examples are the c-myc oncogene (48), the mouse P450 genes (49), the tubulin genes in *Chlamydomonas* (50), the maize alcohol dehydrogenase gene (51) and a number of different soybean seed storage protein genes (52). In addition, comparisons of run-on transcription data with RNA measurements were confirmed by *in vitro* kinetic labelling experiments in mouse cells and indicated that differential RNA stability may be a primary control in determining the levels of many abundant mRNAs in eukaryotic cells (53). The observation that rbcS RNA levels did not parallel the dramatic changes in transcription suggests that RNA turnover plays a major role in controlling plant gene expression in response to light.

Interpretation of the studies described herein assumes that run-on transcription assays in isolated nuclei reflect the *in vivo* situation for the soybean rbcS genes and that these assays provide a minimum estimate of changes in transcription in response to light. Criticism of run-on assays has generally pertained to analysis of genes expressed at low levels which has resulted in underestimation of changes in transcription rates (54). However, use of nuclear run-on assays in a number of different plants has provided evidence for significant levels of change in rbcS transcription in response to light (16, 22, 23, 24, 25). In the experiments reported herein differences in levels of rbcS transcription in soybean plants grown under different light conditions were normalized to rRNA transcription (Figures 1, 2 and 3), which accounts for approximately 50% of transcription in soybean (25). These experiments also showed that transcription of soybean actin genes, which like the rbcS genes are transcribed by RNA polymerase II, did not change in response to light relative to rRNA transcription. Furthermore, even in plants treated with only 15 min of far-red light, which should have little effect on the physiology of plant nuclei, rbcS transcription was reduced at least four fold from the level measured in light-grown plants. In addition, discrepancies were detected not only in the amount of increase in transcription compared to the increase in RNA in response to light, but in the timing of the induction. All these data support the assumption that a minimum value for changes in rbcS transcription *in vivo* is determined using run-on transcription assays. The unlikely possibility still remains that isolation of plant nuclei from light-grown leaves results in derepression or induction of transcription of the rbcS genes and not actin or 18S rRNA genes. Although it is not possible to

accurately measure the *in vivo* transcription rates of individual genes by pulse-labeling of RNA in plant leaves, it may be possible to verify the relative rates of transcription in light and darkness by measuring the amount of nascent hnRNA for rbcS in nuclei or by immunoprecipitation of RNA polymerase II-bound rbcS genes (55).

Potential mechanisms for the light regulation of rbcS RNA stability

Several potential sites and mechanisms can be proposed for RNA turnover immediately following transcription such as degradation during splicing or transport out of the nucleus. However, experiments in pea (12) and soybean (B.W. Shirley and R.B. Meagher, unpublished data) indicate that there are no differences in the accumulation of either unspliced or mature rbcS RNA in nuclei of light- and dark-grown plants. A more compelling possibility is that light-dependent degradation of rbcS RNA is linked to translation. Several recent studies point to a role for ribosome-associated ribonucleases in the translation-dependent degradation of specific RNA species. For example, Graves *et al.* (1) suggest that degradation of histone mRNA, which requires translation to the termination codon, involves recognition of the 3′ end of the mRNA by a system involving factors associated with the ribosome. An exonucleolytic activity has been identified that is bound to ribosomes and specifically degrades histone mRNA in vitro (6). Similarly, work by Wilson and Treisman (56) indicates that degradation of c-fos mRNA requires ongoing translation of the mRNA. This system appears to involve a 3′ untranslated AU-rich sequence that has been found to control the stability of a variety of cytokine, lymphokine and proto-oncogene RNAs (57). Another example is the post-transcriptional mechanism that controls the stability of β-tubulin mRNAs in response to the concentration of unpolymerized β-tubulin subunits (2, 58). Yen *et al.* (2) show that β-tubulin RNA turnover in mammalian cells requires a specific 13 nucleotide sequence encoding the first four amino acids of β-tubulin, binding to ribosomes, and translation of the mRNA to codon 41. This mechanism may also control b-tubulin RNA levels in chicken, in sea-urchin embryos and in yeast (2, 58). Yen *et al.* (2) point out that, despite differences in specific mechanisms, a common theme of RNA degradation coupled to ribosome attachment and translation emerges from these studies.

One simple model for light-regulated RNA turnover would link all RNA turnover to polysome assembly and translation. In fact, in the leaves of soybean seedlings a general increase in RNA turnover may result from the overall increase in the assembly of RNAs into polysomes in the light. In preliminary experiments a rapid and dramatic decrease was observed in the proportion of RNA present in polysomes when soybean seedlings were shifted to darkness (B.W. Shirley and R.B. Meagher, unpublished data). In addition the amount of total RNA recovered per leaf increased approximately two-fold within 12 h, despite an approximately two fold reduction in total *in vitro* transcription rates (25; data not shown). Thus all mRNAs may be destabilized in the light due to an increase in polysome assembly and translation.

Another possiblity is that light-dependent rbcS RNA degradation is determined by specific factors that control the rate of rbcS RNA translation. This model would predict that the rate of translation and associated RNA degradation would be higher for rbcS RNA in the light than in darkness. Berry *et al.* (59) recently showed that even though rbcS translation rates decrease rapidly when amaranth plants are shifted to darkness, rbcS RNA remains associated with ribosomes for several hours. It will be of interest to determine whether the apparent light-dependent degradation of rbcS RNA in soybean leaves is reflected in the proportion of rbcS RNA in polysomes or in the rate of translation in response to darkness. The apparent difference in the effects of darkness and far-red light on rbcS RNA levels (Figure 3) might also be reflected in rates of translation.

CONCLUSIONS

In the majority of systems where RNA turnover has been examined stabilization of mRNA contributes positively to gene expression and correlates with the higher demand for the gene product (2, 56, 60, 61). Differential RNA stability has also been shown to contribute to the control of levels of stored mRNA in development (62). The possibility that rbcS gene expression is negatively regulated by light-dependent turnover of rbcS RNA is in direct contrast to the light-induced expression and activity of Rubisco protein. Thus differential turnover of rbcS RNA in soybean seedlings may represent a third and novel mode of control in which increased RNA degradation is concomittant with light-induced expression. Considering that in plants light controls the expression of hundreds of genes (63), the contribution of such a post-transcriptional mechanism could be extremely significant.

Expression of the small subunit for the highly abundant protein, Rubisco, was previously known to be regulated at the levels of gene transcription, translation and protein turnover in response to light. This study indicates that differential RNA stability may exert a negative control over the light regulation of rbcS gene expression in soybean seedlings. Future work will focus more specifically on the mechanisms involved in the post-transcriptional regulation of rbcS gene expression in soybean. The possibility that this control mechanism is universal in higher plants and that it is under developmental control is examined in a companion manuscript which explores transcription rates and RNA levels in mature petunia and soybean leaves (64).

ACKNOWLEDGEMENTS

We would like to thank Gregory Schmidt and Gerald Plumley for the generous gift of the anti-SSU and anti-LSU antibodies. We also thank Carol Condit, Deborah Thompson and Julie Senecoff for constructive criticism and editing of the manuscript. This work was supported by D.O.E. Grants DE-AS09-83ER13077 and DE-FG09-86ER13543 and by a N.I.H. Predoctoral Training Grant to B.W.S. This work serves as partial fulfillment of a Ph.D. degree in Genetics for B.W.S.

REFERENCES

1. Graves,R.A., Pandey,N.P., Chodchoy,N., and Marzluff,W.F. (1987) Cell, **48**, 615–626.
2. Yen,T.J., Gay,D.A., Pachter,J.S., and Cleveland,D.W. (1988) Mol. Cell. Biol., **8**, 1224–1235.
3. Ross,J., Kobs,G., Brewer,G., and Peltz,S.W. (1987) J. Biol. Chem., **19**, 9374–9381.
4. Tobin,E.M., and Silverthorne,J. (1985) Ann. Rev. Plant Physiol., **36**, 569–593.
5. Watson,J.C. (1989) In Kung,S.D., and Arntzen, C.J. (eds), Plant Biotechnology. Butterworths, Boston, pp. 161–205.
6. Berry,J.O., Nikolau,B.J., Carr,J.P., and Klessig,D.F. (1985) Mol. Cell. Biol., **5**, 2238–2246.
7. Tobin,E.M. (1978) Proc. Natl. Acad. Sci. USA, **75**, 4749–4753.

8. Nelson,T., Harpster,M.H., Mayfield,S.P., and Taylor,W.C. (1984) J. Cell Biol., **98**, 558–564.
9. Zhu,Y.S., Kung,S.D., and Bogorad,L. (1985) Plant Physiol., **79**, 371–376.
10. Thompson,W.F., Everett,M., Polans,N.O., Jorgensen,R.A., and Palmer,J.D. (1983) Planta, **158**, 487–500.
11. Coruzzi,G., Broglie,R., Edwards,C., and Chua,N.-H. (1984) EMBO J., **3**, 1671–1679.
12. Smith,S.M, and Ellis,R.J. (1981) J. Mol. Appl. Genet., **1**, 127–137.
13. Dean,C., Dunsmuir,P., and Bedbrook,J. (1987) In Bruening,G., Harada,J., Kosuge,T. and Hollaender,A.A. (eds), Tailoring Genes for Crop Improvement: An Agricultural Perspective. Plenum Press, New York, pp. 59–68.
14. Turner,N.E., Clark,W.G., Tabor,G.J., Hironaka,C.M, Fraley,R.T., and Shah,D.M. (1986) Nucl. Acids Res., **8**, 3325–3342.
15. Fourcroy,P., Klein-Eude,D.,and Lambert,C. (1985) Plant Sci. Lett., **37**, 235–244.
16. Ernst,D., Pfeiffer,F., Schefbeck,K., Weyrauch,C., and Oesterhelt,D. (1987) Plant Mol. Biol., **10**, 21–33.
17. Berry-Lowe,S.L., McKnight,T.D., Shah,D.M., and Meagher,R.B. (1982) J. Mol. Appl. Genet., **1**, 483–498.
18. Grandbastien,M.A., Berry-Lowe,S., Shirley,B.W., and Meagher,R.B. (1986) Plant Mol. Biol., **7**, 451–465.
19. Poulsen,C., Fluhr,R., Kauffman,J.M., Boutry,M., and Chua,N.-H. (1986) Mol. Gen. Genet., **205**, 193–200.
20. Goldschmidt-Clermont,M., and Rahire,M. (1986) J. Mol. Biol., **191**, 421–432.
21. Stiekma,W.J., Wimpee,C.F., Silverthorne,J., and Tobin,E.M. (1983) Plant Physiol., **72**, 717–724.
22. Silverthorne,J., and Tobin,E.M. (1984) Proc. Natl. Acad. Sci. USA, **81**, 1112–1116.
23. Gallagher,T.F., and Ellis,R.J. (1982) EMBO J., **1**, 1493–1498.
24. Shirley,B.W., Berry-Lowe,S.L, Rogers,S.G., Flick,J.S., Horsch,R., Fraley,R.T., and Meagher,R.B. (1987) Nucleic Acids Res., **15**, 6501–6514.
25. Berry-Lowe,S.L., and Meagher,R.B. (1985) Mol. Cell. Biol., **5**, 1910–1917.
26. Morelli,G., Nagy,F., Fraley,R.T., Rogers,S.G., and Chua,N.-H. (1985) Nature, **315**, 200–204.
27. Timko,M.P., Kausch,A.P., Castresana,C., Fassler,J., Herrera-Estrella,L., Van den Broeck,G., Van Montagu,M., Schell,J., and Cashmore,A.R. (1985) Nature, **318**, 579–582.
28. Facciotti,D., O'Neal,J.K., Lee,S., and Shewmaker,C.K. (1985) Bio/Technology, **3**, 241–246.
29. Shaw,C.H., Sanders,D.M., Bates,M.R., and Shaw,C.H. (1986) Nucleic Acids Res., **14**, 6603–6612.
30. Kirk,M.M., and Kirk,D.L. (1985) Cell, **41**, 419–428.
31. Berry,J.O., Nikolau,B.J., Carr,J.P., and Klessig,D.F. (1986) Mol. Cell. Biol., **6**, 2347–2353.
32. Schmidt,G.W., and Mishkind,M.L. (1983) Proc. Natl. Acad. Sci. USA, **80**, 2632–2636.
33. Hightower,R.C., and Meagher,R.B. (1985) EMBO J., **4**, 1–8.
34. Eckenrode,V.K., Arnold,J., and Meagher,R.B. (1985) J. Mol. Evol., **21**, 259–269.
35. Dretzen,G., Bellard,M., Sassone-Corsi,P., and Chambon,P. (1981) Anal. Biochem., **112**, 295–298.
36. Feinberg,A.P., and Volgelstein,B. (1983) Anal. Biochem. 132, 6–13 and Addendum. (1984) Anal. Biochem., **137**, 266–267.
37. Maniatis,T., Fritsch,E.F., and Sambrook,J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor.
38. Johnson,D.A., Gautsch,J.W., Sportsman,J.R., and Elder,J.H. (1984) Gene Anal. Techn., **1**, 3–8.
39. Meagher, R.B., Berry-Lowe, S., Rice, K. (1989) Genetics, **123**, 845–863.
40. Shirley, B.W., Ham, D.P., Berry-Lowe, S.L., Senecoff, J., Zurfluh, L.L., Shah, D.P., and Meagher, R.B. (in press)
41. Hall,T.C., Ma,Y., Buchbinder,B.U., Pyne,J.W., Sun,S.M., and Bliss,G.A. (1978) Proc. Natl. Acad. Sci. USA, **75**, 3196–3200.
42. Fyrberg,E.A., Mahaffey,J.W., Bond,B.J., and Davidson,N. (1983) Cell, **33**, 115–123.
43. Fitzwater,T., Chang,X.-Y., Elble,R., and Polisky,B. (1988) EMBO J., **7**, 3289–3297.
44. O'Farrell,P. (1975) J. Biol. Chem., **250**, 4007–4021.
45. Towbin,H., Stachelin,T., and Gordon,J. (1979) Proc. Natl. Acad. Sci. USA, **76**, 4350–4354.
46. Plumley,F.G., Kirchman,D.L., Hodson,R.E., and Schmidt,G.W. (1986) Plant Physiol., **80**, 685–691.
47. Tobin,E.M., and Turkaly,E. (1982) J. Plant Growth Regul., **1**, 3–13.
48. Blanchard,K.-M., Piechaczyk,M., Dani,C., Chambard,J.-C., Franchi,A., Pouyssegur,J., and Jeanteur,P. (1985) Nature, **317**, 443–445.
49. Kimura,S., Gonzalez,F.J., and Nebert,D.W. (1986) Mol. Cell. Biol., **6**, 1471–1477.
50. Baker,E.J., Schloss,J.A., and Rosenbaum,J.L. (1984) J. Cell. Biol., **99**, 2074–2081.
51. Rowland,L.J., and Strommer,J.N. (1986) Mol. Cell. Biol., **6**, 3368–3372.
52. Walling,L., Drews,G.N., and Goldberg,R.B. (1986) Proc. Natl. Acad. Sci. USA, **83**, 2123–2127.
53. Carneiro,M., and Schibler,U. (1984) J. Mol. Biol., **178**, 869–880.
54. Watson,G., and Paigen,K. (1988) Mol. Cell. Biol., **8**, 2117–2124.
55. Gilmour,D.S., and Lis,J.T. (1986) Mol. Cell. Biol., **6**, 3984–3989.
56. Wilson,T., and Treisman,R. (1988) Nature, **336**, 396–399.
57. Shaw,G., and Kamen,R. (1986) Cell, **46**, 659–667.
58. Gong,Z., and Brandhorst,B.P. (1988) Mol. Cell. Biol., **8**, 3518–3525.
59. Berry,J.O., Carr,J.P., and Klessig,D.F. (1988) Proc. Natl. Acad. Sci. USA, **85**, 4190–4194.
60. Baker,E.J., Keller, L.R., Schloss,J.A., and Rosenbaum,J.L. (1986) Mol. Cell. Biol., **6**, 54–61.
61. Mangiarotti, G., Giorda, R. Ceccarelli, A. and Perlo, C. (1985) Proc. Natl. Acad. Sci. USA, **82**, 5786–5790.
62. Rao, T.R. and Slobin, L.I. (1988) Mol. Cell. Biol., **8**, 1085–1092.
63. de Vries, S.C., Springer, J., Wessels, J.G.H. (1982) Planta, **156**, 129–135.
64. Thompson, D.M. and Meagher, R.B. (submitted Nucleic Acids Res.)