Light-Mediated Control of Translational Initiation of Ribulose-1,5-Bisphosphate Carboxylase in Amaranth Cotyledons

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In cotyledons of 6-day-old amaranth seedlings, the large subunit (LSU) and the small subunit (SSU) polypeptides of ribulose-1,5-bisphosphate carboxylase are not synthesized in the absence of light. When dark-grown seedlings were transferred into light, synthesis of both polypeptides was induced within the first 3 to 5 hr of illumination without any significant changes in levels of their mRNAs. In cotyledons of light-grown seedlings and of dark-grown seedlings transferred into light for 5 hr (where ribulose-1,5-bisphosphate carboxylase synthesis was readily detected in vivo), the LSU and SSU mRNAs were associated with polysomes. In cotyledons of dark-grown seedlings, these two mRNAs were not found on polysomes. In contrast to the SSU message, mRNAs encoding the nonlight-regulated, nuclear-encoded proteins actin and ubiquitin were associated with polysomes regardless of the light conditions. Similarly, mRNA from at least one chloroplast-encoded gene (rp12) was found on polysomes in the dark as well as in the light. These results indicate an absence of translational initiation in cotyledons of dark-grown seedlings which is specific to a subset of nuclear- and chloroplast-encoded genes including the SSU and LSU, respectively. Upon illumination, synthesis of both polypeptides, and possibly other proteins involved in light-mediated chloroplast development, was induced at the level of translational initiation.

INTRODUCTION

In higher plants, light serves both as a primary energy source and as an environmental stimulus which regulates development. In particular, the development of photosynthetically active chloroplasts from proplastids or etioplasts is light dependent. Many, but not all, dark-grown plants lack thylakoid organization, chlorophyll, and several of the polypeptides associated with photophosphorylation and CO₂ fixation. Illumination of these dark-grown plants causes the accumulation of chlorophyll and induces the development of thylakoid components into photosynthetically functional units. Light also induces changes in the synthesis of a number of nuclear- and plastid-encoded proteins, including in some species ribulose 1,5-bisphosphate carboxylase (RuBPCase) (Apel, 1979; Schroder et al., 1979; Santel and Apel, 1981; Sims and Hague, 1981; Smith and Ellis, 1981; Tobin and Silverthorne, 1985; Berry et al., 1985).

RuBPCase is found in the chloroplasts of all higher plants and is a principle enzyme in photosynthetic carbon fixation. This enzyme is composed of eight large (51 to 58 kD) and eight small (12 to 18 kD) subunits. The large subunit (LSU) is encoded on the chloroplast genome and translated on 70S chloroplast ribosomes (Coen et al., 1977; Ellis, 1981), while the small subunit (SSU) is nuclear encoded and translated on free cytoplasmic ribosomes as a 20-kD precursor (Cashmore et al., 1978; Chua and Schmidt, 1978; Highfield and Ellis, 1978). The precursor is processed to its final size during transport into the chloroplast, where it assembles with LSUs to form the active holoenzyme.

The control of RuBPCase production and activity in a number of plant species is very complex with regulation occurring at many levels. Control at the level of LSU or SSU mRNA accumulation has been well documented (for review, see Tobin and Silverthorne, 1985). In several cases alterations in transcriptional activity have been shown to be responsible for changes in SSU mRNA levels (Tobin and Silverthorne, 1985; Fluhr et al., 1986). At the other end of the spectrum, regulation at the post-translational level via turnover of the protein (Mishkind and Schmidt, 1983) as well as by activation (Mizioruk and Lorimer, 1983; Portis et al., 1986) or inhibition (Gutteridge et al., 1986) of the enzyme’s activity has also been reported.

We have previously described the effects of environmental (light) (Berry et al., 1985, 1986, 1988) and developmental (Berry et al., 1985; Nikolau and Klessig, 1987) signals on the expression of LSU and SSU genes in the C₄ dicotyledonous plant Amaranthus hypochondriacus.
The expression of these genes is regulated not only at the level of mRNA accumulation but also post-transcriptionally. In amaranth cotyledons, rapid and dramatic alterations in the synthesis of the LSU and SSU polypeptides occur in response to changes in illumination without corresponding changes in mRNA levels. Because the stability of these polypeptides and the functionality in vitro of their respective mRNAs were not affected by alterations in illumination, the expression of these genes appears to be regulated, in part, at the translational level. Light-mediated translational regulation of this enzyme has also been observed in other plant species including *Lemna* (Slovin and Tobin, 1982), *Volvox* (Kirk and Kirk, 1985), pea (Inamine et al., 1985), and barley (Klein and Mullet, 1986; Klein et al., 1988; Gamble et al., 1989). In addition, translation appears to play a role in regulating LSU and SSU gene expression during the latter stages of leaf development in amaranth (Nikolau and Klessig, 1987).

We have recently shown that when cotyledons of light-grown amaranth seedlings were transferred to total darkness, both LSU and SSU mRNAs remained bound to polysomes but were not translated in vivo, suggesting that under these conditions control was exercised, in part, at the translational elongation step (Berry et al., 1988). In this report, we show that the RuBPCase LSU and SSU mRNAs are not associated with polysomes in dark-grown (etiolated) cotyledons but are rapidly recruited onto polysomes upon illumination. In contrast, mRNAs encoding constitutive (nonlight-regulated) polypeptides are associated with polysomes in cotyledons of both dark-grown and light-shifted seedlings. These results demonstrate a selective absence of translational initiation (and therefore synthesis) of both RuBPCase subunits when amaranth seedlings are grown in the absence of light.

**RESULTS**

*Effect of Light on LSU and SSU Gene Expression*

The light-mediated stimulation of RuBPCase synthesis in cotyledons was examined by illuminating 6-day-old, dark-grown amaranth seedlings. Similar to our previous studies using 8-day-old seedlings (Berry et al., 1986), synthesis of the LSU and SSU in 6-day-old seedlings increased from nearly undetectable amounts in the dark to levels similar to that observed in light-grown seedlings within 3 to 5 hr post-illumination, as shown in Figure 1A. While total protein synthesis was enhanced twofold to fivefold by 5 hr post-illumination (data not shown), LSU and SSU synthesis was specifically increased 20-fold or more above general protein synthesis during light induction.

Figure 1B shows that levels of the LSU and SSU mRNAs were lower in cotyledons grown in darkness than those maintained in the light. Although synthesis of the LSU and SSU polypeptides was dramatically enhanced during the first 5 hr of illumination, the corresponding mRNA levels increased only modestly (twofold to threefold) during this time period (Figure 1B). Therefore, the marked increase in synthesis of the RuBPCase proteins was not accompanied by similar changes in LSU and SSU mRNA levels.

**Association of LSU and SSU mRNAs with Polysomes**

The above results, as well as our previous studies (Berry et al., 1986), indicated that the rapid induction of RuBPCase synthesis, which occurs when dark-grown
seedlings are transferred to light, may be due to regulation at the translational level. To confirm this, changes in the association of these mRNAs with polysomes during light induction were examined. Figure 2A shows 10% to 40% sucrose gradient profiles of polysomes obtained from cotyledons of light-grown and dark-grown seedlings and from dark-grown seedlings transferred to light for 5 hr. Comparison of polysome profiles indicates that a smaller proportion of the ribosomes are engaged in translation in dark-grown versus light-grown seedlings. When the etiolated seedlings were transferred to light, an increasing proportion of these ribosomes shifted from the monosome regions to the polysome regions of the gradient and presumably began actively synthesizing proteins. These moderate changes in the polysome profiles were consistent with the differences in rates of general protein synthesis observed under the various illumination conditions.

Comparison of these three polysome profiles suggests that there may be differences in the populations of polysome-associated mRNAs in seedlings subjected to the various illumination conditions. To test this, the sucrose gradient profiles were analyzed by dot blotting using specific probes (Figure 2B). Both LSU and SSU mRNAs were associated with polysome regions of the sucrose gradient (fractions 1 to 4, Figure 2B) from light-grown seedlings. However, with polysomes isolated from dark-grown seedlings (when little, if any, RuBPCase synthesis was detected), only small amounts of these two transcripts were detected in the polysome fractions. Within 5 hr of transfer into light (after RuBPCase synthesis was initiated), dramatic increases in the levels of both mRNAs were found in the polysome fractions.

One interpretation of these results is that the LSU and SSU mRNAs from cotyledons of dark-grown seedlings were associated with very large polysomes. This might occur if translational elongation/termination was inhibited in the dark so that ribosomes continued to accumulate on the mRNA. Under the sedimentation conditions used here, the very large polysomes might be pelleted at the bottom of the gradient and thus escape detection. This was unlikely because the gradients contained a dense 1.75 M sucrose cushion at the bottom to prevent pelleting. To further rule out this possibility, polysome extracts were also sedimented through denser 15% to 60% sucrose gradients. Under these conditions, again only small amounts of LSU and SSU mRNA were found in the heavy portion of the gradient (data not shown), thus confirming that very few of the RuBPCase mRNAs were associated with polysomes in cotyledons of dark-grown seedlings.

Very few LSU or SSU mRNAs were detected in any region of the gradient in extracts from cotyledons of dark-grown seedlings compared with those from light-shifted seedlings (Figure 2B). This was unexpected because only very modest changes in the levels of these two mRNAs were observed by RNA gel blot analysis (Figure 1B). A likely explanation for this discrepancy is the manner of...
preparation of the crude polysome fraction. Polysome isolation from plant tissue involves centrifugation of the initial crude extract through a dense sucrose cushion (Leaver and Dyer, 1974; Larkins and Davies, 1975; Jackson and Larkins, 1976). This serves to concentrate the polysomes, monosomes, and free mRNPs and to separate them from debris which can interfere with their subsequent analysis. A major problem with this sedimentation procedure is that it tends to enrich for polysomes, while leaving monosomes and free mRNPs underrepresented in the pellet (Leaver and Dyer, 1974; Larkins and Davies, 1975). Presumably, the levels of LSU or SSU mRNA in the profiles for dark-grown cotyledons were underrepresented because the majority of the mRNA was not associated with polysomes and therefore sedimented through the sucrose cushion less rapidly and efficiently than the denser, polysome-associated messages. In fact, when polysomes were purified using a shorter sedimentation time (3 hr as opposed to the 18 hr used for Figure 2, data not shown), even lower amounts of the nonpolysome-associated LSU and SSU transcripts were recovered from the dark-grown seedlings. Therefore, although recovery of the LSU and SSU mRNAs in crude polysome pellets from dark-grown seedlings was not complete, analysis of the crude polysome preparations confirmed that very few of the LSU and SSU transcripts were associated with polysomes in darkness but upon illumination were recruited onto polysomes. These results suggest that light-induced LSU and SSU synthesis in etiolated seedlings is regulated, in part, at the level of translational initiation.

Translational Runoff Analysis of Polysomes In Vitro

The association of LSU and SSU mRNAs with polysomes was further examined by in vitro polysome runoff analysis. Crude polysomes were added to either an *Escherichia coli* (for LSU) or rabbit reticulocyte (for SSU) cell-free translation system containing aurin tricarboxylic acid (ATA) or edeine/7-methylguanosine monophosphate, respectively, to inhibit initiation. As previously described (Berry et al., 1988), these inhibitors had little or no effect on polysome-directed protein synthesis but reduced RNA-directed synthesis by >90% (Figures 3A and 3B), indicating that the drugs were functioning as specific inhibitors of initiation.

Both LSU and SSU polypeptides were produced in vitro when polysomes from either cotyledons of light-grown or light-shifted seedlings were used to program cell-free translation extracts in the absence (−) or presence (+) of the initiation inhibitors (Figures 3A and 3B). In contrast, polysomes isolated from cotyledons of dark-grown seedlings did not direct the synthesis of detectable amounts of either subunit in runoff translation (Figures 3A and 3B). These results confirm that in the light, when LSU and SSU synthesis was readily detected, both RuBPCase transcripts were associated with translationally active polysomes. In dark-grown seedlings, where synthesis of these proteins was not detected, the LSU and SSU mRNAs were

![Figure 3. Translational Runoff of Polysomes in Vitro.](image)

**Figure 3.** Translational Runoff of Polysomes in Vitro. 

(A) Translational runoff of chloroplast polysomes. Polysomes isolated from light-grown seedlings (L), dark-grown seedlings (D), or dark-grown seedlings shifted to light for 5 hr (D+5hL) were incubated in an *Escherichia coli* cell-free translation system in the absence (−) or presence (+) of the initiation inhibitor ATA. In the controls shown at left, the extracts were programmed with total amaranth RNA in the absence or presence of the inhibitor. LSU was immunoprecipitated from equal aliquots and analyzed by SDS-PAGE.

(B) Translational runoff of cytoplasmic polysomes. Polysomes were added to a rabbit reticulocyte cell-free system in the absence (−) or presence (+) of the initiation inhibitors edeine/m7GMP. In the controls shown at left, the extracts were programmed with amaranth polyadenylated RNA in the absence or presence of the inhibitors. SSU precursor was immunoprecipitated from equal aliquots and analyzed by SDS-PAGE.
not associated with polysomes and therefore were not translated.

**Specificity of Initiation on Cytoplasmic and Chloroplastic Ribosomes**

Is the lack of polysome association and active translation of the LSU and SSU mRNAs simply a reflection of the reduction of polysome number and rate of general protein synthesis in the dark? The greater severity of repression of their translation in comparison with general protein synthesis (20-fold or more) suggests that this is not the case. However, to directly test this, the expression of several nuclear or chloroplast encoded genes, whose expression was unlikely to be light regulated, was examined. As controls for cytoplasmic protein synthesis, the translation and polysome association of mRNAs for actin, a cytoskeletal protein (DeRosier and Tilney, 1984), and ubiquitin, a protein involved in the degradation of other polypeptides (Ciechanover et al., 1984), were analyzed. In contrast to the SSU, the levels of actin mRNA (Figure 4B) and its rate of translation (Figure 4A) were similar in the absence or presence of light. In addition, actin mRNA was associated with polysomes isolated from cotyledons of dark-grown seedlings as well as those from light-grown and light-shifted seedlings (Figure 4C). Like actin, ubiquitin mRNA was associated with polysomes at similar levels in darkness as well as in light (data not shown). Thus, the absence of translational initiation on cytoplasmic ribosomes is not general but is specific to light-regulated genes such as those encoding the SSU.

To determine the specificity of LSU initiation on chloroplasmic ribosomes, the distribution on polysomes of another chloroplast-encoded mRNA was examined. The mRNA for the ribosomal protein L2 (which is encoded by the chloroplast gene rp/2) was present in cotyledons of dark-grown, light-grown, and light-shifted seedlings (Figure 5A). Although similar levels of L2 mRNA were found in dark-grown plants before and after transferring into light, this mRNA was less abundant (about 10-fold) in light-grown seedlings. Therefore, the lack of polysome association observed for the LSU transcripts in dark-grown seedlings is not characteristic of this chloroplastic mRNA.

**DISCUSSION**

We have previously shown that whereas RuBPRCase synthesis occurs continuously in cotyledons of light-grown amaranth seedlings, in dark-grown (etiolated) seedlings LSU and SSU synthesis occurs only as a burst between 2 and 5 days after planting (Berry et al., 1985). Although no RuBPRCase synthesis is observed after day 5 in the dark, mRNAs for both subunits are maintained through day 7. The results presented here indicate that in 6-day-old, dark-grown amaranth cotyledons, where RuBPRCase synthesis is not observed, the majority of the RuBPRCase mRNAs were not associated with polysomes. When these dark-
grown seedlings were transferred into light (light-shifted), both the LSU and SSU mRNAs were rapidly mobilized onto polysomes and synthesis of the two polypeptides was initiated.

Cotyledons from dark-grown seedlings exhibited a lower polysome/monosome ratio than either light-grown or light-shifted seedlings. This suggests that in addition to the RuBPCase messages, the association of a number of other mRNAs with polysomes is affected by light. Increased polysome/monosome ratios in response to red light illumination has been reported for a number of plant species (Yamamoto et al., 1975; Smith, 1976; Fourcroy et al., 1979). In mustard seedlings, this shift is due to the increased synthesis of new mRNAs (Mosinger and Schopfer, 1983), while in bean leaves, there is evidence for the light-induced mobilization of stored mRNA onto polysomes (Giles et al., 1977). In amaranth cotyledons, LSU and SSU mRNA levels increased only slightly following illumination, suggesting that light induced translational initiation on mRNAs that were already present in the dark. With regard to the LSU, these results are consistent with recent studies which indicate that post-transcriptional mechanisms play important roles in regulation of plastid gene expression (Deng and Grussem, 1987; Mullet and Klein, 1987).

At least two nuclear-encoded mRNAs were associated with polysomes in dark-grown seedlings when the SSU transcripts were not found on polysomes. Actin and ubiquitin mRNAs, both of which are translated on cytoplasmic ribosomes, were on polysomes in both dark- and light-grown seedlings. Furthermore, actin was translated at similar levels in darkness as well as in light. Similarly, the mRNAs encoding the plastid ribosomal protein L2 were associated with chloroplast polysomes under all three illumination conditions. While it is interesting that the L2 transcripts levels were lower in light-grown than in either dark-grown or light-shifted seedlings, the important point is that these chloroplast-encoded mRNAs were polysome associated in the dark when the LSU transcripts were not. Therefore, the absence of translational initiation on both cytoplasmic and chloroplastic ribosomes is not a general occurrence in the dark but is selective for the RuBPCase transcripts, and possibly other mRNAs involved in light-regulated chloroplast development as well.

Although the chloroplast-encoded L2 mRNA was polysome associated in the dark, we do not know whether it was actually being translated. Klein et al. (1988) found that chloroplast-encoded mRNAs for psaA-psaB, psbA, and LSU were associated with polysomes in young (4.5 days old), dark-grown barley seedlings. In these barley seedlings, the LSU was synthesized in the dark; however, the synthesis of the psaA-psaB and psbA polypeptides was either arrested in translation elongation or the newly made proteins were rapidly degraded. Similarly, we have found that when light-grown amaranth seedlings are transferred to darkness, LSU synthesis is depressed without any corresponding changes in the polysome association of the LSU mRNAs (Berry et al., 1988). Both of these studies indicate that chloroplastic protein synthesis can be regulated at the level of translational elongation as well as initiation, and this may also be the case for L2. Further characterization of L2 protein synthesis awaits the production of specific antisera.

The distribution on polysomes of the light-regulated and nonlight-regulated transcripts described here for the differ-
...ent light conditions may not be representative of all the mRNAs in the cotyledons. It is very likely that there are chloroplast mRNAs, those encoding subunit IV of cytochrome b_6f (petD) and the \( \beta \) subunit of chloroplast ATPase (atpB), were associated with polysomes in dark-grown cotyledons to a lesser extent than the ribosomal protein L2 mRNA but to a greater degree than LSU mRNA (data not shown).

The results presented here demonstrate that during light-mediated chloroplast development in amaranth the synthesis of both RuBPCase subunits is rapidly induced at the level of translational initiation. This induction occurs coordinately on both cytoplasmic and chloroplastic ribosomes. Similar observations of activation of chloroplastic translational initiation have recently been reported by Gamble et al. (1989) using 8-day-old, dark-grown barley seedlings. When the dark-grown barley seedlings were illuminated, an overall increase in translational initiation occurred for the rbcL, psbA, and psaA-psaB gene products. While Kraus and Spremulli (1986) have demonstrated that the level of a chloroplast translation initiation factor is enhanced by light, it is not known if or how this factor might specifically control the translation of light-regulated chloroplastic mRNAs. Alternatively, the difference in polysome association for the various chloroplastic RNAs may simply reflect differences in ribosome binding affinities of the mRNAs (Lodish, 1974). These differences may become particularly apparent if ribosomes or translation factors are limiting in the absence of light. For example, if LSU mRNA has a weak ribosome binding site, discrimination against it could be relieved as the ribosomes or factors become less limiting in the presence of light.

Interestingly, the translation of LSU and SSU transcripts appears to be regulated by different mechanisms in plants exposed to different light regimes. During a light-to-dark transition, the rapid shutdown of LSU and SSU synthesis appears to be due to regulation of translational elongation, while during a dark-to-light transition, synthesis of both RuBPCase subunits appears to be induced at the level of translational initiation. Translation appears to be only one level at which LSU and SSU gene expression is controlled in amaranth. Changes in mRNA levels, due to altered transcription or mRNA stability, also occur and appear to be responsible for long-term changes in expression of the RuBPCase genes (Berry et al., 1986). Furthermore, changes in the synthesis of the chloroplast-encoded LSU and the nuclear-encoded SSU occur in parallel, being coordinately induced by light and depressed by darkness. Therefore, an extensive pattern of communication must exist between the biosynthetic machinery of the nucleus, cytoplasm, and chloroplast which is used to coordinately regulate the expression of these two genes. Clearly, much more work is needed to define the intricate pattern of regulation for this essential gene system.

**METHODS**

**Plant Material and Growth Conditions**

*Amaranthus hypochondriacus* var R103 was grown as described previously (Berry et al., 1985). For the light induction experiments, seeds were germinated and plants were grown in lightproof boxes which were placed in a darkroom. Extreme care was taken to avoid any exposure of the dark-grown seedlings to light. After 6 days, these dark-grown seedlings were transferred into an illuminated growth chamber, and cotyledons were harvested at the appropriate times. Cotyledons from dark-grown seedlings were harvested under a Kodak No. 7 green safelight.

**Analysis of Protein Synthesis**

Rates of in vivo protein synthesis were determined by radioactive labeling with \(^{35}\)S-methionine. The procedures for labeling, extraction of proteins, immunoprecipitation of LSU and SSU polypeptides, and analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were described previously (Berry et al., 1985). Actin was immunoprecipitated with antisera kindly provided by James Lessard (Childrens Hospital, Medical Center, Cincinnati, OH) according to the method of Lin (1981).

**Probes Used in RNA Gel Blot and Dot Blot Analyses**

LSU and SSU mRNAs were detected by using \(^{32}\)P-labeled cloned amaranth LSU (pAlsl) or SSU (pAssl) genes as probes (Berry et al., 1985). The clones for actin (pSAc3) and for ubiquitin (pXlg20) were generously provided by Richard Meagher (University of Georgia, Athens, GA) and Mark Dworkin (Columbia University, New York, NY), respectively. Clones for the chloroplast genes *rps16* (pXWD14), *rpl2* (pUC8c1), and *petD* (pXWD9) were the gift of Wilhelm Gruissem and David Stern. The probe for the \( \beta \) subunit of chloroplast ATPase (atpB) was kindly provided by Nam-Hai Chua.

**Analysis of mRNA**

Total RNA was isolated from cotyledons as previously described (Berry et al., 1985). Five micrograms per lane of total RNA was fractionated on agarose-formaldehyde gels and transferred to nitrocellulose paper for RNA gel blot analysis by the method of Maniatis et al. (1982). Hybridization conditions for the actin and ubiquitin probes were as described by Klessig and Berry (1983), while those for the other probes were according to Berry et al. (1985).

**Analysis of Polysomes**

Polysomes were isolated as previously described (Berry et al., 1988). Crude polysomes were purified from initial extracts by pelleting through a 1.75 M sucrose cushion (Jackson and Larkins, 1976) at 60,000 rpm using a Ti80 rotor (Beckman) for either 3 hr or 18 hr. The crude polysome preparations were resuspended,
clarified by centrifugation for 1 min in a microfuge at 4°C to remove any remaining debris, and stored at -70°C. Three $A_{260}$ units of the purified polysome preparation were loaded onto a 10% to 40% sucrose gradient (containing 40 mM Tris-HCl (pH 8.4) 20 mM KCl, 10 mM MgCl$_2$) which was overlayed on top of a 1.75 M sucrose cushion (0.5 mL). Polysomes were also analyzed using denser 15% to 60% sucrose gradients. The gradients were centrifuged at 39,000 rpm in an SW41 rotor (Beckman) for 150 min at 4°C. The gradient profiles were determined by using a Perkin-Elmer Lambda 3A spectrophotometer with a flow cell. Gradient fractions were collected and immediately diluted with an equal volume of 50 mM Tris-HCl (pH 8.0), 50mM EDTA, 1% SDS, then phenol extracted and ethanol precipitated. The RNA fractions were characterized by dot blot analysis (Bethesda Research Laboratories, Hybri-Dot manifold) on nitrocellulose or by RNA gel blot analysis using the previously described clones as probes. Hybridization conditions were the same as described above for RNA gel blot analysis.

Translational runoff was performed as described previously (Berry et al., 1988) except that the total crude polysomes obtained after 3 hr sedimentation through a sucrose cushion were used to program the cell-free lysates. Five micrograms or 10 $\mu$g of polysomes were incubated in nuclelease-treated rabbit reticulocyte lysates (Pelham and Jackson, 1976) (Green Hectares, Oregon, WI) or cell-free extracts of Escherichia coli (Zubay, 1973), respectively. Reticulocyte lysates (50 $\mu$L final volume), containing 25 $\mu$g/mL chloramphenicol to suppress translation by chloroplastic polysomes, were supplemented with 200 $\mu$M 7-methylguanosine monophosphate (m$^7$GMP) (Darzynkiewicz et al., 1987) and 10 $\mu$M ecdyone (Kozak and Shatkin, 1978) to block translational initiation, or with an equivalent volume of water (controls). Similarly, E. coli lysates (50 $\mu$L final volume), containing 50 $\mu$g/mL cycloheximide to block translation by cytoplasmic polysomes, were supplemented with the inhibition aurin tricarboxylic acid (ATA, 50 $\mu$M) (Grollman and Stewart, 1968) or an equivalent volume of water. 35S-Methionine-labeled translation products were analyzed by SDS-PAGE with or without prior immunoprecipitation. All drugs were obtained from Sigma except for ecdyene, which was a generous gift from Dr. P. Walter.

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