Polynucleotide phosphorylase of \textit{Escherichia coli} induces the degradation of its RNase III processed messenger by preventing its translation

Murielle Robert-Le Meur and Claude Portier*
Institut de Biologie Physico-Chimique, CNRS (URA1139), 13 rue Pierre et Marie Curie, 75005 Paris, France

Received September 23, 1993; Revised and Accepted December 20, 1993

ABSTRACT
Polynucleotide phosphorylase, a 3' to 5' proccessive exoribonuclease is post-transcriptionally autocontrolled and it was previously shown that this control is dependent on a 5' processing by RNase III. In this paper, the mechanism of regulation is analyzed by studying the properties of a \textit{pnp-lacZ} translational gene fusion. It is shown that this message is stable, even when processed by RNase III, and that the degradation rate is directly linked to the intracellular concentration of polynucleotide phosphorylase or to the \textit{pnp-lacZ} messenger translation rate. Mutations able to decrease the level of repression are all located in the ribosome loading site. Taken together, these results suggest that polynucleotide phosphorylase is able to recognize specifically the processed messenger and to prevent its translation, thus allowing degradation of the message.

INTRODUCTION
In post-transcriptional regulation, expression of the messenger is generally controlled by modulation of the translation initiation rate. Among the numerous mechanisms observed, three classes can be defined. (a) Inhibition of initiation by binding of one of the protein products of their operon in the vicinity of the ribosome loading site (translational autocontrol (1,2). (b) Processing of the message by a specific endonuclease (e.g. shut off of some early message in the phage T4 (3,4). (c) Formation of a secondary structure preventing the ribosome binding either by the presence of an alternative structure or by the hybridization with an antisense message [e.g. control of Tn10 transposase (5)]. In some cases, a combination of several of these different mechanisms is present (e.g. expression of very toxic proteins (6).

An unusual variation of translational autocontrol is observed in the case of polynucleotide phosphorylase which is one of the two major 3' to 5' exonucleases present in \textit{E.coli}, the other being RNase II. Post-transcriptional regulation mediated by polynucleotide phosphorylase is observed, but only after RNase III processing of the \textit{pnp} mRNA at 82 nt upstream of the initiation codon (7). Previous experiments using a translational fusion have indicated that the region involved in the control is located in the 5' part of the processed \textit{pnp} mRNA, suggesting that the RNase III cleavage either creates a new entry site for a ribonuclease or induces a \textit{trans} conformation of the messenger which is recognized by polynucleotide phosphorylase (Fig.1) (8). It should be possible to locate more precisely the region of the message involved in the translational autocontrol by mutating or by shortening the size of the \textit{pnp} fragment of a \textit{pnp-lacZ} translational fusion. Any change in the level of repression of the \textit{pnp-lacZ} fusion by polynucleotide phosphorylase would mean that the mutation has modified the presumed control site.

It has been shown that this autoregulation induced a rapid decay of the message and a corresponding drop in polynucleotide phosphorylase synthesis, suggesting that the \textit{pnp} message is rapidly and irreversibly inactivated. The mechanism of mRNA inactivation is an important question which is still not easy to answer because it is generally very difficult to distinguish between translational, structural or nucleolytic effects. Interestingly, Takata and co-workers (9) have shown that the processed \textit{pnp} messenger is much more sensitive to specific endonucleolytic cutting. These considerations raise several questions. What is the consequence of mRNA cleavage by RNase III? How is mRNA degradation triggered to polynucleotide phosphorylase action? Does polynucleotide phosphorylase bind to the \textit{pnp} mRNA? Are inactivation and degradation linked to the mRNA translation rate? Or is polynucleotide phosphorylase directly involved in message degradation? At first view, polynucleotide phosphorylase is able to discriminate between the native and the processed form of its own mRNA. On the other hand, this enzyme is able to degrade any RNA exhibiting a free 3' OH end and it is believed that it possesses no substrate specificity. In this study, several hypotheses are examined and the results obtained suggest that this regulatory mechanism is likely to be different from those already described.

*To whom correspondence should be addressed
Figure 1. Regulation of polynucleotide phosphorylase synthesis. The *pnp* message (wavy line) is cut as soon as it is synthesized by RNase III at a site within the hairpin located 81 nucleotides upstream of the initiation codon (indicated by bound ribosomes). The processed messenger is presumed to take a new conformation which makes it susceptible to further degradation and thus hinders translation (indicated by free ribosomes). The model proposes that polynucleotide phosphorylase enhances degradation by binding to a specific site located in the 5' part of the processed messenger, which would hinder translation of its messenger.

### MATERIALS AND METHODS

#### Strains, phages and media

Most of the strains, phages and constructions have been described previously (8). New constructions are described under the Figures 2 and 4 and the genotype of the strains used listed in Table I. All the mutations introduced in the fusions were transferred into a λ vector and then inserted into the chromosome, as described previously (8). The cells were grown in LB, 2xYT or Mops buffer as previously described. [³H]uridine (30 Ci/mMole), [γ²P]ATP (3000 Ci/mMole) and [³³S]dATP (600 Ci/mMole) were obtained from Amersham. Restriction enzymes, T4 DNA ligase, Klenow enzyme were purchased from Bohringer (Germany), Biolabs (USA) or Bethesda Research Company (USA). Sequenase was from USB (USA) and T7 DNA polymerase from Pharmacia (Sweden).

#### Site directed mutagenesis and deletions

Site directed mutagenesis was performed on the single-stranded DNA of a derivative M13GF8 (8). Then, the EcoRI—*HindIII* fragment was ligated to λ arms as described under Fig. 2. The resulting phage was used to lysogenize strains IBPC5322TR and IBPC5325.

#### DNA sequencing

All the constructs or mutants isolated were sequenced using the dideoxy chain termination method (11). DNA sequencing was done on M13mp8 and M13mp18 phages after cloning the fragment at the polylinker sites according to the protocol of USB (USA).

#### Labeling, extraction of RNA, DNA—RNA hybridization and measurement of the RNA decay-rate

The procedures were as already published (8). Briefly, cultures in MOPS buffer at an A₆₅₀ of 0.5 were labelled for 2 min with [³H]uridine. Then, rifampicin was added at t = 0 to stop transcription and 2 ml aliquots of the culture were taken at the times indicated; bulk RNA was phenol extracted and alcohol precipitated. Specific single stranded probes derived from M13mp8 containing either a lacZ insert of 1956 nt or a thrS insert of 856 nt were immobilized on nitrocellulose filters and mixed together in a small vial for hybridization at 66°C during 16 h with gentle agitation. The filters were rinsed, RNase-treated, dried and the amount of specific messengers present at different times quantitated by scintillation counting. Non-specific hybridization corresponding to the amount of RNA hybridized to M13 DNA carrying the insert sequence in the reverse orientation was subtracted from the results given for the corresponding specific probe. Hybridization to a thrS probe was used as an internal control in all the experiments. The final yield of labelled RNA was corrected for losses during purification by comparison with the quantity of [³H]uridine incorporated immediately after lysis. This method has the advantage of being fully quantitative since the formation of the different DNA—RNA hybrids occurs in the same conditions and minimizes losses during electrophoresis or blotting to membranes.

#### β-galactosidase activity

β-Galactosidase activity of monolysogens were measured in exponential growing cells in Mops medium by the method of Miller (12). The values indicated are the mean of at least four measures.
junction of this translational fusion between the proximal part of the naked message to nucleases. If this hypothesis is true, the cleavage of the processed form of the pnp mRNA, like the chromosomally encoded pnp mRNA, is not prevented by RNase EH should have no effect on the message stability in the absence of polynucleotide phosphorylase. mRNA of strain GF5322, which is devoid of polynucleotide phosphorylase, was hybridized with specific lacZ probes (see Materials and Methods). As shown in Figure 3A, this fusion (pGF, see 8), the pnp-lacZ mRNA extracted from strain GF5322/pBR322 (half-life around 2.6 min) shown in Fig. 3C and 1439 units respectively, as measured previously (8). Likewise, the reduced stability of pnp-lacZ mRNA from GF5321/pBR322 (half-life around 2.6 min) shown in Fig. 3C

RESULTS

Effect of polynucleotide phosphorylase on the pnp mRNA decay

In a previous study (8), it was shown that all the regulatory properties observed for pnp mRNA are also exhibited by a pnp-lacZ translation gene fusion. In this fusion (pGF, see 8), the pnp messenger is co-expressed with the S15 message from the rpoO promoter P1 and it is also expressed from the pnp promoter P2, about 160 nucleotides upstream of pnp initiation codon (Figure 2). The fusion, carried by a λ phage, was inserted into the att site of a bacterial chromosome carrying a lac deletion. The junction of this translational fusion between the proximal part of pnp and the distal part of lacZ is located at amino acid 61 of polynucleotide phosphorylase. It has been shown that the pnp-lacZ mRNA, like the chromosomally encoded pnp mRNA, is unstable after processing by RNase III in the leader region. One explanation for the mRNA instability is that polynucleotide phosphorylase prevents translation of the processed form of the mRNA which, in turn, increases drastically the sensitivity of the naked message to nucleases. If this hypothesis is true, the cleavage by RNase III should have no effect on the message stability in the absence of polynucleotide phosphorylase. mRNA of strain GF5322, which is devoid of polynucleotide phosphorylase, was pulse-labelled with [3H]uridine, rifampicin added and mRNA extracted after different incubation times. The labelled mRNA was hybridized with specific lacZ and thrS probes (see Materials and Methods). As shown in Figure 3A, this message is stable (half-life around 13 min) in strain GF5322/pBR322 and exhibits the same stability as pnp-lacZ mRNA extracted from strain GF493/pBR322 (lacking RNase III) (Fig. 3B, open circles). The presence of a stable mRNA correlates with the high β-galactosidase levels in GF5322 or GF493 [2175 and 1439 units respectively, as measured previously (8)].

Figure 2. The pnp-lacZ gene fusion and shortened derivatives. The construction of GF5311 and the phage M13GF18 used for site directed mutagenesis were described previously (8). After the creation of a Nael site and verification of the sequence, double-stranded DNA of the mutant was cleaved by EcoRI and Nael and the fragments ligated to the EcoRI-HincII sites of the polylinker of plasmid pUC8 (22). Then a EcoRI–HindIII fragment of the derived plasmid was ligated between the arms of phages IG14 and SKS107 as described previously (8) and the resulting phage used to lysogenise IBPC5311, giving strain MF1. A HindIII site was also created on M13GF18 DNA by site directed mutagenesis. A EcoRI–HindIII fragment was ligated to the arms of λ as described above. Lysogenisation of strain IBPC5311 by the resulting phage gave strain SF1. P1 and P2: Promoters. t1: terminator. RII: RNase III sensitive site. pnp' and lacZ': proximal and distal parts of the respective genes. rpoO: gene encoding the ribosomal protein S15. The genes are represented by horizontal bars and deletions by a space between them.

Table I.

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In the strain GF493 even overproduction of polynucleotide phosphorylase induced by a plasmid carrying \( pnp \) has no effect on the \( pnp-lacZ \) messenger decay [compare in Fig. 3B strain GF493 with pBR322 (open circles) to the same strain carrying pBD10, a multicopy plasmid with \( pnp \) expressed only from promoter P2 (diamonds Fig. 3B)]. On the other hand, in the \( rnc^+ \) strains, the higher the polynucleotide phosphorylase level, the shorter the message half-life (Figure 3A and C): compare the effect of only one chromosomal copy of \( pnp \) gene (Fig. 3C, open circles) to the presence of several \( pnp \) copies expressed from the promoter P2 (Fig. 3A, diamonds) or from both promoters P1 and P2 (Fig. 3A and C, filled circles). The mRNA half-lives correspond respectively to 2.9 min, 1.7 min and 1.2 min. The fact that these differences in \( pnp \) mRNA decay rate are real is shown by the observation that the decay rate of \( thrS \) mRNA, used as an internal control for each experiment, was not significantly altered (Fig. 3A—C, squares and crosses). In conclusion, when the amount of polynucleotide phosphorylase present in \( rnc^- \) cells is increased by introducing a plasmid carrying the \( pnp \) gene, no effect is observed on the \( pnp-lacZ \) messenger degradation rate and the messenger is stable. Similarly, cleavage by RNase III has no effect on message stability in the absence of polynucleotide phosphorylase, thus suggesting an essential role of polynucleotide phosphorylase in the inactivation of the processed messenger. However, in the presence of a functional \( rnc \) gene, the instability of \( pnp-lacZ \) mRNA increases in proportion to the polynucleotide phosphorylase concentration as shown by the more rapid decay in the presence of overproduced \( pnp \) in \( trans \).

**Effect of translation on the \( pnp-lacZ \) messenger stability**

It has been shown previously (8) that both RNase III processing and the presence of polynucleotide phosphorylase are necessary, but that neither alone is sufficient for the polynucleotide phosphorylase mediated control of \( pnp-lacZ \) expression. A 3' to 5' degradation by this exonuclease is then excluded. Another possibility is that the effect of polynucleotide phosphorylase is targeted to the translational rate of the message. This step might be the key factor in \( pnp \) mRNA inactivation and degradation. To determine if the translation rate affects the mRNA degradation rate, a stop codon was created in the 12th codon of the polynucleotide phosphorylase part of the gene fusion (Figure 4) and the level of \( \beta \)-galactosidase measured. The results are given in Table II. In the fully derepressed lysogenic strain GF5322TR (\( rnc^+, pnp^- \)) used as a control, the \( \beta \)-galactosidase level of the fusion is 1525 units. When a \( \lambda \) carrying the fusion with this stop codon is introduced into the corresponding parental strain IBPC5322TR (giving GF5322STOP), the \( \beta \)-galactosidase level drops to 9 units, indicating that the amber codon created is very efficient. This very low level, for a derepressed strain, increases to 239 units, about one tenth of the wild type, after introduction of the suppressor, \( supE44 \), into the strain GF5322STOP (giving GF5325STOP). The effect of this early translation termination on the stability of the \( pnp-lacZ \) message was analyzed by hybridization of \([\text{H}]\text{juridine pulse-labelled mRNA to a lacZ probe.} \) This probe (1956 nt) is located at more than 1.3 kb downstream from the stop codon. mRNA was labelled for 1 min and after preventing initiation of a new round of mRNA synthesis by addition of rifampicin to the medium, the percentage of labelled lacZ mRNA remaining versus time was measured. Figure 5 shows that the amount of this messenger decreases drastically which indicates that the naked message is very unstable. The half-life is about 9-fold lower than the wild-type message. Conversely,

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**Figure 3.** Stability of the \( pnp-lacZ \) mRNA in the absence of polynucleotide phosphorylase or RNase III. Different plasmids, pBP111, expressing \( pnp \) from the two promoters, P1 and P2 (see Fig.1), pBP110, expressing \( pnp \) from one promoter, P2, or the plasmid control pBR322 were introduced into strain GF5322 (\( rnc^-, pnp^- \), GF493 (\( rnc^-, pnp^+ \)) or GF5321 (\( rnc^-, pnp^- \)). The resulting strains, growing exponentially, were radiolabelled with \([\text{H}]\text{juridine for 2 min and then treated with rifampicin. The bulk RNA was extracted at the times indicated and the amount of specific \( pnp-lacZ \) and \( thrS \) mRNAs measured by hybridization with specific \( lacZ \) and \( thrS \) probes (see Materials and Methods). The lines show the best fit values for the different decay rates. Decay rates of the control \( thrS \) mRNA are indicated by lines with an arrowhead. (A) GF5322 (\( rnc^-, pnp^- \)) carrying either plasmid pBR322: \( \circ \), \( pnp-lacZ \), \( \times \), \( thrS \); or plasmid pBP110: \( \bullet \), \( pnp-lacZ \), \( \Delta \), \( thrS \); or plasmid pBP111: \( \oplus \), \( pnp-lacZ \), \( \blacksquare \), \( thrS \); (B) GF493 (\( rnc^- \), \( pnp^- \)) and (C) GF5321 (\( rnc^- \), \( pnp^- \)) carrying pBR322, pBP110 or pBP111 as indicated in (A). (open circles), is associated with a much lower value of \( \beta \)-galactosidase [326 units, (8)]. When the activity of \( \beta \)-galactosidase increases 5—6-fold, the mRNA decay rate decreases 5-fold, indicating a tight coupling between the two phenomena.
Figure 4. Positions of the mutations located in the pnp leader region. The deletions are boxed. The Shine–Dalgarno sequence is underlined and the initiation codon framed by a dotted line. Short arrows indicate the cleavage positions by RNase III. Point mutations are circled. GFT5311 was derived from GFX5311 (which carries the 3 mutations $T_{-2}$, $T_{+10}$ and $T_{+m}$) by restoring C at +10 position. The two mutations necessary for creating the HindIII site are indicated.

partial suppression of the amber codon increases the half-life 3.5-fold when compared to the untranslated message. These results suggest that translation has a strong stabilizing effect on the processed hybrid pnp-lacZ message.

Mapping of the regulatory site
To determine the downstream limit of the regulatory site, two shorter fusions were constructed by displacing the junction of the pnp-lacZ fusion upstream by using two newly created restriction sites (NaeI and HindIII) (Figs 2 and 4). The MF1 fusion extends to the 29th residue and the shorter, SF1, to the 8th. After transfer to a λ phage and insertion into the chromosome of AB5311, the β-galactosidase level of the new strains MF1 and SF1 in the absence and in the presence of pnp in trans was monitored (Table III). The ratio observed between the value corresponding to the absence of pnp in trans and the value obtained in its presence (repression ratio) was unchanged for MF1, despite a 2-fold increase in the translational level indicating that the translational efficiency was better. On the contrary, for the shortest fusion, SF1, the repression ratio drops from 9 to 2.4 and the translational rate has increased slightly (Table III). This result shows that the coding phase of pnp mRNA at least up to the 24th nucleotide and maybe as far as the 87th, is implicated in the regulatory region.

The formation of a particular secondary structure in the processed pnp mRNA leader might also play a role. A deletion was created to remove a putative stable stem-loop structure, upstream of the Shine–Dalgarno sequence, at position (−43) to (−26). The β-galactosidase expression of the resulting mutant GFΔloop (Fig. 4) has increased about 2-fold compared to the control level and the repression ratio is slightly reduced (about 1/3 of the control) (Table III). Another deletion, GFΔpur (Fig.

Figure 5. Decay rate of the pnp-lacZ mRNA after shut-off of its translation. A stop codon was introduced in the beginning of the pnp coding frame (see Fig. 4) and the stability of the corresponding mRNA was analyzed in a strain with and without suppressor. The method used is as described in Fig. 3. pnp-lacZ mRNA: O, GF5322TR (mc+, pnp-); □, GF5322STOP; ΔGF5325STOP (+supE). Controls (thrS mRNA) are indicated by an arrow as in Fig. 3: X, GF5322TR (mc+, pnp-); ■, GF5322STOP; ○, GF3325STOP.

4), removing 8 nucleotides in a purine tract located immediately upstream of the Shine–Dalgarno sequence, decreases both the translation efficiency and the repression ratio (Table III). These results imply that, in addition to a part of the translated RNA frame, sequences located in the non coding part of the message are also involved in the regulatory site.

Effect of changes in translational initiation signals on the control
If polynucleotide phosphorylase acts by interfering with the translation initiation rate, like most of the translational autocontrol repressors, there should exist some kind of competition between
the ribosome and polynucleotide phosphorylase for occupying the ribosome loading site. Any increase in the affinity of the ribosome for this site should decrease the repression ratio and increase the translation level. To verify this prediction, the Shine–Dalgarno sequence was modified (Fig. 4), thus creating a longer complementary sequence AAGGAGGU instead of AAUUGAU and the initiation codon was changed to AUG by inserting an A between the two Us of the wild type UUG initiation codon. As expected (Table III), the translational level of the lysogenic strain carrying this fusion, GFSDIC, is increased (about 2-fold) and the repression ratio strongly decreased (3.2 instead of 9). When the mutation affects only the initiation codon, by changing UUG for AUG, (strain GFIC), an increase in β-galactosidase expression was observed, of the same magnitude as observed for the double mutant GFSDIC, when both the Shine–Dalgarno and the initiation codon were mutated. In the case of the UUG to AUG change however, only a limited effect compared to the UUG start codon. For this mutant, the repression ratio is reduced to 3.7. [Both GFT5311 and GFX5311 have an additional mutation (C → T) at position +171. It is considered that the mutation located at position +171 has probably no effect because it is located in a region which can be deleted without affecting the regulation (see MF1.) A comparison of the GFX5311 and GFT3311 strains indicates that the mutations at position (−2) and (+10) have cumulative effects and that both are involved in the regulatory region. Altogether these results show that the regulation is affected by mutations located in the ribosome loading site. The derepression is generally stronger, but never complete, with multiple mutations in this region (for example, compare GFX5311 and GFSDIC with GFIC).

**DISCUSSION**

Messenger processing and polynucleotide phosphorylase are required for pnp mRNA degradation

It was shown previously (8) that the regulation of pnp expression can be studied by a pnp-lacZ gene fusion and that the wild-type and the chimaeric messages exhibit the same regulatory characteristics. Control is exerted in two steps: at first, the pnp message must be processed in the 5′ leader by RNase III before polynucleotide phosphorylase is able to autocontrol translation of its message. Inactivation of the message is followed by its rapid degradation (13). In this paper, it is shown that the sensitivity of the 5′ leader to decay is strictly dependent upon both RNase III processing of the leader and the presence of polynucleotide phosphorylase. If either one of these two factors is lacking, the message remains quite stable and high levels of polynucleotide phosphorylase are synthesized. These observations imply the RNase III cleavage does not create an entry for another nuclease because, even after this cleavage, the messenger remains stable and active. Only the presence of polynucleotide phosphorylase induces inactivation of the message, as revealed by rapid mRNA degradation and decreased protein expression. On the other hand, it also shows that polynucleotide phosphorylase is not able to interact with its message before it is processed at its 5′ end.

**Tight coupling between pnp message translation and pnp message degradation**

In trying to locate the regulatory region of the mRNA involved in this mechanism, several partially deregulated mutants were isolated. The mutations map in a region overlapping the ribosome loading site and are located between nucleotides (−40) to (+87). The precise downstream limit in the coding sequence has not been defined, but presumably it lies between nucleotide (+24), corresponding to the deregulated SF1 fusion junction, and nucleotide (+87), the site of the MF1 fusion which is still normally regulated. The deletion of the stem loop structure enhances translational efficiency while deletion of the adjacent purine rich tract actually decreases it. The effect of the two mutations on regulation was almost identical. Two other mutations, GFIC, affecting the initiation codon, and GFSDIC, modifying both the initiation codon and the Shine–Dalgarno sequence have identical effects on translational efficiency but very different effects on regulation. Thus these results support the hypothesis that the initiation region and the beginning of the coding region are important in regulation.

The stability of the message was demonstrated to be strongly dependent upon translation by introducing a stop codon in the beginning of the reading frame. The large alteration in the message half-life results from an decreased mRNA stability,
although some induced transcriptional polarity cannot be excluded to account for this effect. If transcriptional polarity due to lack of translation can reduce the amount of distal lacZ mRNA detected by the probe located 1.3 kb downstream of the initiation codon, it has theoretically only a minor effect on the mRNA half-life, which is thus intrinsically much less stable, presumably due to the absence of translating ribosomes. This is similar to the situation for wild-type lacZ mRNA. In the experiments of Yarchuk et al. (14), mRNA stability was tightly coupled to translation initiation rate. In the case of the pnp-lacZ fusion, an additional factor—namely polynucleotide phosphorylase—is involved, which increases the degradation rate of the processed pnp-lacZ mRNA. From its presence and from the location of the deregulated mutations, it is deduced that it functions by impeding translation initiation.

How does polynucleotide phosphorylase function as a translational repressor?

Generally, the repressor protein recognizes a specific structure on the mRNA called a translational operator. In the polynucleotide phosphorylase case, RNase III cleavage might create a new secondary (or tertiary) structure which would allow the binding of the repressor. However, if processing of the pnp mRNA per se induces a conformational modification, it has no inhibitory effect on translational efficiency as indicated by the comparison of strains with (GF5322: rnc+, pnp-*) or without active RNase III (GF493: rnc-, pnp*): the β-galactosidase level is even higher for the strain GF5322 (8). Thus, the pnp message is equally translatable, whether matured or not. However polynucleotide phosphorylase must be able to discriminate between the new structure created by processing of the full length mRNA. On binding to the processed form, it exerts a drastic effect on the expression of the fusion as demonstrated by comparison between rnc+ strains with or without polynucleotide phosphorylase.

There are two possible modes of action for polynucleotide phosphorylase. Either it acts directly by inhibiting translation after binding to a specific RNA structure created after RNase III cleavage or indirectly by interacting with some essential factor involved in translation initiation. Attempts to isolate any mutation in the proximal part of pnp mRNA which completely abolishes the control were unsuccessful. Only the absence of the RNase III cleavages, either due to a rnc mutation or removal of the RNase III sensitive site, abolishes the regulation by polynucleotide phosphorylase. A possible explanation for the inability to eliminate regulation with mutations in the mRNA is that the binding site is multipartite. One hypothesis is that the 5' end created by RNase III cleavage is an essential part of the composite recognition site. Evidence supporting this hypothesis is given by the electron microscopic studies of Sulewski et al. (15). These authors show that polynucleotide phosphorylase is bound to each end of a ribopolymer, suggesting a specific binding not only at the 3' end, but also at the 5' monophosphate end. On the other hand, polynucleotide phosphorylase was shown to carry, at its C-terminus, a region showing a strong homology with the RNA binding sites of the ribosomal protein S1 (16). This suggests that the two molecules compete for the same target. Since protein S1 is primarily involved in the discrimination of the translation initiation site, this observation would account for the effect of mRNA mutations in the vicinity of the ribosome loading site. In addition, the recent discovery of a complex between polynucleotide phosphorylase and RNase E, a specific endoribonuclease, (17) might account for a coupling between the polynucleotide phosphorylase mediated inhibition of translation and the subsequently enhanced mRNA degradation rate.

ACKNOWLEDGEMENTS

We thank L. Dondon and M. Graffe for help in the technical part of this work. We are grateful to M. Grunberg-Manago for her constant interest in this work and we thank J. Plumbridge for very careful reading of and constructive advice on the manuscript. This work was supported by grants from the Centre National de la Recherche Scientifique (URA1139), from the Institut National de la Santé et de la Recherche Médicale (Contrat de Recherche Externe no. 891017), from the Fondation pour la Recherche Médicale and from the Commission des Communautés Européennes [contrat C.E.E. no. CII-0790-M (DSCN and SCI*0194-C(AM))].

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