The Fate of the Initiator tRNAs Is Sensitive to the Critical Balance between Interacting Proteins*

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Formylation of the initiator tRNA is essential for normal growth of *Escherichia coli*. The initiator tRNA containing the U35A36 mutation (CUA anticodon) initiates from UAG codon. However, an additional mutation at position 72 (72A → G) renders the tRNA (G72/U35A36) inactive in initiation because it is defective in formylation. In this study, we isolated U1G72/U35A36 tRNA containing a wobble base pair at 1–72 positions as an intragenic suppressor of the G72 mutation. The U1G72/U35A36 tRNA is formylated and participates in initiation. More importantly, we show that the mismatch at 1–72 positions of the initiator tRNA, which was thus far thought to be the hallmark of the resistance of this tRNA against peptidyl-tRNA hydrolase (PTH), is not sufficient. The amino acid attached to the initiator tRNA is also important in conferring protection against PTH. Further, we show that the relative levels of PTH and IF2 influence the path adopted by the initiator tRNAs in protein synthesis. These findings provide an important clue to understand the dual function of the single tRNA in initiation and elongation, in the mitochondria of various organisms.

Organisms have evolved with two distinct species of methionyl tRNAs. Of these, the initiator recognizes the initiation codons (AUG, GUG, AUU, UUG, etc.), and the elongator de-
determined in Miller units (17, 24) using 30 μg of cell-free extracts and then normalized with respect to the β-lactamase activity. The assays were done at least twice, and the values did not vary by more than 10%.

The average values are shown in Tables III and IV. For the experiments requiring overexpression of plasmid borne in vivo (IF2) or the chromosomal lacZ (β-galactosidase), log phase cultures were induced with 1 mm isopropyl-β-D-1-thio-β-D-galactopyranoside for 4 h (21, 25).

**Northern Blot Analysis**—Total tRNA from various transformants was isolated under acidic conditions, separated on 6.5% acid urea gels at 4 °C, and electroblotted onto a Nytran membrane (26). The 5′-32P end-labeled oligodeoxyribonucleotides used as probes for different blots were complementary to positions 29–47 of tRNA2fMet (U35A36) and 2–44 of tRNAfMet. Conditions used for hybridization of the blots have been described (26). The 19-mer oligomer probe is complementary to the anticodon region to the U35A36 tRNAs, and it possesses two mismatches in the middle when compared with the wild type tRNA sequence. Thus the signals on the Northern blots correspond to the initiator tRNA mutants and not to the endogenous host initiator tRNA (Formyl tRNA). For detection of tRNAfMet or its derivatives with the wild type anticodon (CAU), an oligomer complementary to positions 40–56 of the tRNAfMet was used. This oligomer contains a single mismatch in middle when compared with the tRNAfMet. Further to avoid signals from the host initiator tRNA, we have used E. coli B105 as host, which contains only the genes corresponding to tRNAfMet.

The formylated tRNAs were treated with 100 mM Tris–HCl (pH 9.0) or 100 mM CuSO4 to generate deacylated and formylated tRNAs, respectively. The CuSO4 deacylates both (27, 28).

**In Vitro Aminoacylation**—Glutaminyl tRNA synthetase was purified as before (29). Initiator tRNAs were overproduced in E. coli B105 from respective genes cloned on moderate copy plasmid pBR322. Total RNA from 400-ml log phase cultures were prepared by phenol chloroform extraction method described before (30). The mutant tRNAs were purified further from native polyacrylamide gels (30) and used in aminoacylation method described before (25). The bands corresponding to aminoacylated and deacylated tRNA were visualized by autoradiography, and the corresponding gel pieces were quantified using a scintillation counter. The percent aminoacylation was calculated as (counts in the aminoacylated band/counts in the aminoacylated band + counts in the remaining deacylated band) × 100 and was used to determine pmol of product formed/μg of GlnRS, which in turn was plotted as a function of time. Initial velocities of aminoacylation were determined from the slopes of the straight lines corresponding to the initial phase of the reaction.

**RESULTS**

Isolation of the U1G72/U35A36 Intragenic Suppressor—The binary plasmid system used to carry out in vivo initiation assays is shown in Fig. 1A. The ACYC origin of replication based plasmid, pACQS, harbors glutaminyl-tRNA synthetase gene. The ColE1-based plasmid, pCATam1metYCUA, carries the mutant initiator tRNA (U35A36 or G72/U35A36) and the CAT reporter, CATam1 genes (16). The mutants of initiator tRNA with U35A36 (CAU to CUA anticodon change) are aminoacylated with glutamine. The U35A36 tRNA initiates from the UAG initiation codon of the reporter mRNA and confers chloramphenicol resistance. However, the G72/U35A36 tRNA fails to initiate because it is defective in formylation.

During the course of characterizing suppressors for the G72/U35A36 tRNA (see “Experimental Procedures”) we isolated a chloramphenicol-resistant clone, which contained a single nucleotide change (C→T) in the G72/U35A36 tRNA gene. The mutation corresponded to position 1 of the mature tRNA and resulted in the replacement of the ACYC origin of replication by a CAT am1 tRNA gene. DNA sequence analysis of the translation initiation region of the CAT am1 gene did not reveal any new mutations.

**Initiation Activity of U1G72/U35A36 tRNA**—The cell-free extracts prepared from the transformants harboring the U35A36 or U1G72/U35A36 tRNA were assayed for the relative CAT activity (normalized to β-lactamase activities). Compared with the U35A36 tRNA (100%), the U1G72/U35A36 tRNA was ~56% active in initiation (Table III).

**In Vivo Status of U1G72/U35A36 tRNA**—Total tRNAs were isolated under acidic conditions and analyzed on acid urea gels to determine in vivo status of the G72/U35A36 and U1G72/U35A36 tRNAs. As reported (26), the G72/U35A36 tRNA was distributed between deacylated and aminoacylated forms (Fig. 2, lane 3), whereas the U1G72/U35A36 tRNA accumulated in deacylated and formylated forms (Fig. 2, lanes 1 and 2). Presence of the formylated form of U1G72/U35A36 tRNA explained its activity in initiation.

However, considering that the U1G72/U35A36 tRNA lacked

### Table I

**Description of E. coli strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>CA274 B105</td>
<td>Hfr H, lacZam, trpEmm, lacks endogenous tRNA2met, used for overproduction of tRNAs, and Northern blot analysis involving tRNA2met</td>
<td>18</td>
</tr>
<tr>
<td>CP78</td>
<td>F- arg-, leu-, thr-, his-, thi- derivative of CPF78</td>
<td>14</td>
</tr>
<tr>
<td>pCATam1metYCUA</td>
<td>Renamed from pRSVCATam1.25tm/MU35A36. A pBR322 derivative harboring CAT reporter gene with UAG as an initiation codon and expressing various tRNA2met derivatives with CUA anticodon (U35A36 mutation).</td>
<td>16</td>
</tr>
<tr>
<td>pCATam1metYCUA/pTh</td>
<td>The pth gene was subcloned from pBSA53 (20), into the HpaI site of pCATam1 tRNA plasmids.</td>
<td>This work</td>
</tr>
<tr>
<td>pCATmetY</td>
<td>Renamed from pRSVCAT2.5tm/M. A pBR322 derivative harboring CAT and the tRNA2met</td>
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<tr>
<td>pACQS</td>
<td>pAC1 derivative overexpressing GlnRS.</td>
<td>16</td>
</tr>
<tr>
<td>pACDQS</td>
<td>pACD derivative overexpressing GlnRS.</td>
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<tr>
<td>pACDQS-In/F</td>
<td>pACD derivative overexpressing GlnRS and IF2</td>
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</tr>
<tr>
<td>pACMS</td>
<td>pAC1 derivative overexpressing MetRS</td>
<td>22</td>
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</tbody>
</table>
a Watson-Crick base pair at the 1–72 positions and that the analysis was carried out under GlnRS overproduction, it was intriguing (31) to note that a substantial amount of this tRNA accumulated in the deacylated form (Fig. 2, lanes 1 and 2). The presence of a predominant single band corresponding to Tyr-tRNATyr showed that the tRNAs were not deacylated during preparation. Therefore, the accumulation of the deacylated form could either be because of the inefficient aminoacylation or because the fGln-tRNA^{fMet} carrying the U35A36 mutation and E. coli tRNA^{fMet}.

![Fig. 2](image)

**Fig. 2.** Northern blot analysis of the steady state levels of the mutant tRNAs isolated from *E. coli* CA274 in the presence of overproduced GlnRS. The blot was hybridized simultaneously with 5'-32P-labeled oligonucleotides complementary to *E. coli* tRNA^{fMet} car-rying the U35A36 mutation and *E. coli* tRNATyr.

Table II presents the efficiencies of aminoacylation of tRNAs by GlnRS. Consistent with earlier observations, the relative efficiency of aminoacylation of G72/U35A36 tRNA was ~6% with respect to the U35A36 tRNA. Rate of aminoacylation of U1G72/U35A36 tRNA was substantially better than that of the G72/U35A36 tRNA and only ~2-fold worse than that of the U35A36 tRNA. Interestingly, the aminoacylation efficiency of the U1G72/U35A36 tRNA was as good as the U1/U35A36 tRNA containing the U1-A72 base pair similar to the one found in the tRNAGln (31).

### Table II

<table>
<thead>
<tr>
<th>tRNA</th>
<th>Efficiency of aminoacylation (%)</th>
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<tbody>
<tr>
<td>U35A36</td>
<td>100</td>
</tr>
<tr>
<td>U1G72/U35A36</td>
<td>57.1</td>
</tr>
<tr>
<td>U1/U35A36</td>
<td>50</td>
</tr>
<tr>
<td>G72/U35A36</td>
<td>6.1</td>
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**Hydrolysis of fGln-tRNA (U1G72/U35A36) by Peptidyl-tRNA Hydrolase—*In vivo* status of the U1G72/U35A36 tRNA was analyzed in two additional strains of *E. coli*, CP78 and AA7852, in the presence or absence of GlnRS overproduction (Fig. 3). The two strains are isogenic except that AA7852 carries a temperature-sensitive mutation in *pth* (permissive at 30 °C and nonpermissive at 37 °C). The U1/U35A36 tRNA, with a Watson-Crick base pair at positions 1–72 (U1-A72), is a good substrate for PTH and was used as a control.

In the CP78 strain, expectedly, the U1/U35A36 tRNA accumulated only in deacylated form irrespective of the levels of GlnRS or the growth temperature (Fig. 3, lanes 1–4). As before (Fig. 2), the U1G72/U35A36 tRNA was found distributed between the formylated and deacylated forms (Fig. 3, lanes 5–8). However, in the absence of GlnRS overproduction, the tRNA accumulated somewhat more in the deacylated form (compare lanes 5 with 6, or 7 with 8) irrespective of the temperature at
which the cells were grown (compare lanes 5 and 6 with 7 and 8, respectively).

On the other hand, in the AA7852 strain, at the permissive temperature (30 °C) the U1/U35A36 was found to be distributed between the deacylated and formylated forms, and as expected its accumulation in the formylated form increased substantially when GlnRS was overproduced (compare lanes 9 and 10). Although at the nonpermissive temperature (37 °C) both the deacylated and the formylated forms were seen, all of the deacylated form was converted into the formylated form in the presence of GlnRS overproduction (compare lanes 11 and 12). This observation suggested that although PTH is a major reason for the U1/U35A36 tRNA to accumulate into the deacylated form, its poor aminoacylation by GlnRS also contributed to it. Similar to U1/U35A36 tRNA, U1G72/U35A36 tRNA was distributed between the formylated and deacylated forms at the permissive temperature in the AA7852 strain. However, it was seen to accumulate entirely in the formylated state at the nonpermissive temperature, irrespective of the state of GlnRS overproduction. These observations suggested that U1G72/U35A36 tRNA is a substrate for PTH. However, to our surprise, the U35A36 tRNA (Fig. 4A, lane 2). A band corresponding to its formylated form was not detectable showing that in vivo, U1G72/U35A36 is a substrate for PTH. However, to our surprise, the U35A36 tRNA with a mismatch at the 1–72 positions accumulated entirely into the deacylated form upon PTH overproduction (Fig. 4B, compare lanes 3 and 4 and 5 and 6). The marker, lanes 1 and 2, correspond to the deacylated and the formylated forms of the U35A36 tRNA, respectively. Complete lack of accumulation of aminoacylated form of U35A36 tRNA (as opposed to U1G72/U35A36 tRNA) is most likely because this tRNA is a very good substrate for formyltransferase, and as soon as it is formylated, it is hydrolyzed by PTH or utilized in initiation through IF2 (as shown below). These results suggested that the mismatch at the 1–72 positions, which is considered a hallmark for the resistance of the initiator tRNA toward PTH, is not sufficient in preventing its hydrolysis by PTH. Thus the presence of a minor band corresponding to the deacylated form of the U35A36 tRNA in the absence of GlnRS overproduction (Fig. 4B, lane 3) could be because of a combined effect to lower rates of aminoacylation and the partial hydrolysis of the formylated tRNA by PTH.

Role of the Amino Acid Attached to the Initiator tRNA in Its Recognition by Peptidyl-tRNA Hydrolase—In nature, initiation occurs with fMet. For efficient initiation, it is desirable that the fMet-tRNAfMet is prevented from its wasteful hydrolysis because of PTH. Our observation, that the CxA mismatch at 1–72 positions of the tRNAfMet is not sufficient in conferring resistance to PTH, was made with a tRNA-carrying fGln. Could it be that the amino acid attached to the tRNA also plays a role in preventing its hydrolysis by PTH? Therefore, we examined the steady state levels of tRNAfMet (U1G72) and tRNAfMet (with CAU anticodon, hence aminoacylated with Met) in the presence or absence of PTH and/or MetRS overproduction in E. coli B105, which lacks endogenous tRNA2fMet (19). Because all our tRNA mutants are derivatives of tRNA2fMet, it was possible to detect the tRNA2fMet free from the host background of tRNAfMet by the use of an oligomeric probe spanning the variable loop. This region harbors adenosine at position 46 in tRNAfMet as opposed to 7-methyl guanosine in tRNA2fMet (32). Both the tRNA2fMet and the corresponding tRNAfMet (U1G72) were completely formylated with or without overproduction of MetRS (Fig. 5, lanes 1, 3, 5, and 7). And, as would be relevant from the physiological considerations, the fMet-tRNAfMet remained formylated even when PTH was overproduced (lanes 2 and 4). On the other hand, overproduction of PTH resulted in the appearance of the aminoacylated and deacylated forms of tRNAfMet (U1G72) (lane 6), suggesting that the tRNAfMet (U1G72) is a substrate for PTH even when fMet was attached to it. Expectedly, when MetRS was overproduced, the deacylated form was converted into the aminoacylated form (lane 8). The fact that even upon PTH overproduction a considerable level of tRNA (U1G72) is detected in the formylated form and suggested that the initiator tRNAs are more resistant to PTH when they carry fMet than when they carry fGln. Nevertheless, these observations show that both the amino acid and the 1–72 positions are important in recognition or rejection of the initiator tRNAs by PTH.

Table III

<table>
<thead>
<tr>
<th>tRNA</th>
<th>Overproduction of PTH</th>
<th>Overproduction of fMet</th>
</tr>
</thead>
<tbody>
<tr>
<td>U35A36</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>U35A36</td>
<td></td>
<td>47.7</td>
</tr>
<tr>
<td>U1G72/U35A36</td>
<td>+</td>
<td>56.14</td>
</tr>
<tr>
<td>U1G72/U35A36</td>
<td>–</td>
<td>3.85</td>
</tr>
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</table>
PTH and IF2 Compete for the fGln-tRNA\textsuperscript{Met} Species—As the formylated forms of the U1G72/U35A36 (A) or U35A36 (B) tRNA mutants in the presence (+) or absence (−) of PTH overproduction and with (+) or without (−) overproduction of MetRS. The tRNAs were isolated under acidic conditions, separated on acid urea gel, and probed with a 5'-\textsuperscript{32P}-labeled oligonucleotide complementary to the variable loop of the tRNA\textsuperscript{Met}.

FIG. 4. Northern blot analysis of tRNAs isolated from E. coli CA274 harboring U1G72/U35A36 (A) or U35A36 (B) tRNA mutants in the presence (+) or absence (−) of PTH. The tRNAs were isolated under acidic conditions and resolved on acid urea gels. A mixture of 5'-\textsuperscript{32P}-labeled oligonucleotides complementary to tRNA\textsuperscript{Met} (U35A36) and tRNA\textsuperscript{fMet} were used to detect the corresponding tRNAs. B, the tRNAs were treated with either alkali (lane 1) or CuSO\textsubscript{4} (lane 2) to generate markers for uncharged and formylated tRNAs, respectively.

Fig. 5. Analysis of steady state levels of tRNA\textsuperscript{Met} and tRNA\textsuperscript{Met} (U1G72) (CAU anticodon) isolated from the E. coli B105 strain in the presence (+) or absence (−) of PTH overproduction and with (+) or without (−) overproduction of MetRS. The tRNAs were isolated under acidic conditions, separated on acid urea gels, and probed with a 5'-\textsuperscript{32P}-labeled oligonucleotide complementary to tRNAfMet (U35A36).

Similarly, overproduction of IF2 resulted in the presence of a single band corresponding to the formylated form of U1G72/U35A36 tRNA (compare Fig. 4A, lane 1, to Fig. 6, lane 3). However, as the U1G72/U35A36 tRNA is a good substrate for PTH, the absence of the formylated form in the presence of PTH overproduction, even when IF2 was simultaneously overproduced (Fig. 6, lane 4), was not unexpected. It is very likely that as soon as the tRNA is formylated it is hydrolyzed by PTH and the protection conferred by IF2 binding is not detectable by Northern analysis of the total tRNA, which detects the steady state levels of the different forms.

Effect of PTH Overproduction on the Path Adopted by tRNA\textsuperscript{Met}—The CxA mismatch at positions 1–72 of the alternate fates of initiator tRNA in cell.
tRNA\text Supercircled {\textsuperscript{ Met}} and the formylation of the initiator tRNAs prevent their binding to EFTu and therefore their participation in elongation. Creation of a base pair at the 1–72 position allows the initiator tRNAs to bind to EFTu and participate at the elongation step (25, 26). Recently, using tRNA\text Supercircled {\textsuperscript{ Met}} (U35A36) it was shown that upon EFTu overproduction, it participated in elongation even though it contains the CxA mismatch at 1–72 positions (3).

E. coli CA274 has an internal amber mutation in the lacZ gene. This amber codon can be suppressed by tRNAs with the CU anticodon, and the β-galactosidase thus produced provides a measure of their elongation activities (33). As shown in Table IV, the U35A36 tRNA (carrying the CxA mismatch at the 1–72 positions) showed a basal level of β-galactosidase activity (77.7 units). Upon PTH overproduction, this activity increased to 511.9 units. Similarly, although the U1G72/U35A36 tRNA served as a better elongator (1517.1 units), its activity in elongation increased further when PTH was overproduced (3413.8 units). Interestingly, when IF2 was simultaneously overproduced, these elongation activities decreased to 70.1, 113.7, 213.1, and 774.7 (Table IV).

**Table IV**

<table>
<thead>
<tr>
<th>tRNA</th>
<th>Overproduction of PTH</th>
<th>Overproduction of IF2</th>
</tr>
</thead>
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<tr>
<td>U35A36</td>
<td>77.7</td>
<td>70.1</td>
</tr>
<tr>
<td>U35A36</td>
<td>511.9</td>
<td>113.7</td>
</tr>
<tr>
<td>U1G72/U35A36</td>
<td>1517.1</td>
<td>213.1</td>
</tr>
<tr>
<td>U1G72/U35A36</td>
<td>3413.8</td>
<td>774.7</td>
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**DISCUSSION**

Our studies with a formylation-defective initiator tRNA (G72/U35A36) resulted in isolation of an intragenic suppressor (U1G72/U35A36). The presence of the U1G72/U35A36 tRNA in formylated form (Fig. 2) explains its activity in initiation and highlights the significance of formylation in protein synthesis in E. coli. More importantly, the biochemical and the in vivo analyses involving U1G72/U35A36 tRNA have allowed us to elaborate on the distribution of the initiator tRNAs with various proteins that interact with them. We show that, in vivo, the tRNAs with a wobble base pair (U1-G72) at the top of acceptor stem are substrates for PTH. Also, we discovered that the mismatch (C1xA72) alone at these positions of the initiator tRNA, which was thus far considered to be a hallmark for its resistance toward hydrolysis by PTH, is not sufficient in preventing the wasteful hydrolysis of the formylated tRNA because of PTH. The role of the amino acid attached to the tRNA becomes evident from the observation that initiator tRNA (with C1xA72 mismatch) is completely resistant to hydrolysis by PTH when charged with fMet but not when it was charged with fGln (Figs. 4 and 5).

The crystal structure of PTH shows that its active binding site accommodates the C-terminal end (Lys\textsuperscript{191}-Ala-Gln\textsuperscript{195}) of the neighboring PTH molecule via several electrostatic interactions (34). Our observations that (i) fGln-tRNA\text Supercircled {\textsuperscript{ Met}} (CUA) is a substrate for PTH and the fMet-tRNA\text Supercircled {\textsuperscript{ Met}} is not and (ii) the fGln-tRNA\text Supercircled {\textsuperscript{ Met}} (U1G72/U35A36) is a better substrate than the fMet-tRNA\text Supercircled {\textsuperscript{ Met}} (U1G72) for PTH (Figs. 4 and 5) do suggest that the side chains of the amino acids influence the contacts between the enzyme and the substrate and thereby affect the esterase activity of the enzyme. In addition, an earlier observation that AcMet-tRNA\text Supercircled {\textsuperscript{ Met}} (U1G72) was not a substrate for PTH (35) may be extended to suggest that the nature of modification of the α-amino group of the amino acid attached to the initiator tRNA may also contribute to its recognition by PTH. However, considering that the physiological role of PTH demands nonselectivity toward its substrate, these effects may be subtle for the tRNAs attached to larger size peptides.

Overproduction of IF2 led to an ~5-fold increase in the efficiency of initiation with fGln (Table III). Importantly, when IF2 was overproduced simultaneously with the PTH, it rescued the decrease in initiation activity that resulted upon the overproduction of PTH alone. The protective effect of IF2 on the formylated tRNAs against their hydrolysis by PTH was also clearly discernible from the steady state accumulation of the different forms of tRNA in the cell (compare Figs. 4 and 6).

Our data suggest that after its aminoacylation the initiator tRNA is distributed between the formyltransferase and EFTu. The majority of the aminoacyl-tRNA\text Supercircled {\textsuperscript{ Met}} bound to formyltransferase is formylated and committed for the purpose of initiation (Fig. 7, bold arrows). Evidently, when this equilibrium is favored for binding of the initiator tRNAs to EFTu by its overproduction (3), their participation in elongation is detectable and significant. Thus, a small population of the initiator tRNA must bind to EFTu (Fig. 7, thin arrows) even when EFTu is not overproduced. Understandably, the mutants of initiators, which are poor substrates for formyltransferase, would partition more with EFTu. This interpretation is corroborated by an earlier study wherein the mutant initiator tRNAs such as U1/U3A70/U35A36, G3C70/U35A36, G72/U35A36, G72G73/U35A36, and U1/U3A70/U35A36 etc., which are poor substrates for the formyltransferase, function as good elongators (17).

In the present study, we observed that overproduction of PTH led to substantial increase in elongation activity of U35A36 and U1G72/U35A36 tRNAs. Because the EFTu binds to the aminoacyl tRNA and the PTH binds to the formylated form of the tRNA, PTH overproduction is unlikely to have any direct effect in facilitating better binding of the aminoacyl tRNA to EFTu. A simple interpretation of this observation is that every time an aminoacyl tRNA is released from the aminoacyl-tRNA synthetase, it is subjected to competition for its aminoacyl-tRNA synthetase, some finite fraction of the aminoacyl-tRNA that escapes binding to formyltransferase must partition with EFTu and which in turn participates in elongation. The fact that fGln-tRNA\text Supercircled {\textsuperscript{ Met}} (CUA) is a substrate for PTH, suggests that the overproduction of PTH greatly enhances the turnover of the formylated tRNA (Fig. 7). The cumulative effect of the small finite fractions of the aminoacylated tRNA that bind to EFTu thus results in a significant level of elongation by the initiator.
Alternate Fates of Initiator tRNA in Cell

20367

tRNA. Because both the IF2 and PTH bind to the formylated tRNA, it is reasonable to assume that the two proteins compete with one another for the formylaminocyl-tRNA available in the cell. Thus, our observation that overproduction of IF2 results in steady state accumulation of the formylated tRNA is a consequence of the protection conferred by IF2 against hydrolysis by PTH. However, an implication of this finding is that overproduction of IF2 decreases the levels of the formylated form of the tRNA in the cell that now remains available to bind to PTH for its hydrolysis. Consistent with our hypothesis that it is the increased turn over of formylated tRNA that is responsible for its participation in elongation, IF2 overproduction abates the elongation activity of the initiator tRNA that accrued as a result of PTH overproduction.

In conclusion, our studies with PTH and/or IF2 overproduction show that a critical balance of various proteins that interact with the initiator tRNA is crucial to ensure its appropriate use in the cell. Further, these studies provide an important clue to understand the dual function of a single tRNA\textsuperscript{Met} in initiation and elongation in the mitochondria of various organisms (36).

Acknowledgments—We thank Prof. U. L. RajBhandary for critically reviewing this manuscript as well as for providing us with many of the plasmid constructs used in this study. The recombinant plasmids containing PTH and IF2 genes used to generate various plasmid constructs were originally provided by Drs. G. Guarneros and J. Hershey, respectively.

REFERENCES