Asp[168]) to rearrange to their catalytically effective positions. The 8400-fold discrimination between a methyl and a carboxymethyl side chain is only achieved in an environment in which the substrate is completely solvated by protein groups. Lack of detailed understanding of protein intramolecular mobility may account for other attempts (*27*) to alter enzyme-substrate specificity being less than entirely successful.

Our results show it is possible to design and make a new MDH, which is by any standards a good enzyme and about twice as active as that which is naturally evolved in the organism from which the gene was derived (*28*). In the case of well-studied protein frameworks where new enzymes can be designed and made by small changes in residues close to the protein surface, we may hope it will soon be less laborious to redesign an existing and thermally stable enzyme framework for a new target substrate than to search for a new enzyme activity from natural organisms.

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Translation of Unspliced Transcripts After Heat Shock

H. JOSEPH YOST* AND SUSAN LINDQUIST

Severe heat shocks block the splicing of intervening sequences from messenger RNA precursors. The RNA's that accumulate after a severe heat shock have normal transcription start sites and are uncut at both their 5′ and 3′ splice junctions. Some of these unspliced transcripts leave the nucleus and enter the pool of cytoplasmic messenger RNA. Translation of these RNA's proceeds into their intervening sequences, resulting in the production of abnormal proteins. Thus, the repression of normal transcription, which usually accompanies the heat shock response, may protect the cell from the large-scale synthesis of abnormal RNA's and aberrant proteins.

ALL CELLS RESPOND TO HIGH TEMperatures by inducing the synthesis of heat shock proteins (hsp's) and at least partially suppressing the synthesis of normal cellular proteins (*1*). In *Drosophila melanogaster* cells, transcription of heat shock genes is rapidly induced by a shift from the normal growing temperature of 25°C to temperatures between 29° and 39°C. Heat-shock transcription is maximal at 37°C; at this temperature the transcription of previously active genes is repressed (*2*). Heat shock also elicits a complete change in the specificity of translation. While heat-shock mRNA's are translated with very high efficiencies at 37°C, preexisting mRNA's are translationally inactive. These messages are not degraded, but are quantitatively retained and translated during recovery from heat shock (*3*).

We have shown that another basic aspect of gene expression in *Drosophila* cells is altered by heat shock; the splicing of intervening sequences from mRNA precursors is blocked at high temperatures and remains blocked during the initial stages of recovery at normal temperatures. This block in splicing was first discovered because it greatly reduces the synthesis of the 83-kD heat shock protein (hsp83). The *hsp83* gene is the only hsp gene in *Drosophila* that contains an intervening sequence, and its protein is therefore the only hsp so effected. However, the block in splicing is not specific to *hsp83* transcripts. When the intron-containing alcohol dehydrogenase gene is placed under the control of a heat-inducible promoter, its transcripts are also not spliced at high temperatures (*4*).

As is the case with other toxic effects of heat, the hsp's appear to mitigate the effects of heat on RNA splicing. When *Drosophila* cells are given a mild heat treatment, which induces the synthesis of hsp's, before they are exposed to more severe temperatures, RNA splicing is protected. The protective effect of the prior treatment is blocked when cycloheximide is added before, but not after, the synthesis of hsp's (*4*). The disruptive effects of severe heat shocks on RNA splicing are highly conserved and are found in yeast (*5*), trypanosome (*6*), plant (*7*), and mammalian cells (*8*). The protective effects of mild heat treatment have been demonstrated for both *Drosophila* and yeast cells (*4, 5*).

In all eukaryotes the excision of intervening sequences to form mature messenger RNA's begins with the incorporation of RNA polymerase II transcripts into spliceosome complexes consisting of small nuclear (snRNP) and heteronuclear (hnRNP) ribonuclear protein particles. Two catalytic reactions then occur. First, the precursor RNA is cleaved at the 5′ exon-intron junction with the concurrent formation of a lariat structure, in which the 5′ guanosine of

Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

*Current address: Department of Molecular Biology, University of California, Berkeley, CA 94720.

the intron is joined by a 2′,5′-phosphodiester bond to an adenine near the 3′ end of the intron. Next, the 3′ intron-exon junction is cleaved and the exons are ligated together (*9*).

To determine which step in RNA splicing is blocked by heat shock and how hsp's might function to protect splicing, we have examined the structures and intracellular locations of intron-containing RNA's accumulated during the heat shock response. Our previous results demonstrated that these RNA's are uncut at their 3′ splice sites (Fig. 1, probe A) (*10*). Therefore, two features of the 5′ ends of hsp83 precursor RNA's were examined. First, we asked whether the heat-induced block in splicing occurred before or after 5′ splice-site cleavage and lariat branch formation. Second, because a different transcriptional initiation site might make the RNA a poor substrate for splicing, we determined whether the heat-induced, unspliced hsp83 RNA's had the same 5′ ends as the normal, spliced hsp83 mRNA's.

The 5′ splice sites and 5′ ends of all accumulated hsp83 RNA's were first mapped by nuclease protection analysis with a uniformly labeled probe. This single-stranded DNA probe began with sequences 170 nucleotides (nt) upstream of the normal transcription start site, spanned the 150-nt first exon, and continued for 440 nt into the intron (Fig. 1, probe B). RNA's from control cells, containing constitutively synthesized mature hsp83 mRNA (*11*), protected 150 nt of the probe from nuclease digestion, as expected from previous experiments identifying the 5′ exon sequence (*12*). With RNA's from cells exposed to a severe heat shock, the most prominent protected fragment was 590 nt. Apparently, most of the accumulated hsp83 precursor RNA's were not cleaved at their 5′ splice sites.

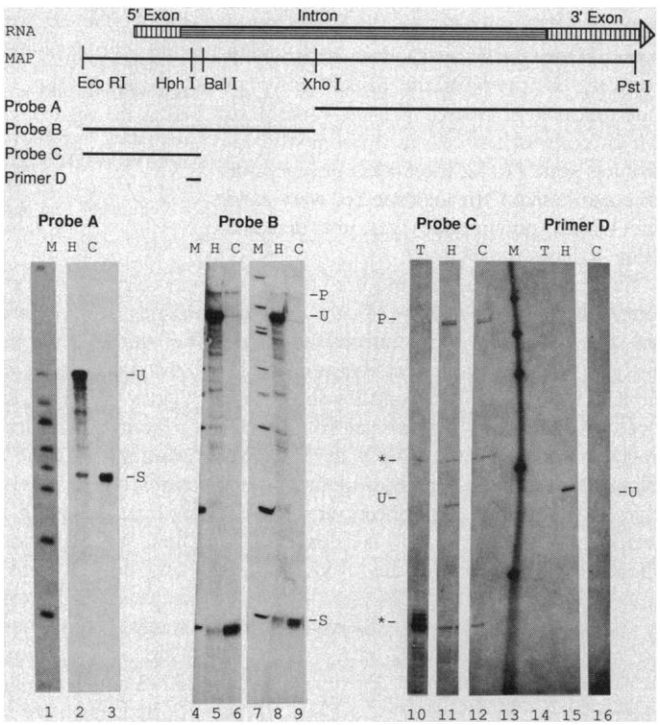

Fig. 1. Structure of hsp83 RNA's. (**Top**) The hsp83 transcript, with (vertically hatched) exons and (horizontally hatched) 1.1-kb intron above restriction sites used to generate probes. (**Bottom**) S1 protection and primer extension analysis. RNA's were extracted from Schneider's line 2 cells that had been heat-shocked at 38°C for 30 min and returned to 25°C for 1 hour (H) or from cells maintained at 25°C (C). The positions of the original probe (P) and the fragments expected from the unspliced precursor (U) and the normal spliced mRNA (S) are indicated at the sides. Molecular size markers (M, lanes 1, 4, 7, and 13) were ^{32}P–end-labeled fragments from Eco RI and Hinf I digestion of pBR322 (988, 632, 517, 506, 396, 344, 298, 221, 220, 154, and 75 nt). (Lanes 1 to 3) Probe A, a continuously labeled, single-stranded DNA probe that spans the 3′ splice site, was hybridized in solution to 10 μg of total cellular RNA's and digested with 80 units of S1 nuclease. (Lanes 4 to 9) Probe B, a continuously labeled, single-stranded DNA probe that spans the 5′ splice site and 5′ end of hsp83 RNA's, was hybridized in solution to 10 μg of total cellular RNA's and digested either with 80 units of S1 nuclease (lanes 5 and 6) or 450 units of mung bean nuclease (lanes 8 and 9). (Lanes 10 to 12) Probe C, which spans the 5′ splice site and 5′ end of hsp83 RNA, was end-labeled at the Bal I site with T4 polynucleotide kinase, with subsequent Eco RI digestion. The gel-purified, double-stranded DNA fragment was hybridized in solution to 10 μg of total cellular RNA's (lanes 11 and 12) or to 10 μg of yeast tRNA's (lane 10) and digested with 450 units of mung bean nuclease. Digestion-artifact bands (*) appeared in samples that contained only tRNA as well as in samples that contained *Drosophila* RNA. (Lanes 14 to 16) Primer D consisted of purified probe C digested with HphI. The end-labeled, double-stranded DNA fragment of 42 nt was denatured and hybridized in solution to 10 μg of total cellular RNA's (lanes 15 and 16) or to 10 μg of yeast tRNA's (lane 14). Nucleic acids were precipitated, and the primer was extended with 15 units of avian reverse transcriptase. Products were electrophoretically separated on gels containing 8*M* urea and 4% (lanes 1 to 3), 6% (lanes 10 to 16) or 8% (lanes 4 to 9) polyacrylamide, and were detected by autoradiography (*25*).

To provide better resolution of the 5′ ends of hsp83 precursor RNA's and confirm that the 5′ splice site was uncleaved, another probe (probe C, Fig. 1) was used. Since this probe was 5′ end-labeled at the Bal I site within the intron sequence, RNA's from control cells did not protect the ^{32}P-labeled portion of the probe. RNA's from heat-shocked cells protected a single ^{32}P-labeled fragment of approximately 200 nt. The bands indicated by an asterisk in Fig. 1 were digestion artifacts since they also appeared in a reaction that contained only the probe and 10 μg of yeast tRNA (lane 10). The majority of the intron-containing transcripts that accumulated during heat shock had been initiated approximately 200 ± 5 nt upstream of the Bal I site, at the previously mapped normal transcription start site (*12*). These RNA's remained uncut, either at the 5′ exon-intron junction or elsewhere in the region.

Both results were confirmed by primer extension analysis. An end-labeled intron-specific primer (primer D, Fig. 1) was hybridized to RNA's from either control or heat-shocked cells and then extended by reverse transcriptase to the end of the RNA. As expected, with RNA's from control cells the primer was not extended. With RNA's from heat-shocked cells, the primer was extended to a total length of approximately 200 nt, representing the distance from the end-labeled nucleotide to the normal transcription start site. Thus no extraneous RNA sequences were present at the 5′ end of the transcript.

We conclude that when RNA splicing is blocked by heat shock, most of newly synthesized hsp83 RNA's accumulate as full-length, uncut transcripts with the same 5′ ends as mature hsp83 mRNA's. These unspliced transcripts accumulate in large numbers during the initial stages of recovery from a severe heat shock. After a few hours, their concentration declines and the concentration of mature hsp83 messages increases (*4, 5*). Whether the messages were produced by processing of the previously accumulated unspliced transcripts or by direct processing of new transcripts was investigated in several ways. For example, either DRB (5,6-dichlorobenzimidazole riboside) or actinomycin D was used to block new transcription during recovery. In both cases, precursor concentrations declined at the same rate as in the absence of the drugs, but there was no corresponding increase in the concentration of mRNA. The results suggest that the accumulated unspliced transcripts are unable to reenter the splicing pathway. However, the interpretation of the results is clouded by the possibility that the drugs interfered with splicing as well as with transcription.

Cell fractionation experiments suggested that most of the unspliced transcripts were located in the cytoplasm, the wrong cellular compartment for splicing. However, the RNA's might have simply leaked from the nucleus during fractionation (*13*).

To rigorously determine if heat-induced intron-containing transcripts enter the cytoplasm, we asked whether they were translated into protein. We constructed a chimeric gene, *JY113*, whose transcripts would encode a protein of one size if properly spliced and a protein of another size if unspliced (diagrammed in Fig. 2). The 5′ half of the *hsp70* gene, including all of the 5′ transcriptional and translational elements required for heat shock expression (*14*), was fused to the 3′ half of the *Drosophila* alcohol dehydrogenase (*adh*) gene, which contains a 70-nt intervening sequence (*15*). Translation of spliced transcripts from this gene would produce a 50-kD protein. Translation of unspliced transcripts would yield a shorter polypeptide, of 40 kD, because of an in-frame nonsense codon in the intervening sequence.

As expected, in stably transformed *Drosophila* tissue-culture cells (*16*), no products of this gene were detected at the normal growing temperature of 25°C (Fig. 3A, lane 1). When cells were shifted to an intermediate heat shock temperature of 35°C, transcription was induced. The transcripts were spliced accurately and transported to the cytoplasm, as evidenced by the abundant synthesis of a novel 50-kD polypeptide (Fig. 3A, lane 2). This polypeptide was reactive on immunoblots with a goat antiserum to ADH. Immunoreactive peptides were not detected in untransformed cells nor in cells transformed with the vector alone.

At 37°C, the 50-kD polypeptide was produced at a lower level and a novel 40-kD polypeptide, of the size expected for translation of the unspliced precursor, appeared (Fig. 3A, lane 4). This 40-kD polypeptide also reacted with the antiserum to ADH. To control for artifactual production of the 40-kD polypeptide, we constructed another gene from an *adh* cDNA clone; *JY112* is identical to *JY113* except that it does not contain the *adh* intervening sequence. When *Drosophila* cells stably transformed with the cDNA gene were exposed to various temperatures, the 50-kD polypeptide was produced, but the 40-kD polypeptide was not detected, even at high temperatures (Fig. 3B). We conclude that the 40-kD polypeptide results from the translation of unspliced, intron-containing RNA's that enter the cytoplasm of heat-shocked cells.

The heat-induced block in RNA splicing persists for 2 hours after exposure to a severe heat shock (*4*). In keeping with this result, when cells with the intron-containing gene were exposed to 38°C for 30 min and then returned to 25°C, only the 40-kD polypeptide, and not the 50-kD polypeptide, was synthesized during the first 2 hours of recovery. During the third and fourth hours of recovery, synthesis of the 50-kD polypeptide gradually increased (Fig. 4, lanes 1 to 4). In contrast, in cells containing the intronless gene *JY112*, the 50-kD polypeptide was synthesized throughout recovery, and the 40-kD polypeptide was not detected (Fig. 4, lanes 5 to 8).

Since the 5′ sequences required for translation during heat shock (*14*) are identical on the intronless and intron-containing RNA's, the relative rates of synthesis of the 50-kD and 40-kD polypeptides should reflect the relative concentrations of the spliced and unspliced RNA's in the cytoplasm. Northern blot analysis demonstrated that total *adh* transcript concentrations were nearly identical in the two cell lines. Since the rate of production of the 40-kD protein in cells carrying the intron-containing gene (lanes 2 and 3) was approximately the same as the rate of production of the 50-kD protein in cells carrying the cDNA gene (lanes 6 and 7), it appears that the intron-containing transcripts exit the nucleus with roughly the same efficiency as the intronless transcripts.

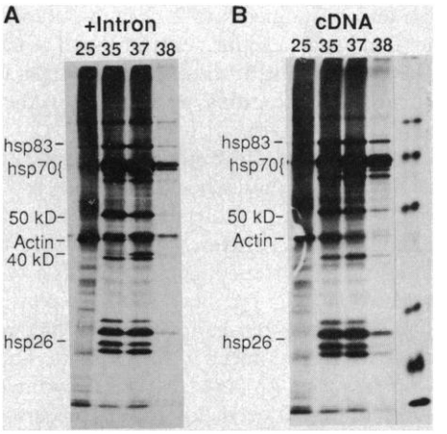

Fig. 3. Protein synthesis at various temperatures in Schneider's line 2 cells stably transformed with the intron-containing gene *JY113* or the cDNA lacking gene *JY112*. (**A**) Cells transformed with *JY113* were maintained at normal growth temperature (25°C, lane 1), or shifted to 35°C (lane 2), 36°C (lane 3), or 37°C (lane 4) for 75 min. [^{3}H]Leucine was added, and incubation was continued at these temperatures for an additional 45 min. (**B**) Cells transformed with *JY112*, treated as for (A). Proteins were extracted and analyzed on 10% polyacrylamide-SDS gels as previously described (*4*). Positions of the hsp, actin, and the novel 50-kD and 40-kD polypeptides are indicated at the right of each panel. In (A), the band migrating just above actin is due to an endogenous virus that is heat shock–resistant. Expression of the virus varies from culture to culture and has no effect on heat shock gene expression (*28*).

Intron splicing is thought to be one of the final steps in transcript maturation, occurring before RNA is transported from the nucleus to the cytoplasm, and it has been suggested that intron sequences might provide a signal for discriminating between RNA's that should or should not be transported. Here we observe efficient splicing at one temperature and a complete block in splicing at another, with both RNA's being transported to the cytoplasm with apparently similar efficiencies. It is possible that high temperatures disrupt both the splicing machinery and a separate nuclear discrimination system that operates to prevent intron-containing RNA's from entering the cytoplasm. A more parsimonious explanation is that once having escaped the splicing machinery, an RNA is free to enter the cytoplasm.

In contrast to most genes in higher eukaryotes, most of the heat shock genes are free of intervening sequences. The signifi-

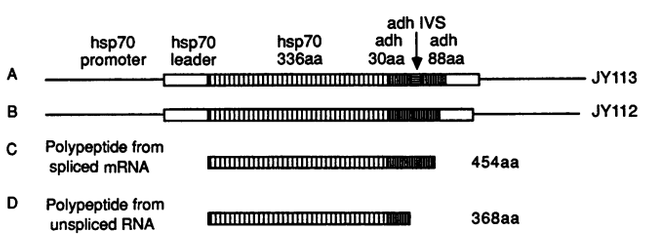

Fig. 2. Diagram of the intron-containing and the cDNA gene constructs and the polypeptides which they encode. (**A**) The intron-containing gene, *JY113*, was constructed by replacing the Bam HI to Sma I fragment of the 3′ half of the *hsp70* gene in pDM300 (*26*) with the Bam HI to Xba I (filled by the Klenow fragment of DNA polymerase) fragment of the 3′ half of the *Drosophila adh* gene in pHAP (*27*). (Solid line) Untranscribed sequences from the *hsp70* gene. (Open boxes) 5′, transcribed, untranslated sequences of the *hsp70* message leader required for translation at high temperatures and 3′, transcribed, untranslated sequences of the *adh* gene. (Broad, vertically hatched boxes) 5′, the first 336 codons of *hsp70*. (Narrow vertically hatched boxes) *adh* coding sequences, 30 codons at 5′ and 88 codons at 3′. (Horizontally hatched box) the 70 nt *adh* intron. (**B**) The corresponding cDNA gene, *JY112*, was constructed in a similar manner from an *adh* cDNA clone, pWX3008. (**C**) The polypeptide predicted from translation of mRNA's, derived either by correct splicing of *JY113* transcripts or from unspliced transcripts of *JY112*, is 454 amino acids (aa), approximately 50 kD. (**D**) The polypeptide predicted from translation of unspliced transcripts of *JY113*, ending at the inframe nonsense codon in the intervening sequence, is 368 amino acids, approximately 40 kD.

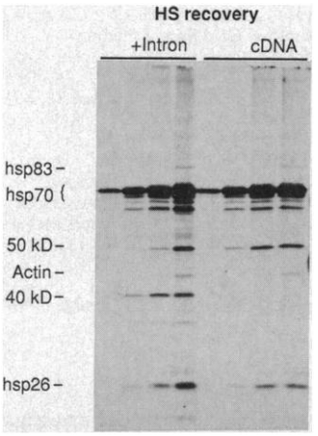

Fig. 4. Protein synthesis in transformed cells during recovery from a 38°C heat shock (HS). Cultures of cells transformed with either *JY113* (lanes 1 to 4) or *JY112* (lanes 5 to 8) were divided into four equal portions and exposed to 38°C for 30 min and returned to 25°C. [^{3}H]Leucine was added to one portion 15 min after return to 25°C, and incubation was continued for 45 min (lanes 1 and 5). The other portions were labeled for 45 min after 2 hours (lanes 2 and 6), 3 hours (lanes 3 and 7), and 4 hours (lanes 4 and 8) of recovery. Proteins were analyzed as in Fig. 3.

cance of this observation is underscored by the fact that other members of the heat shock gene family, those expressed at normal temperatures, do contain introns (*17*). The heat-induced block in RNA splicing and the translation of intron-containing transcripts place two restrictions on gene expression, and these restrictions may have provided selective pressure for the evolution of intronless heat shock genes. First, the presence of an intron in a heat shock gene would restrict synthesis of a hsp when it is presumed to be most needed, during heat shock or during recovery from heat shock. Second, if an intervening sequence was present within the coding region, not only would synthesis of a normal hsp be eliminated, but an abnormal, truncated hsp would be produced. We have found that the synthesis of aberrant hsp's can be detrimental. In particular, certain carboxyl-terminal deletions of hsp70 have the dominant phenotype of prolonging the time required for a cell to recover from heat shock (*18*).

The *hsp83* gene of *Drosophila* is one of the exceptions to the general rule that heat-induced genes lack intervening sequences. In this case, two characteristics may mitigate the effect of the intron. First, the gene is constitutively expressed as well as heat-inducible. Thus, a substantial quantity of hsp83 protein is present in the cell before heat shock. Second, the intervening sequence is positioned immediately in front of the first AUG of the normal coding sequence. If translation is initiated at the first AUG of the intervening sequence, a polypeptide of only seven amino acid residues would be produced.

The repression of normal transcription occurs at temperatures that produce very high rates of heat-shock transcription (*2*). Thus it is not simply a nonspecific toxic effect of heat. Our results suggest that it may have evolved, at least in part, to prevent the accumulation of intron-containing transcripts in the cytoplasm and the subsequent synthesis of abnormal polypeptides. The argument is supported by the fact that the histone genes, which do not have intervening sequences, are the only normal cellular genes in *Drosophila* whose expression is known to continue with heat shock (*19*). Moreover, in yeast cells, the block in normal transcription is apparently not as stringent as it is in *Drosophila* cells, and some intron-containing transcripts of normal cellular genes accumulate in substantial numbers at high temperatures (*5*). Very few yeast genes contain intervening sequences and, in those that do, the introns are located near the 5′ ends of the transcripts, which would therefore produce only short polypeptides.

That a heat-induced block in splicing can lead to the production of abnormal polypeptides may have other biological consequences. For example, it might be responsible for certain developmental anomalies that are produced by short, high-temperature heat shocks. To take a case in point, when *Drosophila* embryos at the cellular blastoderm stage are exposed to a short, severe heat shock, the adult flies that emerge several days later display developmental anomalies, with patches of tissue developing as though they belonged to other segments (*20*). These transformations, known as phenocopies, are very precise and closely resemble those seen in flies carrying certain dominant gain of function mutations in the *fushi tarazu* (*ftz*) segmentation gene (*21*). In both cases, the most common transformation is of first abdominal segment tissues to third abdominal segment tissues, with transformation of thoracic tissues appearing less commonly. One of the *ftz* mutations, ftz^{Rp1}, is associated with a chromosomal breakpoint that would result in the production of a truncated *ftz* polypeptide (*22*). Since the single intron of the *ftz* gene is located in the middle of the protein coding region, a truncated polypeptide would also be produced from unspliced *ftz* transcripts if a short high-temperature heat shock disrupted splicing before transcription could be repressed. Thus, the phenocopies induced by heat-shock in cellular blastoderm embryos might be due to the production of a truncated *ftz* polypeptide that is dominant over the wild-type *ftz* polypeptide. We do not suggest that this mechanism is responsible for all heat-induced developmental anomalies, but it may make a significant contribution to other heat-induced toxicities.

Mild prior heat treatments reduce the incidence of developmental abnormalities and of lethality following a severe heat shock (*23*). They also alleviate the disruption of RNA splicing, a protective effect that appears to be dependent upon the synthesis of hsps themselves (*4*). Here we have shown that the heat-induced block in splicing occurs prior to the first catalytic reaction in the splicing pathway. Presumably then, hsps exert a protective effect on an early step in the pathway. They might act indirectly by preserving a general nuclear structure required for splicing. An alternative possibility is suggested by the fact that hsp70 and its low-temperature relatives are involved in the proper assembly and disassembly of protein complexes (*24*). The hsp70 protein might play an essential role in spliceosome assembly and disassembly at high temperatures. Clearly, the roles of the hsp's in protecting RNA splicing and in preventing heat-induced toxicities are subjects for further investigation.

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Accuracy of in Vivo Aminoacylation Requires Proper Balance of tRNA and Aminoacyl-tRNA Synthetase

ROBERT SWANSON, PATRICIA HOBEN, MARTIN SUMNER-SMITH,* HARUKI UEMURA,† LYNLEY WATSON, DIETER SÖLL‡

The fidelity of protein biosynthesis in any cell rests on the accuracy of aminoacylation of tRNA. The exquisite specificity of this reaction is critically dependent on the correct recognition of tRNA by aminoacyl-tRNA synthetases. It is shown here that the relative concentrations of a tRNA and its cognate aminoacyl-tRNA synthetase are normally well balanced and crucial for maintenance of accurate aminoacylation. When *Escherichia coli* Gln-tRNA synthetase is overproduced in vivo, it incorrectly acylates the *supF* amber suppressor $tRNA^{Tyr}$ with Gln. This effect is abolished when the intracellular concentration of the cognate $tRNA_2^{Gln}$ is also elevated. These data indicate that the presence of aminoacyl-tRNA synthetase and the cognate tRNAs in complexed form, which requires the proper balance of the two macromolecules, is critical in maintaining the fidelity of protein biosynthesis. Thus, limits exist on the relative levels of tRNAs and aminoacyl-tRNA synthetases within a cell.

AMINOACYL-TRNA SYNTHETASES catalyze the acylation of tRNAs with their respective amino acids, a set of reactions whose specificity is critical for fidelity in the biosynthesis of proteins (*1*). In *Escherichia coli*, each of these enzymes must select its few cognate tRNAs from among approximately 80 tRNA species. Nevertheless, the low frequency of errors found in protein sequences (for example, ovalbumins and globins) indicates that aminoacyl-tRNA synthetases display an extremely high degree of substrate discrimination (*2*). In fact, the term "superspecificity" has been applied to aminoacyl-tRNA synthetases when compared with other enzymes involved in amino acid metabolism, for example, proteases (*3*). Superspecificity can be explained in part by a number of proposed proofreading mechanisms that prevent incorrectly recognized amino acids entering the aminoacyl-tRNA pool of the cell (*4*). The accuracy of tRNA recognition by an aminoacyl-tRNA synthetase is an intrinsic part in achieving the specificity of aminoacylation. However, an additional factor is very important. As we show in this report, competition effects between different tRNA–aminoacyl-tRNA synthetase complexes lead to enhanced binding specificity and are critical for accurate tRNA charging in vivo.

To investigate the accuracy of in vivo aminoacylation, we have made use of a sensitive assay for misrecognition of tRNA by *E. coli* Gln-tRNA synthetase (GlnRS). Certain nonsense mutants are suppressed (that is, a functional protein is made) in the presence of some suppressor tRNAs, but not of others, depending on the amino acids carried by the tRNA. For example, the β-galactosidase amber mutant $lacZ_{1000}$ can be suppressed by *supE* $tRNA^{Gln}$ (which inserts Gln in response to amber codons) but not by *supF* $tRNA^{Tyr}$ (which inserts Tyr), presumably because the mutant enzyme with Tyr at the amber mutation site is inactive. Thus, the *E. coli* strain BT32 ($lacZ_{1000}$, *supF*) is normally phenotypically Lac^-. However, if GlnRS can aminoacylate the *supF* suppressor $tRNA^{Tyr}$ with Gln (forming suppressor Gln-$tRNA^{Tyr}$), active β-galactosidase is made in this strain. With this test system we showed that an altered form of GlnRS can misacylate (mischarge) *supF* $tRNA^{Tyr}$ with Gln (*5, 6*).

We found that wild-type GlnRS also possesses a certain affinity for some noncognate *E. coli* tRNAs and can mischarge these species in vitro (*6*), and thus we investigated the conditions under which such reactions might take place in vivo. The most likely factor to influence the extent of mischarging was the concentration of GlnRS present in the cell, so we varied in vivo GlnRS levels by constructing strains with different copy numbers of *glnS*, the structural gene for GlnRS. The mischarging indicator strain BT32 (described above) contains only the chromosomal copy of *glnS*. The λ-lysogen BT32(λ*glnS*), which carries a single extra copy of *glnS* as part of the prophage, contains two copies of the gene. In addition, a multicopy plasmid clone of *glnS* was introduced into BT32 to form BT32/pBR322*glnS*. Liquid cultures of these strains were grown to mid-log phase, and the levels of $tRNA^{Gln}$, GlnRS, and β-galactosidase (arising from suppression of $lacZ_{1000}$) were determined (Table 1). We found that BT32 makes significant levels of active β-galactosidase only when it markedly overproduces GlnRS because of the presence of *glnS* on the multicopy plasmid. Little enzyme activity was observed in strains BT32 and BT32(λ*glnS*), and also in the control strain BT32/pBR322. Since a $lacZ_{1000}$ mutant synthesizes active β-galactosidase when Gln, but not Tyr, is inserted in response to the $lacZ_{1000}$ amber codon, it appears that the *supF* $tRNA^{Tyr}$ suppressor in BT32 can be charged with Gln by wild-type GlnRS, given sufficiently high intracellular GlnRS concentrations. The transformed strain BT32/pBR322*glnS* yielded dark red colonies on lactose MacConkey agar plates. GlnRS charged *supF* tRNA less efficiently than its cognate *supE* suppressor tRNA, as can be seen by comparing the amounts of β-galactosidase activity found in BT32 and in the BT32(λ*supE*) control. In another strain (BT32/pBR322*supF*), the *supF* amber sup-

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511.

*Present address: Allelix, Inc., Missisauga, Ontario, Canada.
†Present address: Department of Pharmacology, School of Dentistry, Nagasaki University, Nagasaki, Japan.
‡To whom correspondence should be addressed.