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Nonsense Codons Can Reduce the Abundance of Nuclear mRNA without Affecting the Abundance of Pre-mRNA or the Half-Life of Cytoplasmic mRNA

JIU CHENG AND LYNNE E. MAQUAT*

Department of Human Genetics, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, New York 14263

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The abundance of the mRNA for human triosephosphate isomerase (TPI) is decreased to approximately 20% of normal by frameshift and nonsense mutations that cause translation to terminate at a nonsense codon within the first three-fourths of the reading frame. Results of previous studies inhibiting RNA synthesis with actinomycin D suggested that the decrease is not attributable to an increased rate of cytoplasmic mRNA decay. However, the step in TPI RNA metabolism that is altered was not defined, and the use of actinomycin D, in affecting all polymerase II-transcribed genes, could result in artifactual conclusions. In data presented here, the nonsense codon-mediated reduction in the level of TPI mRNA is shown to be characteristic of both nuclear and cytoplasmic fractions of the cell, indicating that the altered metabolic step is nucleus associated. Neither aberrancies in gene transcription nor aberrancies in RNA splicing appear to contribute to the reduction since there were no accompanying changes in the amount of nuclear run-on transcription, the level of any of the six introns in TPI pre-mRNA, or the size of processed mRNA in the nucleus. Deletion of all splice sites that reside downstream of a nonsense codon does not abrogate the reduction, indicating that the reduction takes place independently of the splicing of a downstream intron. Experiments that placed TPI gene expression under the control of the human c-*fos* promoter, which can be transiently activated by the addition of serum to serum-deprived cells, verified that there is no detectable effect of a nonsense codon on the turnover of cytoplasmic mRNA.

The translation of human triosephosphate (TPI) mRNA normally terminates at codon 249 within the final exon, exon 7. In previous experiments that were designed to examine the effect of TPI gene frameshift and nonsense mutations on TPI RNA metabolism, mouse L cells were transiently cotransfected with two plasmids. One plasmid was a pMT-TPI construct, in which the TPI allele harboring the mutation to be tested was driven by the mouse metallothionein (MT)-1 promoter. The other plasmid served to control for variations in cell transfection efficiency, RNA recovery, or both, and was either another pMT-TPI construct or pMT-Gl, in which all but the 5′ untranslated region of the TPI allele in pMT-TPI was replaced with the corresponding region of a hybrid human-mouse β-globin allele. By using primer extension to quantitate the coexpression of two different MT-TPI alleles or by using Northern (RNA) blot hybridization to quantitate the coexpression an MT-TPI allele and the MT-Gl allele, it was found that nonsense codons that cause translation to terminate prematurely at or upstream of position 189 in the penultimate exon result in a fivefold reduction in the level of MT-TPI mRNA (9, 10). The reduced level was also evident when the MT promoter was replaced by the immediate-early promoter of cytomegalovirus (10). Furthermore, the reduced level was found to be characteristic of both total cell and cytoplasmic RNA but, as evidenced by studies inhibiting RNA synthesis with actinomycin D, was not attributable to an increased rate of cytoplasmic mRNA decay (9). These data imply that nonsense codons reduce the abundance of TPI mRNA by affecting the metabolism of newly synthesized RNA. Nonsense codons of a second type that cause translation to terminate prematurely at or downstream of position 208 within the penultimate exon did not affect the level of MT-TPI mRNA (9).

Urlaub et al. (38) have obtained similar data for the endogenous dihydrofolate reductase (DHFR) RNA of Chinese hamster ovary cells, i.e., that nonsense codons that reside upstream of the proximal end of the penultimate exon (except for those that reside within the first two exons, which allow for translation reinitiation within the proper reading frame) reduce mRNA abundance without affecting either the rate of nuclear run-on transcription or the rate of cytoplasmic mRNA decay in the presence of actinomycin D. In explanation of these data, nonsense codons were hypothesized to alter the metabolism of nuclear RNA by one of two mechanisms (38). In one mechanism, dubbed translational translocation, the cytoplasmic translation of an RNA that is in the process of being exported from the nucleus to the cytoplasm provides the driving force by which that RNA is completely processed and translocated through the nuclear pore. According to the model, the premature termination of translation that is brought about by a nonsense codon inhibits the removal of introns that reside downstream of the nonsense codon and, thus, results in an abnormally low abundance of cytoplasmic mRNA that manifests a normal half-life. In the other mechanism, dubbed nuclear scanning, nuclei are envisioned to have the capability of recognizing a nonsense codon that resides in frame with the translation initiation codon, and recognition triggers an alteration in the nuclear metabolism specifically of the nonsense codon-containing RNA. Consequentially, an abnormally low amount of mRNA is transported to the cytoplasm, all of which manifests a normal half-life.

Here, we demonstrate by Northern blot hybridization that

* Corresponding author.

the nonsense codon-mediated reduction of either MT-TPI mRNA or fos-TPI mRNA (i.e., mRNA that derives from a plasmid harboring the human c-*fos* promoter in place of the TPI promoter) is evident in both the cytoplasmic and nuclear fractions of the cell. The reduction is apparently not attributable to an altered rate of RNA synthesis or splicing since nonsense codons do not affect the rate of gene transcription, the level of any of the six introns in MT-TPI or fos-TPI pre-mRNA, or the size of the spliced product in either the nucleus or the cytoplasm. The reduction is also not attributable to a block in the splicing of introns that reside downstream of the nonsense codon since deletion of the donor, lariat, and acceptor splice sites of the final intron do not abrogate the reduction in mRNA abundance that is brought about by a nonsense codon in the penultimate exon. Furthermore, nonsense codons do not decrease the half-life of cytoplasmic mRNA based on measurements of fos-TPI mRNA in the cytoplasmic cell fraction immediately after the transient induction of *fos*-TPI gene expression by the addition of serum to serum-deprived cells. Our findings are incompatible with the part of the translational translocation model that envisions translation facilitating RNA splicing and are more consistent with nonsense codons interfering with the metabolism of newly synthesized yet fully processed mRNA that is either within or being exported from the nucleus. Thus, the effect of nonsense codons on TPI RNA metabolism is distinct from an effect on RNA splicing, as for nonsense codons within the minute virus of mice (29), or an effect on cytoplasmic mRNA half-life, as exemplified by nonsense codons within certain *Saccharomyces cerevisiae* mRNAs (15, 25) and the mRNAs that derive from either the endogenous β-globin gene of human bone marrow cells (27) or human β-globin transgenes in mouse erythroid cells (23, 24).

MATERIALS AND METHODS

Plasmid constructions. A 34-bp deletion of the lariat and acceptor sites (Δ5′ TCTTGACCAAGCCCTTGTTCTGCTC CCTTCCCAG 3′ of the sense strand) within TPI gene intron 6 was introduced into subclones of the 660-bp *Pst*I-*Eco*RI fragment from pMT-TPI^{Norm} (9) by using the mutagenic oligonucleotide 5′ CCCAGTCACAGAGCΔATGGGATGA GTCTGCC 3′ and the method of Kunkel et al. (19) as previously described (9). Subsequently, a 6-bp deletion of the donor site (Δ5′ GTGAGT 3′ of the sense-strand) of this intron was introduced by using the mutagenic oligonucleotide 5′ CCGGGAACCAAAGCCΔCTCCATAAATGA 3′. All mutagenized fragments were sequenced in their entirety before being used to reconstruct pMT-TPI$^{Norm,\Delta(int\ 6\ scs)}$ and pMT-TPI$^{189Ter,\Delta(int\ 6\ scs)}$ (9).

p*fos*-TPI plasmids that harbored either a normal TPI allele or the nonsense 189 allele were constructed from the corresponding pMT-TPI plasmids. The 750-bp *Bam*HI-*Bam*HI fragment that contains the mouse MT-1 promoter was substituted for the 750-bp *Xba*I-*Nae*I fragment of p*fos* (originally referred to as PUCFOS1 by Kabnick and Housman [17]) that contains the promoter of the human c-*fos* gene, including the serum response element, and 41 bp of the adjacent 5′ untranslated region. Both the c-*fos* promoter fragment and the promoterless pMT-TPI derivatives into which the fragment was inserted were Klenow filled prior to the ligation reaction.

Cell culture, DNA-mediated cell transfection, and RNA purification. Human fibroblasts and mouse Ltk$^-$ cells were grown in minimal essential medium α medium containing 10% calf serum and 5% fetal calf serum. L cells (3×10^7/15-cm dish) were transfected with one or more plasmid DNAs (a total of 20 μg) by using DEAE-dextran as previously described (10). For the serum induction of *fos*-TPI gene expression, cells were washed four times with TS buffer (28 mM Tris [pH 7.4], 0.7 mM Na_2PO_4, 5 mM KCl, 0.137 M NaCl, 0.68 mM $CaCl_2$, 0.49 mM $MgCl_2$ [30]) 12 h after transfection and maintained in minimal essential medium α medium containing 0.5% calf serum for 24 h. The low-serum medium was then replaced with medium containing 15% fetal calf serum, and cells were harvested at specified times. Total, nuclear, and cytoplasmic RNAs were isolated as published elsewhere (10). Briefly, nuclei were washed twice in iso-hi-pH (0.01 M Tris [pH 8.4], 0.14 M NaCl, 1.5 mM $MgCl_2$, 0.5% [vol/vol] Nonidet P-40) and once in iso-hi-pH that was supplemented with 0.1 volume of a Tween-deoxycholate solution, pelleted through a 2.1 M sucrose pad, and washed once more in iso-hi-pH. No remnants of the cytoplasm were evident upon visualization by light microscopy.

Northern blot analysis. RNA was electrophoresed in a 1.5% agarose gel, transferred to a nylon membrane (10, 33), and hybridized to DNA that had been ^{32}P labeled by random priming (11). Blots of either L-cell RNA (25 μg from total, nuclear, or cytoplasmic cell fractions) or fibroblast RNA (5 μg) were hybridized to a 299-bp *Nde*I-*Nco*I fragment that derived from the 3′ untranslated region of human TPI cDNA and a 170-bp *Bal*I-*Dra*I fragment from the mouse β^{major}-globin gene that consists of 158 bp of exon 3 plus 3′-flanking sequences (9). The TPI cDNA fragment was used to detect either MT-TPI or fos-TPI RNA and did not cross-react with mouse TPI sequences under the conditions used. In transfections with p*fos*-TPI DNAs, blots were stripped of hybridized DNA by washing them four times at 95°C in 1% sodium dodecyl sulfate (SDS)–0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and subsequently rehybridized to a 610-bp *Pst*I-*Nco*I fragment from the human c-*fos* gene that consists of 50 bp of exon 3, all (115 bp) of intron 3, and 445 bp of exon 4 (17) to quantitate the endogenous c-*fos* mRNA.

Nuclear run-on analysis. Nuclei were prepared (2) from 5×10^6 untransfected or cotransfected L cells. The entire nuclear preparation was used in the run-on reaction (100 μl). Equivalent amounts of ^{32}P-labeled RNA were hybridized in 2 ml for 40 h to plasmid DNAs (4 μg of each) that had been linearized and subsequently immobilized onto nitrocellulose by dot blotting. These DNAs consisted of pTPI 3′ untranslated region, which contains the 643-bp *Nde*I-*Pvu*II fragment of the human TPI gene that includes 366 bp of the 3′ untranslated region and 277 bp of 3′-flanking DNA in pGEM (30a) (none of which cross-reacts with mouse TPI RNA under the hybridizations conditions used); pMT-Gl; p*fos*; and pJUC50, which contains the 595-bp *Hin*cII-*Eco*RI fragment of the human gene for histone H4 in pUC13 (37). The nitrocellulose strips were quantitated by phosphorimaging and autoradiography.

RT-PCR assays. cDNA was made from 0.022 to 5.4 μg of nuclear RNA from untransfected or cotransfected L cells by using Moloney murine leukemia virus reverse transcriptase (RT) (Superscript; BRL) essentially as described by Carothers et al. (8), except that 0.4 μg of random hexamer (Promega) served as the primer. The polymerase chain reaction (PCR) was then used to amplify quantitatively either MT-TPI or fos-TPI cDNA and, as a control, MT-Gl cDNA, following a protocol provided by Chen and Chasin (8a). For each PCR, 6 μl of the 20-μl RT reaction was

brought to 50 μl so as to contain 0.3 mM of each of the four deoxynucleoside triphosphates, 4 μCi of [α-^{32}P]dATP (3,000 Ci/mmol; Amersham), 100 pmol of each of four primers, and 2.5 U of *Taq* DNA polymerase (Promega). All samples were overlaid with 50 μl of mineral oil, and 17 PCR cycles were performed in an automated thermal cycler (Perkin-Elmer Cetus). Each cycle consisted of denaturation at 94°C for 50 s, primer annealing at either 60 or 55°C (depending on the primer pair) for 50 s, and primer extension at 72°C for 70 s. One-tenth of each PCR sample was electrophoresed in a 5% polyacrylamide gel, and the PCR products were quantitated by phosphorimaging. Conditions for accurate quantitations of the introns within either MT-TPI or fos-TPI pre-mRNAs and MT-Gl pre-mRNA were assessed in experiments that used serial dilutions of a standard preparation of nuclear RNA from transfected L cells to determine for each primer pair the times of primer annealing and primer extension and the number of cycles that provided for a linear correlation between the amount of template RNA and the PCR product. Under the PCR conditions ultimately chosen, a linear correlation was obtained by using 0.06 to 1.0 μg of nuclear RNA.

PCR primer pairs. TPI intron 1: 5′ GTCTCATCCCCCT GTGGTACCATC 3′ (sense; distal end of intron 1); 5′ CTCCCCAGTAAAAGCCCC 3′ (antisense; exon 2); PCR product, 153 bp. TPI intron 2: 5′ GGGGAAAGCCAC AGGGTGGG 3′ (sense; intron 2); 5′ CCAAAGACATG CCTTCTCT 3′ (antisense; exon 3); PCR product, 128 bp. TPI intron 3: 5′ GGATGTCTTTTTCCAAGAAGG 3′ (sense; intron 3); 5′ CTCCGAGTCCCTCTGCC 3′ (antisense; exon 4); PCR product, 95 bp. TPI intron 4: 5′ GTGGCCCATGC TCTG 3′ (sense; exon 4); 5′ [GCTCTA]GAGTCTCTGTG GCCCTGGATAGGC 3′ (antisense; intron 4, in which bracketed sequence is not complementary to intron 4); PCR product, 174 bp. TPI intron 5: 5′ AAGGGCATCCAGTC CAGGG 3′ (sense, intron 5); 5′ AGCCACCGCATCAG AGACGTTGGACTTCAGCCA 3′ (antisense; exon 6); PCR product, 204 bp. TPI intron 6: 5′ CCCATTCTTGACC AAGCCC 3′ (sense; intron 6); 5′ GCTGCAGGCTGCTT AGTCCCTGGC 3′ (antisense; exon 7); PCR product, 211 bp. Human β-globin intron 1: 5′ GCCTATTGGTCTATTT TCCC 3′ (sense; intron 1); 5′ CCTGAAGTTCTCAGGATCC 3′ (antisense; exon 2); PCR product, 251 bp.

RESULTS

Nonsense codons reduce the abundance of MT-TPI mRNA in the nuclear cell fraction. In previous experiments, nonsense codons that resided at or upstream of position 189 in MT-TPI DNA (Fig. 1A) were found by using blot hybridization to reduce the level of MT-TPI mRNA in the total and cytoplasmic fractions of the cell to approximately 20 to 25% of normal (9). An indication that this reduction was not attributable to an abnormally fast rate of cytoplasmic mRNA decay derived from the finding that the ratio of MT-TPI mRNA that harbored the nonsense codon at position 189 and MT-TPI mRNA that did not harbor a nonsense codon remained constant (approximately 1:4) throughout a 14-h block in transcription with actinomycin D (10).

To gain additional insight into the mechanism by which nonsense codons alter TPI mRNA abundance, we analyzed MT-TPI RNA in the nuclear cell fraction. L cells were transiently cotransfected with a pMT-TPI construct that harbored a normal TPI allele (MT-TPI^{Norm}) or an allele that directed the premature termination of mRNA translation either at codon 23 within exon 1 (MT-TPI^{23Ter}) or at codon 189 within exon 6 (MT-TPI^{189Ter}) (Fig. 1A) and, as a control, pMT-Gl DNA (Fig. 1D). MT-TPI and MT-Gl mRNAs were subsequently quantitated in nuclear and cytoplasmic cell fractions by Northern blot hybridization (Fig. 2A). In agreement with previous data for the cytoplasmic fraction, the nonsense codon at either position 23 or position 189 resulted in a four- to fivefold reduction in the level of cytoplasmic MT-TPI mRNA (Fig. 2A, lanes 1 to 3). Each nonsense codon also resulted in a similar reduction in the level of MT-TPI mRNA that fractionated with the nuclei (Fig. 2A, lanes 4 to 6). The reduction was not accompanied by the appearance of abnormally sized nuclear species.

One concern with any study of the nuclear fraction is that accurate measurements of the mRNA components of this fraction could be obscured by contamination with the cyto-

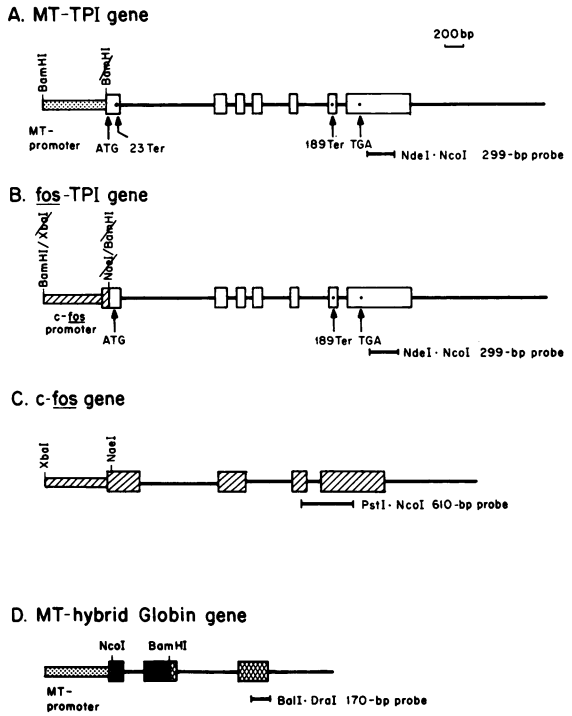

FIG. 1. DNA structures and blot hybridization probes. Narrow boxes represent the various promoter regions. The dotted box represents the 750-bp *Bam*HI-*Bam*HI fragment that harbors the mouse MT-1 promoter within the MT-TPI and MT-hybrid globin (MT-Gl) genes (9); the diagonally striped box represents the 750-bp *Xba*I-*Nae*I fragment that harbors the human c-*fos* promoter within the *fos*-TPI and human c-*fos* genes. The *fos*-TPI gene differs from the MT-TPI gene in that 42 bp that derive from the human c-*fos* 5′ untranslated region replace 8 bp, 7 of which derive from the mouse MT-1 5′ untranslated region and one of which derives from a synthetic linker. Wider boxes and interspersed lines designate, respectively, exons and introns of each gene. Open boxes represent TPI gene exons; diagonally striped boxes represent c-*fos* gene exons; solid boxes represent human β-globin gene exons; and the cross-hatched boxes represent mouse β-globin gene exons. The horizontal line to the right of and contiguous with the final intron of each gene represents 3′-flanking DNA. Arrows below the MT-TPI and fos-TPI structures specify the normal translation initiation codon (ATG), nonsense codons at position 23 (TAG; 23 Ter) or 189 (TGA; 189 Ter), or the normal translation termination codon at position 249 (TGA). The restriction fragments that were used as hybridization probes to detect the RNA products of each gene are diagrammed below each gene structure.

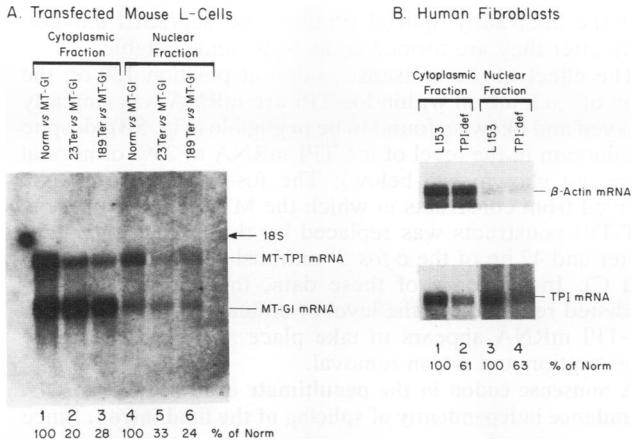

FIG. 2. Northern blot analysis of RNA in the nuclear and cytoplasmic fractions of transfected mouse L cells or fibroblasts from a TPI-deficient patient. (A) L cells (3×10^7/15-cm dish) were cotransfected with 10 μg of the specified pMT-TPI construct and 10 μg of pMT-Gl as a control. RNA was purified from nuclear and cytoplasmic fractions, and MT-TPI and MT-Gl RNAs were quantitated by blot hybridization. RNA (25 μg) was denatured, electrophoresed in agarose, transferred to a nylon membrane, and cohybridized to the ^{32}P-labeled, 299-bp *Nde*I-*Nco*I fragment of TPI cDNA and the ^{32}P-labeled, 170-bp *Bal*I-*Dra*I fragment of the mouse β^{major}-globin gene. The percentage of MT-TPI^{Norm} mRNA (Norm) was calculated for each MT-TPI mRNA after normalization to the level of MT-Gl mRNA and represents the average of three independently performed experiments. For each fraction, the level of normalized MT-TPI^{Norm} mRNA was defined as 100. 18S marks the position of 18S rRNA. (B) RNA was purified from the nuclear and cytoplasmic fractions of the TPI-deficient fibroblasts of patient 1 (26). This patient harbored the nonsense codon at position 189 within one TPI allele (10). RNA (5 μg) was treated as described in panel A except that hybridization was sequentially to a ^{32}P-labeled, 1.2-kbp *Pst*I-*Pst*I fragment of TPI cDNA (lower) and a ^{32}P-labeled, 2.0-kbp *Pst*I-*Pst*I fragment of chick β-actin cDNA (upper). Between hybridizations, hybridized radioactivity was removed by washing in 1% SDS–0.1× SSC four times at 95°C for 15 min. The percentage of Norm was calculated for TPI mRNA relative to the level of β-actin mRNA and is representative of two independently performed experiments. Analyses of RNA from the nuclear fraction were undertaken with six times the number of cells as analyses of RNA from the cytoplasmic fraction.

plasmic fraction, which generally contains more mRNA. With this concern in mind, nuclei were routinely washed twice with buffer containing Nonidet P-40 and a third time in buffer containing Nonidet P-40 and Tween-deoxycholate, pelleted through sucrose, and washed again with buffer containing Nonidet P-40 so as to be free of detectable cytoplasmic tags when visualized by light microscopy. Under the conditions used for cell transfection, subcellular fractionation, and blot hybridization (Fig. 2A), there was more radioactivity hybridizing to MT-Gl mRNA than to MT-TPI mRNA in the cytoplasmic fraction but more radioactivity hybridizing to MT-TPI mRNA than to MT-Gl mRNA in the nuclear fraction. From these data, 5% of MT-TPI mRNA but <1% of MT-Gl mRNA copurified with nuclei. Assuming that the percentage of cytoplasmic MT-Gl mRNA that contaminated the nuclear fraction equals the percentage of cytoplasmic MT-TPI mRNA that contaminated the nuclear fraction, then the maximum amount of contamination for each mRNA can be determined by assuming that all MT-Gl mRNA in the nuclear fraction is due to cytoplasmic contamination. On the basis of this calculation, the conclusion that each of the two nonsense codons reduces the level of nucleus-associated MT-TPI mRNA to approximately 25% of normal still holds (see also below). In fact, however, the extent to which cytoplasmic RNA contaminated nuclear RNA under the fractionation conditions used was minimal since blot hybridization to c-*myc* cDNA was barely detectable in nuclear RNA but as intense as the signal for MT-Gl mRNA in cytoplasmic RNA (data not shown).

The nonsense codon-mediated reduction in nuclear mRNA is not a peculiarity of the transient transfection assay as evidenced by analyzing the effect of the nonsense codon at position 189 on the metabolism of bona fide TPI mRNA in cultured fibroblasts from a TPI-deficient patient. The patient harbored the nonsense 189 mutation in one allele and a missense mutation that does not alter TPI RNA metabolism in the other allele (10, 26). Using the level of β-actin mRNA to control for variations in RNA recovery, the level of TPI mRNA in patient fibroblasts was 63% of normal in the nuclear fraction and 61% of normal in the cytoplasmic fraction (Fig. 2B). These values indicate that the premature termination of translation at codon 189 reduces the level of TPI mRNA that derives from the endogenous gene in the nuclear cell fraction to 26% of the normal level (63% minus the 50% of normal that derives from the missense allele is 13%, which is comparable to 26% of the normal level).

Nonsense codons do not alter the rate of MT-TPI gene transcription or the level of individual introns in MT-TPI pre-mRNA. The abnormally low abundance of MT-TPI^{23Ter} and MT-TPI^{189Ter} mRNAs in the nuclear fraction could be attributable to the inefficient formation of nuclear mRNA, an increased rate of nuclear mRNA decay, or both. The first possibility could be accompanied by an abnormal rate of synthesis, splicing, or degradation of pre-mRNA, any of which would result in an abnormal level of intron-containing RNA. In particular, if translation facilitates splicing, as predicted by the translational translocation model, then the level of introns that reside downstream of a nonsense codon should be abnormally high, while the level of introns that reside upstream of a nonsense should be unaffected.

Initially, to assay for a nonsense codon effect on pre-mRNA synthesis, the rates of transcription of the normal MT-TPI allele and the MT-TPI allele that harbored the nonsense codon at position 189 were compared by run-on analysis of isolated nuclei. There was no detectable difference between the rates of transcription of the two alleles (Fig. 3), indicating that the nonsense codon did not alter the process of transcription initiation. Next, to determine whether nonsense codons altered the abundance of unspliced or partially spliced TPI RNAs, an effect on the levels of each of the six introns within MT-TPI pre-mRNA was assayed. These experiments were particularly important since nonsense mutations within either exon of the gene that encodes the nonstructural proteins NS1 and NS2 of the minute virus of mice were recently shown to inhibit splicing and result in an abnormally high abundance of unspliced RNA (30). The level of each intron within MT-TPI pre-mRNA that derived from either the normal allele or an allele that harbored the nonsense codon at position 23 or position 189 was quantitated by using an RT-PCR assay (6, 32). As an internal control, the level of intron 1 within MT-Gl pre-mRNA was concomitantly quantitated. In the assay, cDNA was made from nuclear RNA by using random hexamers. An aliquot of this reaction mixture was then used as a template for PCR, which contained one primer pair to amplify part of a specific intron plus part of an adjacent exon within MT-TPI

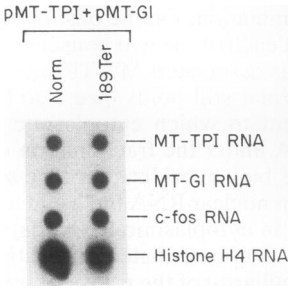

FIG. 3. Nuclear run-on analysis of the nonsense codon effect on MT-TPI gene transcription. Cells were cotransfected with pMT-Gl and either pMT-TPI^{Norm} or pMT-TPI^{189Ter}. After 48 h, nuclei were prepared for run-on analysis. Equivalent counts of ^{32}P-labeled run-on transcripts (3×10^7) were hybridized to nitrocellulose-immobilized DNAs, each of which specifically detected MT-TPI, MT-Gl, c-*fos*, or histone H4 RNA. Hybridized radioactivity was quantitated by phosphorimaging and visualized by autoradiography. In comparing the amount of run-on transcription among genes, the absolute amounts of radioactivity do not reflect the relative amounts of run-on transcription because of variations in the size of the filter-immobilized DNA that is complementary to each transcript.

pre-mRNA and another primer pair to amplify part of intron 1 plus part of exon 2 within MT-Gl pre-mRNA.

In initial experiments, RT and PCR conditions for accurate quantitations of MT-TPI and MT-Gl pre-mRNAs were determined empirically for each primer pair. These conditions were then applied to demonstrate a linear relationship between the amount of each RT-PCR product and the amount of input nuclear RNA (Fig. 4). For each pre-mRNA region analyzed, the size of the RT-PCR product was as expected, and the identity of each product was confirmed by the presence and proper location of a particular restriction enzyme site. Additionally, none of the products were synthesized from either untransfected L-cell RNA (Fig. 4) or transfected L-cell RNA that had been pretreated with RNase A, and product formation was unaffected by pretreatment of transfected L-cell RNA with RNase-free DNase I (data not shown).

The RT-PCR assay was next used to determine the effect of the nonsense codon at position 23 or position 189 on the level of each intron within MT-TPI pre-mRNA. The same preparations of nuclear RNA that had been subjected to Northern blot hybridization (Fig. 2) were analyzed. After each RT-PCR product was quantitated by phosphorimaging, the effect of each nonsense codon on the level of each intron in MT-TPI pre-mRNA was determined by first normalizing the quantity of each product of MT-TPI pre-mRNA to the quantity of the intron 1-exon 2 product of MT-Gl pre-mRNA and subsequently calculating each normalized value as a percentage of the normalized value for the corresponding intron in MT-TPI^{Norm} pre-mRNA, which was defined as 100. Results indicated that neither nonsense codon significantly altered the level of any of the introns (Fig. 5A) and, thus, the rate of removal of any of the introns. Therefore, the previous finding with the same RNA samples that the level of the spliced product was reduced (Fig. 2) is not attributable to an altered rate of splicing. Considering also that neither nonsense codon appears to create a splice consensus sequence or alter the size of nuclear mRNA, it is probable that the presence of a nonsense codon does not affect the accuracy of intron removal. An effect on accuracy would require that the rate of aberrant splicing equal the rate of normal splicing and that the aberrantly spliced products be degraded immediately after they are formed so as to be undetectable.

The effect of the nonsense codon at position 189 on the level of each intron within fos-TPI pre-mRNA was similarly assayed and likewise found to be negligible (Fig. 5B), despite a reduction in the level of fos-TPI mRNA to 25% of normal (data not shown; see below). The fos-TPI RNAs mRNA derived from constructs in which the MT-1 promoter of the MT-TPI constructs was replaced by the human c-*fos* promoter and 42 bp of the c-*fos* 5′ untranslated region (Fig. 1B and C). In summary of these data, the nonsense codon-mediated reduction in the level of either MT-TPI mRNA or fos-TPI mRNA appears to take place at a step after gene transcription and intron removal.

A nonsense codon in the penultimate exon reduces mRNA abundance independently of splicing of the final intron. Since the presence of a nonsense codon does not result in the accumulation of introns and, in particular, those introns that reside downstream of a nonsense codon, the part of the translational translocation model that envisions translation facilitating the removal of downstream introns does not seem to apply to TPI RNA. Considering that this conclusion is valid only if the cell does not immediately degrade any pre-mRNAs that have accumulated downstream introns, an alternative experimental approach that does not rely on the detectable accumulation of partially processed pre-mRNAs was also undertaken. It was rationalized that if translation does not facilitate the splicing of downstream introns, then deletion of the splice consensus sequences within the final intron should not abrogate the reduction in mRNA abundance that is brought about by a nonsense codon in the penultimate exon, provided that the remains of the final intron become a part of the final exon. However, if translation does facilitate splicing, then these deletions should abrogate the reduction in mRNA abundance. To ascertain which possibility is accurate, we introduced a 6-bp deletion that removed the intron 6 donor site and a 34-bp deletion that removed the intron 6 lariat-acceptor sites into the normal MT-TPI allele and the MT-TPI allele that harbored nonsense 189. The constructs were designated pMT-TPI$^{Norm,\Delta(int\ 6\ scs)}$ and pMT-TPI$^{189Ter,\Delta(int\ 6\ scs)}$, respectively, where scs specifies splice consensus sequences. Measurements of the MT-TPI^{Norm} derivative controlled for any effects on mRNA abundance that were brought about by the deletions independently of the nonsense codon. Initially, the scs deletion was shown to abolish the removal of the remaining nucleotides of intron 6 by sequencing the RT-PCR products that derived from the mRNA of either the normal or nonsense codon-containing allele (30a). Thus, the requirement that the remains of intron 6 be recognized as a part of the final exon was fulfilled. Next, the level of mRNA that derived from each Δscs construct was quantitated by Northern blot hybridization. While the deletions in the context of the normal allele reduced the level of mRNA to 33% of normal, the nonsense codon in the presence of the deletions reduced the level of mRNA to 5% of normal (Fig. 6). Thus, the deletion did not abrogate the nonsense codon effect. It can be concluded that the nonsense codon-mediated reduction in mRNA abundance is not attributable to the inhibition of splicing of introns that reside downstream of the nonsense codon. By inference and consistent with the finding that nonsense codons do not affect the level of any intron (Fig. 5), translation must not facilitate intron removal.

Nonsense codons do not increase the decay rate of fos-TPI mRNA in the cytoplasmic cell fraction. All data indicate that nonsense codons do not affect the quantity of nuclear

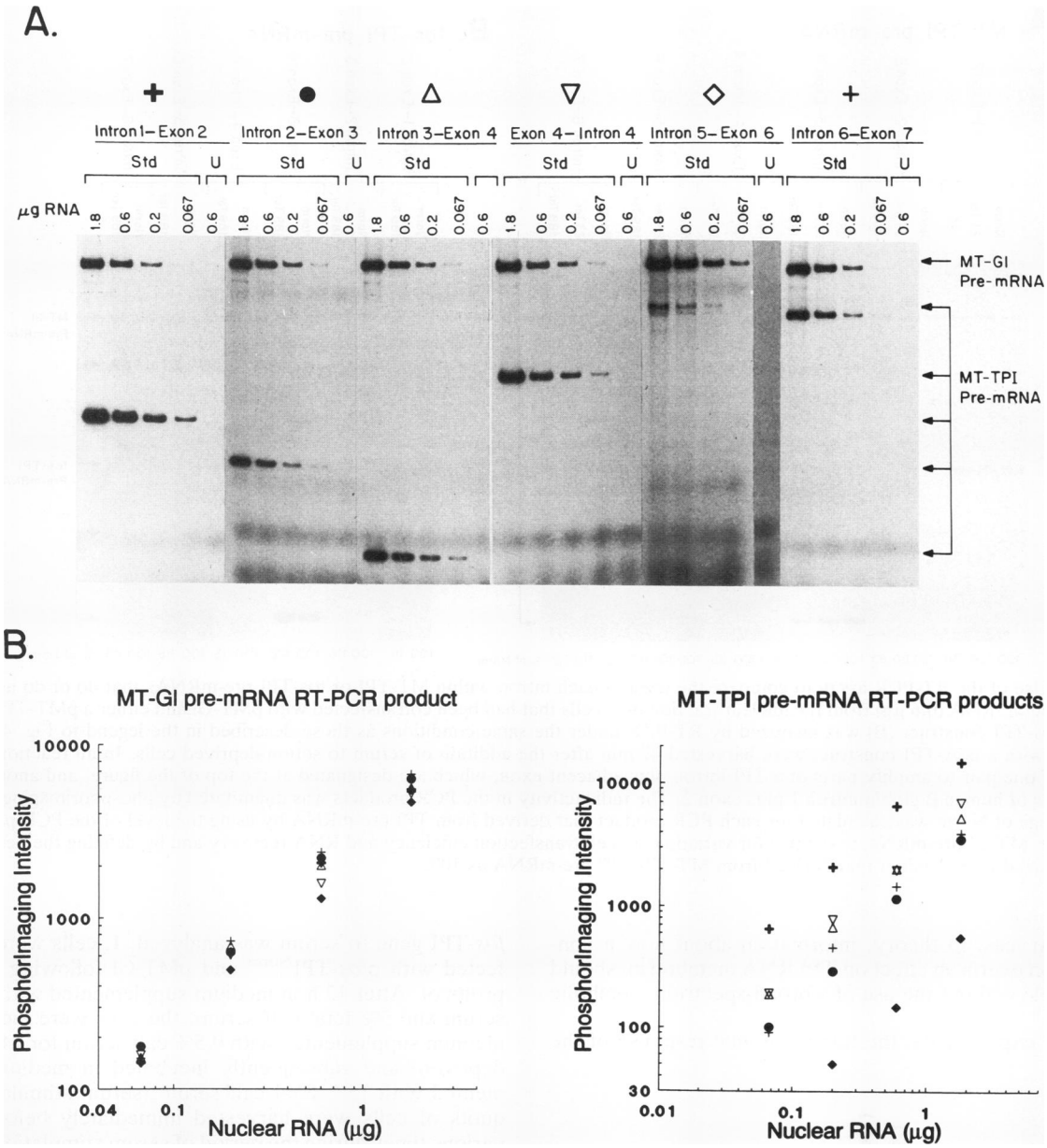

FIG. 4. Characterization of the quantitative RT-PCR assay. (A) Quantitative analysis of intron 1 within MT-GI pre-mRNA and individual introns within MT-TPI^{Norm} pre-mRNA. Specified amounts of nuclear RNA from either transfected (Std) or untransfected (U) L cells were amplified by RT-PCR, and the resulting products were resolved by electrophoresis in a 5% polyacrylamide gel and measured by phosphorimaging. The particular region of MT-TPI^{Norm} pre-mRNA that was analyzed is indicated at the top of the autoradiographic image. The lanes in which each region was analyzed have been labeled with a symbol. (B) Plot of the radioactivity in each of the RT-PCR products of panel A as a function of the amount of nuclear RNA. For each of the six reactions, the phosphorimaging intensity of the intron 1-exon 2 product that derives from MT-GI pre-mRNA (left) and each of the six different intron-exon products that derive from MT-TPI pre-mRNA (right) are plotted in arbitrary but relative units. Because of possible variations in the efficiencies with which each intron sequence is reverse transcribed and/or amplified and variations in the RT-PCR product size, the relative abundance of each intron within MT-TPI pre-mRNA cannot be assessed from these data.

pre-mRNA. To confirm without the use of actinomycin D that nonsense codons do not alter the fate of cytoplasmic mRNA, we utilized the *fos*-TPI constructs that harbor either the normal or the nonsense 189 allele. *fos*-TPI gene transcription should be inducible throughout the cell cycle in response to the growth factors that are present in serum (6, 14, 18, 29). If *fos*-TPI gene expression in L cells were to parallel c-*fos* gene expression in NIH 3T3 cells, then transcription should be reduced by serum deprivation and after serum readdition should be induced within 5 min, peak within 10 min, and return to basal level after 30 to 60 min (14, 16). A serum-induced burst and subsequent shutoff of *fos*-TPI gene expression should provide a means of monitoring the rate of fos-TPI mRNA turnover in the cytoplasmic

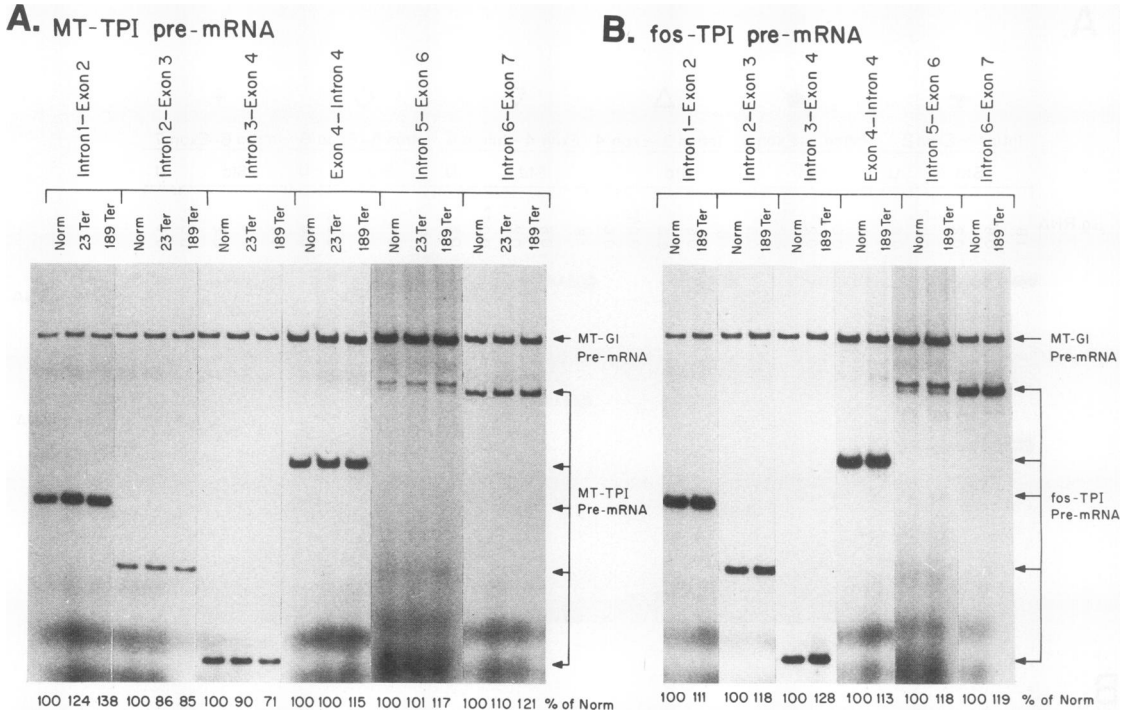

FIG. 5. Use of the RT-PCR assay to compare the level of each intron within MT-TPI or fos-TPI pre-mRNAs that do or do not harbor a nonsense codon. RNA (0.6 μg) from the nuclear fraction of L cells that had been cotransfected with pMT-Gl and either a pMT-TPI construct (A) or a p*fos*-TPI construct (B) was analyzed by RT-PCR under the same conditions as those described in the legend to Fig. 4. The cells transfected with a p*fos*-TPI construct were harvested 47 min after the addition of serum to serum-deprived cells. In all reactions, primers consisted of one pair to amplify parts of a TPI intron plus adjacent exon, which are designated at the top of the figure, and another pair to amplify parts of human β-globin intron 1 plus exon 2. The radioactivity in the PCR products was quantitated by phosphorimaging. As usual, the percentage of Norm was calculated for each PCR product that derived from TPI pre-mRNA by using the level of the PCR product that derived from MT-Gl pre-mRNA to control for variations in cell transfection efficiency and RNA recovery and by defining the percentage of each normalized PCR product that derived from MT-TPI^{Norm} pre-mRNA as 100.

fraction. At least in theory, information about how a nonsense codon exerts an effect on TPI RNA metabolism should be attainable without the use of a broad-spectrum metabolic inhibitor.

In initial experiments, the transcriptional response of the *fos*-TPI gene to serum was analyzed. L cells were cotransfected with p*fos*-TPI^{Norm} and pMT-Gl following the usual protocol. After 12 h in medium supplemented with 10% calf serum and 5% fetal calf serum, the cells were incubated in medium supplemented with 0.5% calf serum for 24 h (serum deprived) and subsequently incubated in medium supplemented with 15% fetal calf serum (serum stimulated). Aliquots of cells were harvested immediately before and at various times during the period of serum stimulation, and the rates of *fos*-TPI gene transcription were measured by nuclear run-on analysis. For comparative purposes, the transcription rates of the transfected MT-Gl gene and the endogenous histone H4 and c-*fos* genes were also measured.

Results indicated that transcription of the *fos*-TPI gene, like transcription of the endogenous c-*fos* gene, increased upon the addition of serum to serum-deprived cells despite a low level of transcription just before serum stimulation (Fig. 7A) (14). The increase (3.5-fold for the *fos*-TPI gene and 7.0-fold for the endogenous c-*fos* gene) was detectable 15 min after the addition of serum, peaked within 30 min, and returned to basal level by 60 min. For reasons unknown, the magnitude of the increase was not as high as that for 3T3 cells (14). The level of cytoplasmic c-*fos* mRNA as measured by Northern blot hybridization was undetectable just before serum stimulation and, after the readdition of serum, increased by 20 min, peaked by 30 min, and was barely detectable by 60 min (Fig. 7B) (14, 17). These measurements are as expected since c-*fos* mRNA has a half-life of <15 min

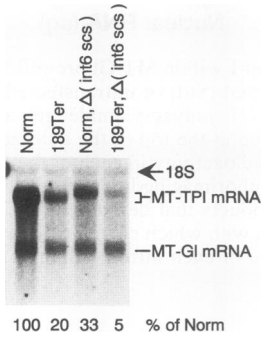

FIG. 6. Northern blot analysis of the role of intron 6 splicing in the nonsense 189-mediated reduction in the level of MT-TPI mRNA. L cells were cotransfected with pMT-Gl and the specified pMT-TPI construct, and MT-Gl and MT-TPI RNAs were quantitated in total cell RNA (25 μg) as described in the legend to Fig. 2. Δ(int 6 scs) refers to the deletion of the donor, lariat, and acceptor splice sites within intron 6. None of the values for percentage of Norm differed between two independently performed experiments by more than 1%.

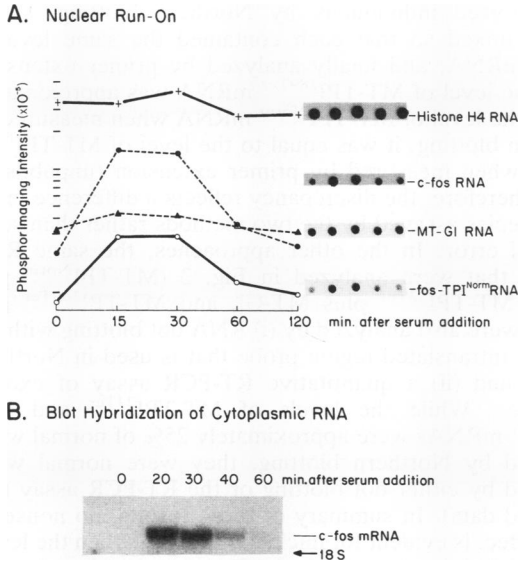

FIG. 7. Analysis of the rates of *fos*-TPI, MT-Gl, and endogenous gene transcription after the serum induced-stimulation of transfected L cells. Cells were either not transfected or cotransfected with p*fos*-TPI^{Norm} and pMT-Gl. After 12 h, the concentration of serum was lowered from 15 to 0.5%. After 24 h, the concentration of serum was returned to 15%, and cells were harvested at various times thereafter. (A) Nuclei were prepared for run-on analysis in which equivalent counts of ^{32}P-labeled run-on transcripts (2×10^7 cpm) were hybridized to nitrocellulose-immobilized DNAs as described in the legend to Fig. 3. (B) RNA was extracted from the cytoplasm, and c-*fos* mRNA in 25 μg was quantitated by Northern blot hybridization. Hybridized radioactivity was quantitated by phosphorimaging and visualized by autoradiography.

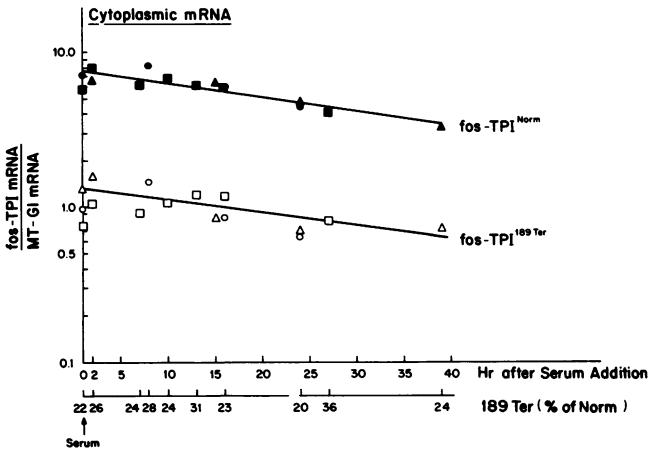

FIG. 8. Quantitations of the effect of nonsense 189 on the half-life of fos-TPI mRNA in the cytoplasmic fraction of transfected L cells. Cells were cotransfected with pMT-Gl and either p*fos*-TPI^{Norm} or p*fos*-TPI^{189Ter}. Serum deprivation and stimulation were as described in the legend to Fig. 7. At various times after the readdition of serum, RNA from the cytoplasmic cell fraction was prepared, and fos-TPI and MT-Gl mRNAs were quantitated by Northern blot hybridization and phosphorimaging. The level of each fos-TPI mRNA was normalized relative to the level of MT-Gl mRNA and plotted as a function of the time after serum readdition. Each of the three symbols specifies the results of three independently performed transfections. Solid symbols represent the normalized amount of fos-TPI^{Norm} mRNA, and open symbols represent the normalized amount of fos-TPI^{189Ter} mRNA. The ratio of normalized fos-TPI^{189Ter} mRNA and normalized fos-TPI^{Norm} mRNA is provided below the *x* axis for each time of analysis.

(17, 35). The rates of MT-Gl and histone H4 gene transcription did not change as appreciably as did the rates of *fos*-TPI nand c-*fos* gene transcription upon the addition of serum to serum-deprived cells (Fig. 7A).

The L-cell cotransfections were repeated in parallel with cotransfections with p*fos*-TPI^{189Ter} and pMT-Gl. RNA was purified from the cytoplasmic cell fraction at various times after the addition of serum to serum-deprived cells, and fos-TPI and MT-Gl mRNAs were analyzed by Northern blot hybridization. The level of fos-TPI mRNA was normalized to the level of MT-Gl mRNA and plotted as a function of time.

It is difficult to detect the small increase in the amount of either fos-TPI^{Norm} or fos-TPI^{189Ter} mRNA in the cytoplasm as a consequence of serum-induced transcription when each is normalized to the level of MT-Gl mRNA (Fig. 8). This is because the burst in *fos*-TPI gene transcription was only 3.5-fold and fos-TPI mRNA is sufficiently long-lived so that the amount that is synthesized during the induction comprises only a small fraction of what preexists. Measurements of the half-life of cytoplasmic mRNA are not dependent on a detectable level of newly synthesized RNA but only on the turnoff of *fos*-TPI gene transcription, which has been sufficiently accomplished (Fig. 7A). The ratio of the normalized levels of fos-TPI^{189Ter} mRNA and fos-TPI^{Norm} mRNA was constant and approximately 25% for each time point between 0 and 39 h, the longest time point after serum readdition (Fig. 8). This finding confirmed the previous results, which were obtained using actinomycin D and measured total cell TPI RNA (10), that there is no effect of a nonsense codon on the half-life of cytoplasmic mRNA. The cytoplasmic half-life of each mRNA was estimated to be 33 h.

DISCUSSION

Nonsense codons are known to be recognized in the context of mRNA during the process of mRNA translation in the cytoplasm. The data presented here demonstrate that nonsense codons within TPI mRNA affect the metabolism of newly synthesized nuclear mRNA without affecting the half-life of cytoplasmic mRNA. While numerous examples of a nonsense codon-mediated alteration in mRNA abundance exist, the alteration often takes place in the cytoplasm concomitantly with mRNA translation. In some cases, mRNA abundance is increased by the presence of a nonsense codon. For example, a nonsense codon may preclude ribosome translocation across a specific sequence in the coding region, a requirement for the degradation of c-*fos*, c-*myc*, and *MATα1* mRNAs (13, 31, 35, 36, 39). As another example, a nonsense codon may preclude ribosome translocation sufficiently close to a particular structure in the 3′ untranslated region, a requirement for the degradation of certain histone mRNAs at the end of the S phase (7, 12, 22). As yet another example, a nonsense codon may preclude the accessibility of specific portion of the nascent peptide to interactions in *trans*, a requirement for the degradation of β-tubulin mRNA that is brought about by unpolymerized microtubule subunits (40, 41). In other cases, mRNA abundance is decreased by the presence of a nonsense codon because the premature termination of translation, for unknown reasons, results in an increased rate of cytoplasmic

mRNA decay. Examples of mRNAs for which this type of decay has been demonstrated include those for Rous sarcoma virus *gag* (3, 3a), rabbit β-globin (36), human β-globin when it is produced from a transgene in mice (23 and references therein), yeast URA3 (25), and yeast HIS4 (15). In yeast cells, the *trans*-acting factor UPF1 has been shown necessary for the enhanced degradation specifically of mRNAs that contain a premature stop codon (21). The presence of a nucleic acid-binding domain in UPF1 suggests that the factor may function by directly interacting with RNA either as an RNase or as a positive effector of an RNase.

A nonsense codon effect on nuclear RNA metabolism has been reported for DHFR mRNA (38), minute virus of mice mRNA (30), and for human β-globin mRNA when it is produced from a simian virus 40-based expression vector that has been integrated into the genome of AF8 cells, a derivative of the BHK syrian hamster cell line (4). The reports for DHFR and β-globin mRNAs were based on studies demonstrating that the presence of a nonsense codon does not affect either the rate of gene transcription or the half-life of mRNA. Transcription rates were measured by nuclear run-on, and mRNA half-lives were determined by inhibiting RNA polymerase II-catalyzed transcription with either actinomycin D or a cell line that harbored a temperature-sensitive RNA polymerase II. It was concluded from these studies that nonsense codons may reduce mRNA abundance by affecting nuclear RNA processing, nuclear mRNA stability, or nuclear mRNA export. Evidence presented here corroborates the findings for DHFR and β-globin mRNAs by demonstrating that nonsense codons within human TPI RNA, either the bona fide TPI RNA of diploid fibroblasts or the MT-TPI and fos-TPI RNAs that were transiently produced in mouse L cells, reduce the level of nucleus-associated mRNA (Fig. 2) (data not shown for fos-TPI RNA). Furthermore, the data on the transcription rates of the MT-TPI and *fos*-TPI genes demonstrate that the reduction is entirely posttranscriptional (Fig. 3). In analyses that have yet to be performed for DHFR and β-globin RNAs, it was found that neither the accuracy nor the efficiency of either MT-TPI or fos-TPI RNA splicing appears to be affected; there was no abnormal accumulation of any of the six introns within pre-mRNAs (Fig. 5), deletion of the splice sites within the final intron did not abrogate the effect of a nonsense codon within the penultimate exon (Fig. 6), and only properly sized mRNA was detected in both the nuclear and cytoplasmic fractions (Fig. 2). In experiments that did not rely on the inhibition of RNA polymerase II, nonsense codons were shown to have no effect on the half-life of cytoplasmic mRNA (Fig. 8).

Using a primer extension assay, we had previously demonstrated that nonsense codons do not reduce the abundance of nuclear TPI RNA (10). We do not completely understand why measurements of the nonsense codon effect on the level of nuclear TPI RNA are different when using the primer extension assay and Northern blotting. The primer extension assay measures the level of exon 6 that exists in any unspliced, partially spliced, fully spliced, or degraded species. Northern blotting measures exclusively fully spliced mRNA, because mRNA is the most abundant and only discrete species detected by this method.

The discrepancy between the results obtained by primer extension and Northern blotting was further investigated by several experimental approaches. In one approach, the nuclear RNAs prepared from L cells transfected with either MT-TPI^{Norm} and MT-Gl or MT-TPI^{189Ter} and MT-Gl were first analyzed individually by Northern blotting, subsequently mixed so that each contained the same level of MT-Gl mRNA, and finally analyzed by primer extension. While the level of MT-TPI^{189Ter} mRNA was approximately 25% of the level of MT-TPI^{Norm} mRNA when measured by Northern blotting, it was equal to the level of MT-TPI^{Norm} mRNA when measured by primer extension (unpublished data). Therefore, the discrepancy reflects a difference in the RNA species assayed by the two methods rather than some technical error. In the other approaches, the same RNA samples that were analyzed in Fig. 2 (MT-TPI^{Norm} plus MT-Gl, MT-TPI^{23Ter} plus MT-Gl, and MT-TPI^{189Ter} plus MT-Gl) were also analyzed by (i) RNA dot blotting with the same 3′ untranslated region probe that is used in Northern blotting and (ii) a quantitative RT-PCR assay of exon 1 sequences. While the levels of MT-TPI^{23Ter} and MT-TPI^{189Ter} mRNAs were approximately 25% of normal when measured by Northern blotting, they were normal when measured by either dot blotting or the RT-PCR assay (unpublished data). In summary of these results, no nonsense codon effect is evident for nuclear TPI RNA when the levels of exon 6 (primer extension), the 3′ untranslated region (dot blotting), or exon 1 (RT-PCR) are individually measured. However, a nonsense codon effect is evident for nuclear TPI RNA when the level of full-length mRNA (Northern blotting) is measured. Since nonsense codons do not affect the level of pre-mRNA, it may be that in the nucleus the degradative products of nonsense codon-containing mRNAs accumulate to a level that compensates for the reduced level of full-length mRNA. However, if degradative products do exist, then they must be too diffuse in size or too small to be detectable by our method of Northern blotting.

Nuclear mRNA in this report is operationally defined as RNA that fractionates with nuclei. In theory, nuclear mRNA molecules could reside entirely within the nucleus or partly in the nucleus and partly in the cytoplasm, as would be expected for those in the process of being exported from the nucleus to the cytoplasm. While it is also possible that at least some of these molecules could reside entirely within the cytoplasm in a compartment that copurifies with nuclei, we do not deem this possibility likely. Great care was taken to prepare nuclei that were free of cytoplasm as judged biochemically by RNA fractionation studies as well as visually by light microscopy. Since the data suggest that nuclear mRNA could be the species that undergoes the nonsense codon-mediated decay, it will be important to localize this RNA by using in situ hybridization.

We have shown recently that the nonsense codon-mediated reduction in the level of TPI mRNA that fractionates with nuclei is abrogated by either a hairpin structure in the 5′ untranslated region or a suppressor tRNA that alters the involvement of ribosomes or tRNA, respectively, in cytoplasmic translation (5). The simplest interpretation of these data and the data presented here is that cytoplasmic translation most likely functions in the recognition of a nonsense codon and triggers the decay of nuclear mRNA. If this interpretation is valid, then the finding that nonsense codons do not affect the half-life of cytoplasmic mRNA may be attributable to differences in either the efficiency or the manner with which TPI mRNA that copurifies with nuclei and TPI mRNA that copurifies with cytoplasm are translated. For example, cytoplasmic MT-TPI mRNA in L cells may be translated with an efficiency that is too low to permit detection of the nonsense codon effect. If this explanation is true, then it must also be true for cytoplasmic TPI mRNA in fibroblasts from the TPI-deficient patient. As another exam-

ple, the nonsense codon-mediated degradation of TPI mRNA could be restricted to mRNA molecules that are being translocated through the nuclear pore. Degradation could be brought about by a block in the export of those molecules that had initiated translation during transit across the nuclear envelope. For this possibility to be realized, mRNA must exit the nucleus 5′ first and immediately associate with ribosomes upon entry into the cytoplasm, as has recently been demonstrated in the dipteran *Chironomus tentans* for the ribonucleoprotein particles called Balbiani ring granules (28). Perhaps, once ribosomes load onto a TPI mRNA that is in the process of being translocated through the nuclear pore, the ribosomes must then progress to within the last one-fourth of the reading frame in order for the mRNA to be completely exported rather than degraded at the pore. Possibly, the initiation of translation displaces a factor that is required for mRNA export so that protein synthesis becomes the driving force which the mRNA is transported to the cytoplasm. Translation is apparently not required for export since reducing the efficiency of translation initiation by inserting a hairpin structure in the 5′ untranslated region did not significantly affect the production of MT-TPI^{Norm} mRNA (5). Thus, mRNA that harbors a nonsense codon could escape the decay process if it was translocated free of ribosomes. If this model is correct, then 20 to 30% of TPI mRNA may normally exit the nucleus in a ribosome-free state as deduced from the finding that nonsense codons, regardless of their position within the first three-fourths of the reading frame, reduce mRNA abundance only to 20 to 30% of normal.

An alternative explanation of how the half-life of nuclear but not cytoplasmic mRNA could be affected by nonsense codons may be that the transcripts of each TPI allele form tracks or conduits from their site of synthesis to one or more nuclear pores. mRNA that maintains an association with the nucleus could be degraded in response to a signal that emanates from a site of cytoplasmic translation during the process of tracking. At least theoretically, such a signal could be mechanically transduced by the continuous cellular network (42), the cytoskeleton and the nuclear matrix, that connects the cytoplasm and the nucleus via the nuclear pore through which the RNA is translocated. Tracks have been demonstrated for a specific Epstein-Barr virus nuclear RNA that originates from integrated viral genomes in Namalwa cells (20) as well as for the endogenous c-*fos* RNA of NIH 3T3 cells (16).

Any proposal of nuclear scanning to explain the nonsense codon effect would be greatly simplified if the scanning process were to take place after intron removal. The finding that nonsense codons do not detectably alter the metabolism of pre-mRNA by no means proves but is compatible with this simplification. Otherwise, the mechanism would have to distinguish between exons and introns, since introns almost without exception contain in-frame nonsense codons (34). Despite these reservations, a scanning mechanism that distinguishes between certain exons and introns seems to fit the data on minute virus of mice RNA since an accumulation of intron-containing RNA is evident with a nonsense codon in either of the exons that flank the accumulated intron (30). The scanning mechanism does not appear to be required for the splicing of minute virus of mice pre-mRNA since deletion of the initiator AUG codon that resides within exon 1 does not alter the efficiency of intron removal (30). Nuclear scanning implicates a functional role for a ribosome or a ribosome-like particle in the nucleus. However, there is as yet no convincing biochemical or structural evidence that ribosomes with properties like those in the cytoplasm assemble on polymerase II-transcribed RNAs in the nucleus, with the possible exception of the thymocyte nucleus (1). Alternatively, and also without supporting evidence, the reading frame of an RNA might be recognized in the nucleus by a non-ribosome-mediated process.

It is now essential to quantitate the effect of a nonsense codon on the half-life of nuclear TPI mRNA. Attempts to maximize the serum-mediated induction of *fos*-TPI gene expression in L cells in order to analyze the half-life of this mRNA have not proved satisfactory. *fos*-TPI gene transcription is nearly completely shut off by serum deprivation as expected. Unexpectedly, however, the export of fos-TPI mRNA appears to be blocked concomitantly with the shutoff of transcription both during serum deprivation and after the burst in transcription during serum readdition (data not shown). The blocks in export result in a level of nuclear fos-TPI mRNA that is sufficiently large to preclude a reliable measurement of mRNA half-life. The expression of the fos-TPI genes in another cell line that manifests a larger transcriptional response to serum is presently being investigated.

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