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Cytoplasmic nonsense-mediated mRNA decay for a nonsense (W262X) transcript of the gene responsible for hereditary tyrosinemia, fumarylacetoacetate hydrolase☆

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Abstract

Messenger RNAs containing premature stop codons are generally targeted for degradation through the nonsense-mediated mRNA decay (NMD) pathway. The subcellular localization of the NMD process in higher eukaryotes remains controversial. While many mRNAs are subjected to NMD prior to their release from the nucleus, a few display cytoplasmic NMD. To understand the possible impact of NMD on the pathogenesis of hereditary tyrosinemia type I, a severe metabolic disease caused by fumarylacetoacetate hydrolase (FAH) deficiency, we examined the metabolism of FAH mRNA harboring a nonsense mutation, W262X, in lymphoblastoid cell lines derived from patients and their parents. W262X-FAH transcripts show a ~20-fold reduction in abundance in mutant cells, which is translation-dependent. Cellular fractionation shows that this down-regulation of the W262X transcript occurs in the cytoplasm. Thus the W262X FAH is another example of nonsense mRNAs subjected to the NMD pathway in the cytoplasm.

Keywords: Tyrosinemia; FAH; Nonsense-mediated mRNA decay

Fumarylacetoacetate hydrolase (FAH; EC 3.7.1.2), the last enzyme of the tyrosine catabolic pathway, catalyzes the hydrolysis of fumarylacetoacetate (FAA) into fumarate and acetoacetate. A deficiency in FAH results in hereditary tyrosinemia type I (HTI; MIM 276700), the most severe disease of the tyrosine catabolic pathway [1]. HTI is an autosomal recessive disorder, characterized by hepatic and renal dysfunctions, as well as by neurological crises. In the absence of FAH, FAA accumulates and this toxic metabolic intermediate has been shown to display mutagenic and cytotoxic effects [2,3], to induce mitotic abnormalities and genomic instability, and to activate the ERK pathway [4]. Despite some advances in the understanding of the molecular bases of the pathogenesis in HTI, the phenotypic diversity of clinical features in the two forms of HTI remains to be clarified. Indeed, patients harboring the same mutation have been reported to display diverse forms of the disease [5]. Immunohistological studies have suggested that the less severe form might be explained by a reversion of

☆ *Abbreviations:* CHX, cycloheximide; FAH, fumarylacetoacetate hydrolase; FAA, fumarylacetoacetate; HTI, hereditary tyrosinemia type I; PTC, premature termination codon; NMD, nonsense-mediated mRNA decay; RT, reverse transcription; PCR, polymerase chain reaction; Cyt, cytoplasm; Nuc, nucleus; RAR, retinoic acid receptor; GAPDH, glyceraldehyde-3-phosphate deshydrogenase; TcR, T-cell receptor; Ig, immunoglobulin; HexA, hexaminidase A; GPx1, glutathione peroxidase.

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the mutation in some liver cells with nodular expansion, thereby restoring FAH activity in parts of this organ [5–7]. The underlying molecular bases of the reversion phenomenon remain unknown.

The *fah* coding gene located on chromosome 15 in the q23–q25 region [8] spans over 35 kb and contains 14 exons [9]. Forty-seven naturally occurring mutations have been identified so far, including 7 nonsense mutations [1,10,11].

We have been interested in characterizing the effects of the W262X mutation, a nonsense mutation first identified in the Finnish population [12], on RNA metabolism. A G → A transition at position 786 introduces a stop codon in replacement of a tryptophan residue. No protein of normal size was detected in fibroblasts and liver obtained from these HTI patients [12], but a truncated protein corresponding to the arrest of translation at amino acid 262 was detected in an in vitro transcription-translation assay [13].

Contrary to what may be predicted on the basis of the type of mutation, introduction of a premature termination (stop) codon (PTC) does not usually give rise to a truncated protein. Rather, nonsense transcripts are rapidly degraded through the nonsense-mediated mRNA decay pathway (NMD; [14–16]). NMD is evolutionarily conserved and has been reported in all the organisms tested, from yeast to human [17]. The process of nonsense transcript degradation is well understood in yeast, where it is believed to take place in the cytoplasm [18]. Despite recent advances, notably through the identification of yeast orthologous *trans*-acting factors [19,20] and more recently through the identification of the nuclease activities responsible for 5′ → 3′ and 3′ → 5′ decay [21–23], many aspects of NMD in higher vertebrates are still unresolved. There is a large set of data suggesting that NMD relies on translating ribosomes [14]. However, biochemical fractionation of the nuclear and cytoplasmic compartments has shown that most defective mRNAs studied are degraded prior to their release from the nucleus [14,24,25]. Splicing, which is a nuclear process, has also been suggested to provide a mark to distinguish between a physiological and a premature stop codon [26–28]. Thus, the exact involvement of each intracellular compartment in the NMD pathway in higher eukaryotes remains to be clarified.

Here we examine whether W262X-containing FAH mRNAs are subjected to NMD. RT-PCR analyses of FAH transcripts in lymphoblastoid cells obtained from HTI patients reveal that W262X-containing mRNAs are strongly down-regulated in the cytoplasm and that this diminution is translation-dependent, as demonstrated by a cycloheximide treatment. Interestingly, a minor alternative transcript of the *fah* gene, del100, in which skipping of exon 8 generates a PTC downstream, is also subjected to NMD. We conclude that, in contrast to many mammalian nonsense mRNAs for which the degradation is nucleus-associated, nonsense FAH mRNAs are subjected to the NMD pathway in the cytoplasm

Experimental procedures

Patients. The four W262X homozygous patients and their parents originated from the Pohjamaa county (Finland). They are ancestrally related at a distance of 5–14 generations [12]. Homozygous patients are referred to as patient 1–4. The detection test of the W262X mutation has been reported elsewhere [29].

Lymphoblastoid cell establishment and cellular culture. The lymphoblastoid cell lines were established from lymphocytes as described in Tremblay and Khandjian [30]. Briefly, blood samples from the Finnish probands and their parents (obtained from Dr. M. Salo, Tempere) were centrifuged and the nucleated cell layer isolated. These cells were resuspended in a solution containing 50% RPMI-1640 culture medium, 10% DMSO and 40% fetal bovine serum, prior to freezing. Frozen lymphocytes were thawed before infection with EBV, in the presence of cyclosporin A. Cultures were incubated at 37 °C in a humidified 5% CO_2/95% air atmosphere. Cells were propagated and maintained in RPMI-1640 culture medium with 15% FBS supplemented with antibiotics. For translation inhibition experiments, 100 μg/ml cycloheximide was added to the culture medium 3 h prior to RNA extraction. The control (wt/wt) lymphoblastoid cell line is T19-EBV (Gift of Dr. A. Darveau, Québec).

RNA extraction from lymphoblastoid cells. The method is adapted from Chomczynski and Sacchi [31]. Cells were first pelleted for 5 min at 1000 rpm and washed twice with PBS. Total RNA was extracted from 1 to 3×10^7 cells with Trizol (InVitrogen) according to the instructions of the manufacturer. RNA was then suspended in 80 μl RNA storage solution (Ambion) and treated with RNAse-free DNAse (DNA-*free*; Ambion). The fractionation of nuclear and cytoplasmic RNAs was reported elsewhere [32]. Briefly, $1–3 \times 10^7$ cells were lyzed in 200 μl lysis buffer (50 mM KCl, 5 mM $MgCl_2$, 10 mM Tris–HCl, pH 8.0, and 40 U/ml of RNAse Out (InVitrogen), containing 0.05% NP-40). The crude pellet was obtained after 2 min centrifugation at 1000 rpm at 4 °C and separated from the supernatant containing the soluble cytoplasmic fraction (cyt1 fraction). The pellet was then resuspended in a second lysis buffer (150 mM NaCl, 1.5 mM $MgCl_2$, 10 mM Tris–HCl, pH 8.0, and 40 U/ml of RNAse Out, containing 0.65% NP-40). The supernatant, resulting from a 2 min centrifugation at 1000 rpm at 4 °C, is designated as cytoplasmic fraction 2 (cyt2 fraction). The pellet is the nuclear cell fraction (nuc fraction). RNA from the three fractions (cyt1, cyt2, and nuc) was extracted with 1 ml of Trizol. The RNA was suspended in 50 μl RNA-storage solution and treated with DNA-*free*. Proteins were extracted from each phenolic fraction according to the manufacturer's recommendations and separated on a 15% SDS–polyacrylamide gel to verify the subcellular fractionation.

Analysis of W262X-FAH transcripts. Two micrograms of RNA were reverse transcribed for 50 min at 37 °C using 1 μg of oligo(d(T)) and 2U of M-MLV reverse transcriptase (Moloney Murine Leukemia Virus, InVitrogen) in a volume of 40 μl. The reverse transcription was followed by 15 min of inactivation of M-MLV at 70 °C. For each sample, a control without reverse transcriptase was included (RT^-).

Four microliters of RT^+ or RT^- products were then amplified in a final volume of 50 μl with 5 μl 10× buffer (50 mM Tris–HCl, pH 9.2, 160 mM $(NH_4)_2SO_4$, 22.5 mM $MgCl_2$, 20% DMSO, and 1% Tween 20), 10 mM dNTP, 200 ng of each primer, and 2.5 U *Taq* polymerase (Pharmacia Biotech). Amplification of the 1.4 kb product was performed by 33 cycles at 95 °C for 30 s, 59 °C for 30 s, and 72 °C for 2′, preceded by a denaturation step of 5 min at 95 °C and followed by a final elongation step of 15 min at 72 °C. PCR conditions were

optimized for the cycle number and the annealing temperature so that nonspecific products were minimal and the FAH transcripts from the homozygous individuals could be seen. One-tenth of the reaction volume was then run on an 1.5% agarose gel stained with ethidium bromide. The primers used for the amplification of the full-length FAH RNA (Fig. 1) are TanR130 (5′-CAT GTC CTT CAT CCC GGT GGC-3′) and RT025 (5′-GGG AAT TCT GTC ACT GAA TGG CGG AC 3-′). Another reverse primer Tan 53 (5′-GTC TAT GGG CTT CGT TCC 3-′), located in exon 13, was used with RT76 to amplify the alternatively spliced region (Fig. 1).

RAR (retinoic acid receptor) cDNA was amplified using RAR-I (5′-CCC GGT GAC ACG TGT ACA CC 3-′) and RAR-C (5′-CAG CAC CAG CTT CCA GTT AG 3-′), to serve as an internal standard for RNA quantity.

The alternative transcripts del100 and del231 were amplified using the specific primers RT84 and RT85 as described in Dreumont et al. (submitted).

Semi-quantitative RT-PCR. The PCR amplification was performed on the cDNAs of FAH and an internal control GAPDH (glyceraldehyde-3-phosphate deshydrogenase). A primary solution of 100 μl was prepared and distributed in 5 aliquots of 20 μl, each corresponding to the five points of the amplification curve measured during the exponential phase of the PCR. The solution was as follows: 8 μl of RT^+ product, 10 mM dNTPs, 200 ng of each primer couple, and 3 U of *Taq* polymerase in 10× buffer. The amplification reaction was performed as follows: after a denaturation step of 5 min at 95 °C, cycles were of 95 °C for 30 s, 59 °C for 30 s, and 72 °C for 2′, with a final elongation step of 72 °C for 15′. FAH was amplified for 28 cycles and GAPDH for 20 cycles. A tenth of the amplification product was electrophoresed on a 1.5% agarose gel and stained with ethidium bromide. The signal intensity was determined using the NIH Image v.1.62 program.

In translation inhibition experiments using cycloheximide, 2 μCi of [α-^{33}P]dATP were added to the PCR mixture, which was the same as above except for the dNTP concentration (5 mM instead of 10 mM). PCR products were runned on a 6% acrylamide gel. Signals were quantitated from a X-ray film using the NIH Image v.1.62 program.

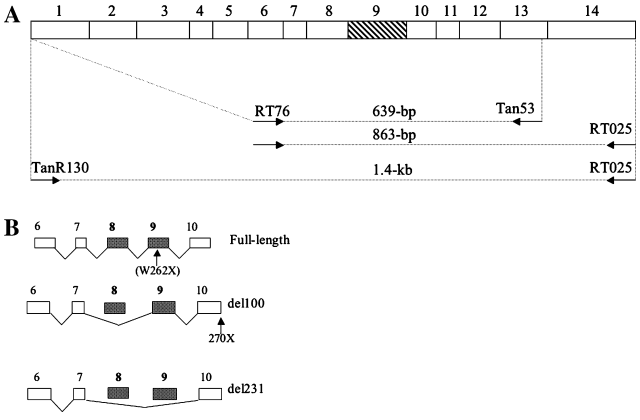

Fig. 1. FAH cDNA and RT-PCR strategies. (A) A summary of RT-PCR experiments is depicted below the schematic representation of the FAH cDNA. For each amplification, the primers and the product length are indicated. Exon 9, which contains the W262X mutation, is represented by a hatched box. TanR130 and RT025 are used to amplify the full-length transcript. RT76, which is located in exon 6, is used with different reverse primers to amplify the alternatively spliced region of the FAH mRNA. (B) Schematic representation of the full-length FAH transcript and the alternative del100 and del231 FAH transcripts. Exons are represented by boxes (dotted when alternative) and introns by lines. Del100 has skipped exon 8 and del231 both exons 8 and 9.

Results and discussion

W262X-containing transcripts are strongly down-regulated

The *fah* gene is predominantly expressed in liver and kidneys but also shows a basic level of expression in most other tissues [33]. The effects of the W262X mutation were studied in lymphoblastoid cell lines established from four Finnish patients and their parents. After transformation, cell lines were genotyped and the presence of the mutation assessed, as previously described ([29]; data not shown). FAH transcripts analysis was first performed by RT-PCR on total RNA. The primer pair TanR130-RT025 extends most of the 1.4 kb of the FAH mature mRNA, from the ATG codon to the poly(A) signal (Figs. 1A and 2).

As shown in Fig. 2A, the W262X FAH mRNA is greatly reduced in patient 3, when compared to his parents (father and mother). An estimation of the amount of FAH mRNA in this patient was obtained by semi-quantitative RT-PCR (Fig. 2B). The number of PCR cycles (28) was determined in order to amplify FAH mRNA in both heterozygous and homozygous individ-

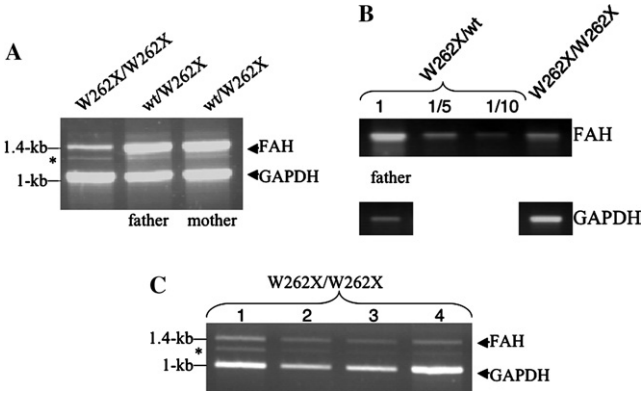

Fig. 2. W262X-containing mRNAs are greatly reduced in homozygous HTI patients. Total RNA was extracted from lymphoblastoid cell lines as described in the Materials and methods, and reverse transcribed. Full-length FAH cDNA was amplified using primers TanR130 and RT025. The expected product is 1368-bp long and is referred to as the 1.4 kb product. (A) cDNA obtained from total RNA was amplified in patient no 3 (W262X/W262X) and his parents (wt/W262X). The FAH 1.4 kb product is greatly decreased in comparison to his father and mother. Moreover, additional products of lower molecular weight are detected in the three individuals (indicated by a star). They are del100 and del231 alternative FAH transcripts (Dreumont et al., submitted; see Fig. 1B). GAPDH serves as an internal standard for RNA quantity. (B) Aliquots were taken at different times during the exponential phase of the PCR and run on a 1.5% agarose gel. The 1.4 kb product of patient no 3 (W262X/W262X) has the same amplification curve as the father cDNA (wt/W262X) diluted to 1/5. GAPDH cDNA serves as an internal standard in order to compare the RNA quantity extracted from each cell line. (C) Amplification was performed in four Finnish homozygotes patients (lanes 1–4, with the amplification from patient 3 loaded in lane 3). Each individual presents with a great diminution of W262X-containing mRNAs and the presence of additional products (indicated by a star).

uals while staying in the exponential phase of the reaction. A 1/5 dilution of the father mRNA gave a PCR product signal equivalent to that of the patient (Fig. 2B, top panel). Considering that approximately 4-fold less RNA was necessary to initiate RT synthesis as estimated by a concomitant amplification of GAPDH (Fig. 2B, bottom panel), this indicates that the patient cell line contains ~20-fold less nonsense FAH mRNA than the heterozygous parents. The diminution of W262X FAH RNA measured in three distinct experiments on RNA from patient no 3 ranged from 20- to 25-fold and was also observed in the three other Finnish HTI patients with the same mutation as demonstrated by amplification of the 1.4 kb cDNA (Fig. 2C; lanes 1, 2, and 4).

The down-regulation of nonsense transcripts has been reported to be in the order of 3–5-fold (see recent example of BRCA1 in [34]), except for the TcR, Ig, and, HexA genes [35,36], for which the diminution rates have been reported to be more than 10-fold. Our results are in agreement with the strong down-regulation observed for these three examples, since we estimate the reduction of FAH nonsense transcripts to be more than 20-fold of the level in an heterozygous carrier (Figs. 2B and 4). The differences in NMD efficiency in different systems could reflect gene- or cell-specificity of the NMD process. Alternatively, it was recently reported that a *cis*-acting sequence located in the rearranging VDJ exon of a Vβ 8.1 TcR mRNA was responsible for the strong down-regulation of TcR nonsense mRNAs [37]. The introduction of this *cis*-acting sequence in another PTC-containing mRNA coding for triose phosphate isomerase resulted in an increase of NMD efficiency to levels comparable to those of TcR transcripts [37]. FAH mRNA could contain such a *cis*-acting sequence, thereby promoting a robust down-regulation of aberrant transcripts.

Additional PCR products were detected when amplifying FAH 1.4 kb mRNAs in W262X individuals (indicated by a star in Fig. 2). These products were identified as alternative transcripts of the *fah* gene (Fig. 1B). Del100 has skipped exon 8, with the appearance of a PTC in exon 10, and del231 has skipped both exons 8 and 9, without any disruption of the reading frame (Fig. 1B; Dreumont et al., submitted). Briefly, these two alternative transcripts are due to minor alternative pathways and are not solely restricted to W262X individuals but are retrieved in wild-type cells too. They are detected with TanR130 and RT025 primers in W262X individuals because of the reduction of full-length nonsense mRNAs.

Diminution of W262X-FAH mRNAs occurs in the cytoplasmic compartment

The subcellular localization of NMD remains controversial [16]. Thus, many mammalian nonsense transcripts are degraded while still associated with the nucleus. This nucleus-associated NMD is not well understood, as biochemical fractionations have not yet determined whether the nonsense transcripts are degraded in the nucleoplasm proper or during the transit through the nuclear pore (when they are still attached to the nuclear membrane). In contrast, a minority of mammalian genes have been described to display a cytoplasmic NMD [36,38,39]. In order to determine to which category FAH transcripts belonged, we next examined the intracellular distribution of normal and nonsense FAH mRNAs after cellular fractionation and RT-PCR analysis (Fig. 3A). The first cytoplasmic fraction (Cyt1) contains soluble cytoplasmic components and tRNAs whereas the second cytoplasmic fraction (Cyt2) contains less soluble components, such as the rRNAs and RNAs associated with membrane-bound polysomes [32]. FAH transcripts were amplified from exons 6 to 14 (Fig. 1, 863-bp) in normal cells (Fig. 3A, wt/wt), patient no. 3 (W262X/W262X) and his father (wt/W262X).

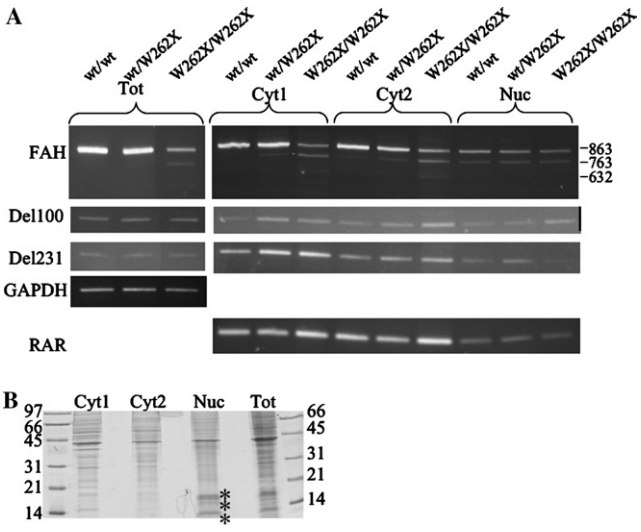

Fig. 3. Diminution of W262X mRNAs is apparent in the cytoplasm, but not in the nuclear fraction. (A) RNA was extracted from fractionated lymphoblastoid cells (patient no 3 W262X/W262X and his father wt/W262X) and normal lymphoblastoid cells (wt/wt) and subjected to RT-PCR. The amplification product extending from exons 6 to 14 is 863-bp long. RAR was amplified as a control for RNA quantity in each cell line. Amplifications on total RNA were performed from exons 6 to 13. The length of FAH PCR products is indicated on the right in base-pairs. Del100 and del231 were amplified using specific primers, as described in Dreumont et al. (submitted). Diminution of W262X mRNA is clearly apparent in the cytoplasmic fractions Cyt1 and Cyt2, but not in the nuclear fraction (Nuc). In the nuclear fraction, FAH seems to be expressed at the same extent in each individual when corrected to RAR levels. The additional PCR product at 763-bp, which is observed in all nuclear fractions and W262X/W262X cytoplasm is del100. (B) Coomassie blue staining of a 15% SDS–PAGE performed on proteins extracted from fractionated wild-type cells. Note that histones (indicated by stars) are only visible in the total (Tot) and nuclear fractions (Nuc), and not in the cytoplasmic fractions (Cyt1 and Cyt2).

RAR was used as an internal standard for RNA quantity, as GAPDH mRNA was only amplified in the cytoplasmic fractions (data not shown), indicating that there is no detectable contamination of the nuclear fraction by the cytoplasm [32]. In addition, the distribution of proteins in each fraction was verified by SDS–PAGE and Coomassie blue staining (Fig. 3B). Histones, which are strictly nuclear, are detected only in the nuclear fraction and not in the cytoplasmic fractions (Fig. 3B, indicated by stars). As shown in Fig. 3A, a decrease of W262X nonsense mRNAs is clearly apparent in the cytoplasmic fractions (Cyt1 and 2) of patient no 3 (W262X/W262X) as compared to a normal control (wt/wt) and the carrier (wt/W262X). In contrast, the amount of W262X FAH mRNA seems to remain unaffected in the nucleus (Nuc, compare lanes for wt/wt control to W262X/W262X patient 3). The cytoplasmic reduction of the W262X nonsense mRNA was also observed in the cell lines of the other three patients (data not shown).

Del100 and del231 were detected in the cytoplasmic fraction of patient 3 (Fig. 3A, W262X/262X, 763-bp, and 632-bp) and del100 was also observed in the nuclear fractions of all individuals (Fig. 3A) when using the RT76 and RT025 primer pair. The alternative transcripts were amplified using specific primers (Dreumont et al., submitted) and were detected in all fractions (Fig. 3A, del100 and del231 panels). Del100 was previously reported to be stabilized following a cycloheximide treatment in total cells contrary to del231, which was unaffected (Dreumont et al., submitted), suggesting that del100 is subjected to NMD. However, given the low amounts of each transcripts (Fig. 3A, del100 and del231 panels), it is difficult to conclude to a cytoplasmic reduction of del100 from the present data. Moreover, we cannot exclude the possibility of an effect of the W262X mutation on the amount of alternative transcripts produced or a different behavior of the del100 and del231 transcripts in the normal or mutated allele.

Since cellular fractionation experiments have shown that most mammalian nonsense transcripts are reduced to the same extent in the nuclear and cytoplasmic fractions [14,24–27,40], it has been proposed that PTC-containing mRNAs are degraded by NMD in the nucleus (the nuclear scanning model; [41]) or while still associated with the nucleus (the cotranslational export model; [14]). Few genes have been reported to display a cytoplasmic NMD, i.e., a cytoplasmic rather than nuclear diminution of nonsense mRNAs: among these are β-globin transcripts in erythroid cells or in transgenic mice [38,42], GPx1 in rat hepatocytes [39] and HexA in lymphoblastoid cells [36]. Our results on FAH suggest a cytoplasmic degradation of W262X-containing transcripts. Indeed the reduction of the nonsense FAH transcript is clearly evident in the cytoplasmic fractions, whereas its amount seems to be unaffected in the nuclear fraction (Fig. 3A, compare Cyt lanes to Nuc lanes). Thus, FAH transcripts may be added to the short list of transcripts, which are subjected to a cytoplasmic rather than a nuclear decay. Interestingly, most studies about the subcellular localization of NMD have been performed using cells transfected with minigenes. However, in the case of both HexA and FAH, the cytoplasmic NMD was observed in lymphoblastoid cells, obtained from patients ([36], this report). Maybe this apparent discrepancy could be explained by the different systems used, i.e., cells obtained from patients versus cells transfected with minigenes.

Translation is required for decay of nonsense FAH mRNAs

As NMD is translation-dependent, we finally assessed the effect of translation on FAH nonsense mRNAs by treating normal and homozygous cells with cycloheximide (Fig. 4A). This translation inhibitor is commonly used to demonstrate that NMD is responsible for the decrease of a given nonsense mRNA [43]. FAH transcripts from total RNA were analyzed by RT-PCR of exons 6–14 (Fig. 1). As seen in Figs. 4A and B, W262X FAH mRNA (the 863-bp band) represented less than 5% of

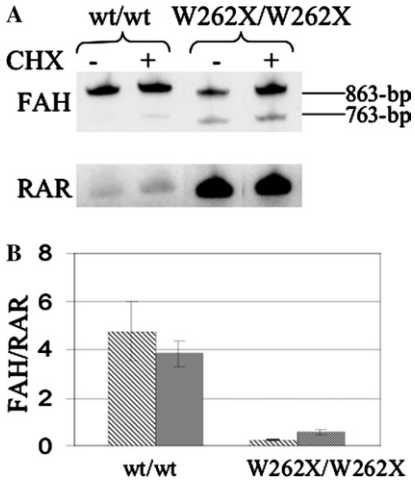

Fig. 4. W262X mRNAs are stabilized following a block of translation. Control cells (T19, wt/wt) and the homozygous cell line from patient no 3 (W262X/W262X) were treated with 100 μg/ml cycloheximide (CHX) for 3 h. Total RNA was extracted and subjected to RT-PCR. FAH was amplified from exons 6 to 14 (product of 863-bp) and RAR serves as a control for RNA quantity in each sample. (A) Amplifications were performed with [α-^{33}P]dATP and products were loaded on a 6% acrylamide gel. The gel was directly exposed to an X-ray film for at least 24 h. The amplification of FAH (from exons 6 to 14) in patient no 3 gives rise to two PCR products: the full-length transcript (863-bp) and del100 (763-bp). (B) Quantitation of the amount of FAH mRNA in A and in three other experiments, normalized to RAR levels. The error bars represent standard deviations. W262X mRNA is stabilized up to 3-fold when homozygous cells (W262X/W262X) are treated with cycloheximide (hatched bars, −cycloheximide; dotted bars, +cycloheximide).

the amount of normal mRNA in untreated cells after correction for RNA loading using RAR. Following a block of translation by cycloheximide, W262X mRNAs were stabilized and the level of this nonsense mRNA increased to 11% of cycloheximide-treated normal FAH mRNA. The >20-fold decrease of W262X mRNAs is only partially reversed by a CHX treatment. W262X transcripts are stabilized about 3-fold in treated cells. This rate is similar to that reported in other studies [43,44]. Moreover, the CHX effect depends on the presence of the PTC, since del100 was also stabilized by the treatment, whereas del231 remained relatively unaffected (Dreumont et al., submitted). This partial reversion of nonsense transcript degradation could reflect a side-effect of the drug, or the existence of another decay mechanism. Hence, FAH transcripts could be subjected to NMD and to another mechanism, which is translation-independent [44].

In conclusion, we have shown that nonsense transcripts of the *fah* gene are eliminated by the NMD pathway in a translation-dependent manner. This down-regulation occurs strictly in the cytoplasm, in contrast to many mammalian genes, for which the reduction is either nuclear, or nucleus-associated.

Acknowledgments

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