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Ribosomal frameshifting in response to hypomodified tRNAs in *Xenopus* oocytes

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ABSTRACT

We used *Xenopus* oocytes as an intracellular system to study ribosomal frameshifting. Microinjection of oocytes with a construct encoding the naturally occurring UUU or AAC codon at the frameshift site demonstrated that the level of frameshifting was similar or lower than found normally in retroviral frameshifting in mammalian cells, suggesting that oocytes are a reliable system to study this event. Phenylalanine (Phe) or asparagine (Asn) tRNAs with and without the highly modified wyebutoxine (Y) or queuosine (Q) base, respectively, were microinjected to assess their ability to promote frameshifting. $tRNA^{Phe}_{+Y}$ inhibited the level of frameshifting, while $tRNA^{Phe}_{-Y}$ promoted frameshifting providing evidence that the hypomodified form does not act only to enhance frameshifting, but is an essential requirement. Both $tRNA^{Asn}_{+Q}$ and $tRNA^{Asn}_{-Q}$ were used indiscriminately in frameshifting, whether the frameshift site contained the wild-type AAC, or the mutant AAU codon, suggesting that Q base modification status does not influence this process.

Many mammalian retroviruses use ribosomal frameshifting to extend their reading frames beyond *gag* and into *pro-pol* or *pol* by shifting the reading frame in the −1 direction (reviewed in [1–3]). The frameshift signal is a heptanucleotide sequence, X XXX YYZ. Unique features of the YYZ codon at the frameshift site are that only certain codons, e.g., UUU or AAC, occur at this position [4] and, when UUU is changed to UUC or AAC is changed to AAU, the level of frameshifting is reduced substantially demonstrating that only one of two synonymous codons effectively promotes this event. These observations led Jacks and Varmus to propose the existence of shifty tRNAs that enhance frameshifting [4]. The phenomenon of preferential synonymous codon translation is unexpected since the same tRNA must decode one of its codewords far better than the other.

The paradox of how synonymous codons are preferentially translated led to the proposal that base modifications in tRNA might account for the shiftiness of different isoforms [5]. The fact that base modifications in tRNAs have a dramatic effect on their coding properties [6–10] also suggested that these bases play a role in frameshifting in mammalian retroviruses.

The first direct evidence that hypomodified tRNAs enhance frameshifting in mammalian cells reported that Phe tRNA lacking the highly modified Wye base at position 37 ($tRNA^{Phe}_{-Y}$) stimulated the frameshift event [11,12]. Recently, Waas et al. [13] reported a stepwise decrease in frameshifting in yeast as the synthesis of Wye base became more abundant. The level of slippage observed in rabbit reticulocyte lysates and yeast cells in the presence of $tRNA^{Phe}_{-Y}$, or for that matter, in the presence of $tRNA^{Phe}_{+Y}$, exceeded that observed naturally with mammalian retroviruses raising concerns whether hypomodified $tRNA^{Phe}$ only enhanced ribosomal frameshifting or was actually required for the event to occur.

Prokaryotic systems were also developed for assessing the role of hypomodified tRNAs in ribosomal frameshifting which showed that base modification had little effect (e.g., see [14–16]). Although it was speculated from these studies that hypomodified tRNAs were not involved in frameshifting in mammalian systems, it was subsequently recognized that bacterial events were different from those of higher eukaryotes and direct comparisons for assessing the role of hypomodified tRNAs were likely not valid [17].

A major limitation in assessing the shiftiness of hypomodified tRNAs in mammalian retroviral ribosomal frameshifting as an essential or only enhancing constituent has been identifying an appropriate in vivo protein synthesis system wherein (1) the endogenous tRNA population contains low amounts of the suspected shifty tRNA in order that supplementation can sufficiently enrich the population to test its shiftiness, and (2) the level of frameshifting without supplemented tRNA is lower than observed in the natural retroviral frameshift event so that addition of the appropriate shifty isoform enhances the phenomenon. Herein, *Xenopus* oocytes were found to meet these criteria providing evidence that $tRNA^{Phe}_{-Y}$ is essential in frameshifting. The data also suggest that $tRNA^{Asn}_{+Q}$ or $tRNA^{Asn}_{-Q}$ are used indiscriminately in

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frameshifting as was reported previously [18,19]. The results of these studies are described herein.

Materials and methods

Materials. [^{35}S]Met (Spec. Act. > 1000 Ci/mmol), [^{3}H]Phe (Spec. Act. 123 Ci/mmol), and [^{14}C]Phe (Spec. Act. 489 mCi/mmol) were purchased from GE Healthcare (Piscataway, NJ), and [^{14}C]Asn (Spec. Act. 195 mCi/mmol) from Moravek Biochemicals (Brea, CA). *Xenopus laevis* females were obtained from Nasco (Fort Atkinson, WI). Rabbit reticulocyte lysate protein synthesis systems and Ribomax large scale transcription kits were purchased from Promega (Madison, WI), QIAfilter kit from Qiagen (Chatsworth, CA) and DH5α-competent cells from GIBCO (Grand Island, NY). Immobilized glutathione columns and reduced glutathione were purchased from Thermo Scientific (Rockford, IL). Freon (1,1,2-trichlorotrifluoroethane) and Protease Inhibitor Cocktail were purchased from Sigma. NuPAGE LDS Sample Buffer and NuPAGE Bis–Tris gels were purchased from Invitrogen.

Constructs encoding frameshift signals and mRNA preparation. Both wild-type and mutant GST-encoding constructs used to assess ribosomal frameshifting have been described in detail elsewhere [11,12]. These GST constructs were amplified in *Escherichia coli* DH5α-competent cells, extracted, and purified using a QIAfilter kit [12]. The purified constructs were digested with EcoR1 and mRNA generated [11] using a Ribomax large scale transcription kit as recommended by the vendor.

Transfer RNA, aminoacylation and RPC-5 chromatography. Total tRNA was isolated from freshly harvested *Xenopus* oocytes [20,21], calf liver [22], *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* [11,18], aminoacylated with labeled phenylalanine or asparagine [22] and the resulting labeled aminoacyl-tRNAs chromatographed [22] on a RPC-5 column [23]. *S. pombe* tRNA^{Asn} containing Q base (designated tRNA$^{Asn}_{+Q(Sp)}$) and lacking Q base (designated tRNA$^{Asn}_{-Q(SP)}$) and *S. cerevisiae* tRNA^{Asn} lacking Q base (designated tRNA$^{Asn}_{-Q(Sc)}$) were purified [11,18] and rabbit reticulocyte tRNA^{Phe} containing Wye base (designated tRNA$^{Phe}_{+Y}$) and mouse neuroblastoma tRNA^{Phe} lacking Wye base (designated tRNA$^{Phe}_{-Y}$) were isolated and purified as given [12].

Xenopus oocytes and microinjections. Forty nanoliters of a mixture containing 0.12 pmol of purified tRNA, and 60 ng of mRNA were injected into the cytoplasm of oocytes and the oocytes incubated in OR-2 buffer (82.5 mM NaCl, 2.5 mM KCl, 1 mM CaCl2, 1 mM $MgCl_2$, 1 mM $NaHCO_3$, 5 mM Hepes, pH 7.8, and 1 μCi/μl [^{35}S]Met) at 22 °C for 16 h. mRNA was prepared as given [11,12,18]. Thirty oocytes were homogenized in 240 μl of protein extraction buffer [ice cold 15 mM Tris–HCl, pH 7.5, 2 mM AEBSF, protease inhibitor cocktail]. Two hundred and forty microliters of Freon was then added (1,1,2-trichlorotrifluoroethane), the samples vortexed, and the upper aqueous phase was separated from the lower freon phase by centrifugation at 14,000 rpm for 10 min at 4 °C. Total protein extracts were electrophoresed on 10% NuPAGE Bis–Tris gels. Protein synthesis in rabbit reticulocyte lysates was carried out and the resulting protein samples electrophoresed as given previously [12,18]. GST-tagged proteins were isolated using an immobilized glutathione column and eluted with reduced glutathione according to the manufacturers instructions, and electrophoresed on 10% NuPAGE Bis–Tris gels. All developed gels were exposed to a PhosphorImager screen.

Results

Characterization of Asn and Phe tRNA populations

Xenopus oocyte Asn and Phe tRNA populations were examined to access the relative amounts of fully modified and hypomodified isoforms. Total tRNA isolated from *Xenopus* oocytes was aminoacylated with [^{3}H]Phe or [^{14}C]Asn and the resulting labeled tRNAs fractionated on a RPC-5 column. [^{3}H]Phe-tRNA^{Phe} from oocytes was co-chromatographed with [^{14}C]Phe-tRNA^{Phe} from calf liver. The tRNA^{Phe} population of calf liver exists primarily as a single, fully modified isoform that contains Wye base ([22], see also Fig. 1A). Phe-tRNA^{Phe} from oocytes fractionated into two major isoforms that contain Wye base and an earlier eluting, hypomodified isoform. The last eluting major isoform from oocytes likely represents an additional modification of the Wye base-containing species. Asn-tRNA^{Asn} was resolved into a major, fully modified isoform and a minor, later-eluting hypomodified isoform as shown (Fig. 1B). Since both of these tRNA populations in oocytes represent about 85% of the Wye and Q base-containing isoforms, it would appear that oocytes are an ideal system to test the ability of the hypomodified isoforms to support ribosomal frameshifting, at least with respect to the endogenous tRNA population.

The *S. cerevisiae* tRNA^{Asn} population contains only tRNA$^{Asn}_{-Q(Sc)}$, which enhances frameshifting [11,18], while the *S. pombe* tRNA^{Asn} population contains both isoforms with and without Q base, i.e., tRNA$^{Asn}_{+Q(Sp)}$ and tRNA$^{Asn}_{-Q(Sp)}$, respectively. All three isoforms were therefore purified from the respective organisms as described in the Materials and Methods. The primary structures of the two yeast asparagine tRNAs, along with that of mouse asparagine tRNA, are compared in Fig. 2. The anticodon stems and loops are shown in larger letters. Although any variations in the effects on ribosomal frameshifting between the three isoforms may be due to a change or changes anywhere in the three molecules, we focused on the anticodon stem and loops. All three isoforms have identical anticodon loops, but vary in their stems. The two yeast isoforms differ from each other in the first two and last two bases in their anticodon stems, while *S. cerevisiae* and mouse tRNAs^{Asn} manifest differences between each other in the second, third, and fourth (and the

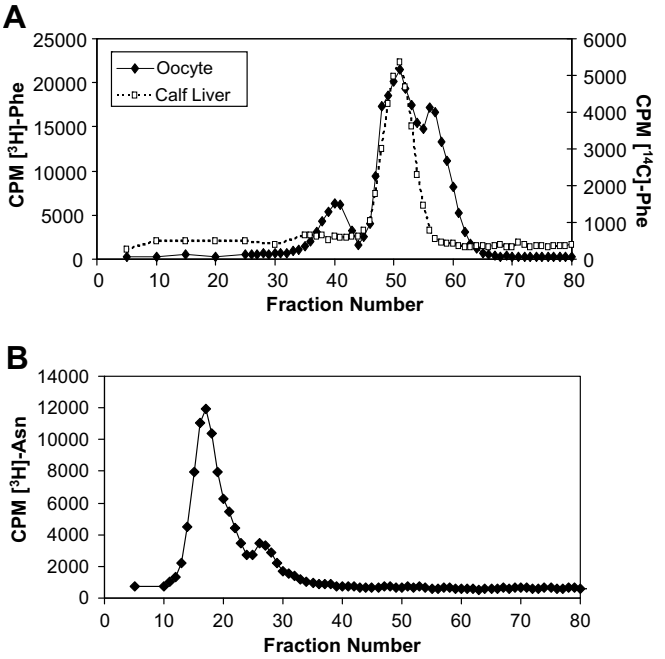

Fig. 1. Examination of the endogenous tRNA^{Phe} and tRNA^{Asn} populations in *Xenopus* oocytes. Total tRNA was isolated from *Xenopus* oocytes, aminoacylated with the appropriate labeled amino acid in the presence of 19 unlabeled amino acids and chromatographed on a RPC-5 column as given in Materials and methods. In A, [^{3}H]Phe-tRNA^{Phe} from oocytes was co-chromatographed with [^{14}C]Phe-tRNA^{Phe} from calf liver, and in B, [^{3}H]Asn-tRNA^{Asn} was chromatographed as described in Materials and methods.

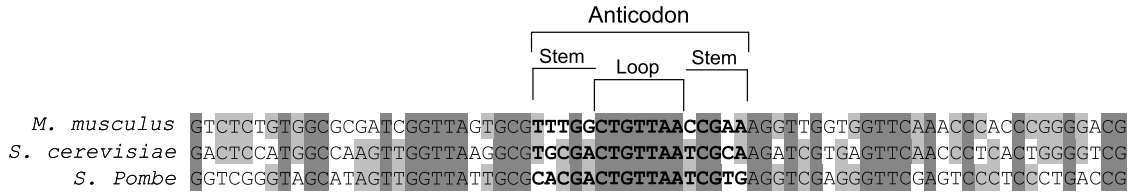

Fig. 2. Primary structures of tRNA^{Asn} from *M. musculus*, *S. cerevisiae*, and *S. pombe*. The anticodon loops and stems of each tRNA are shown in bold. Darker shading indicates homology between all three organisms, while lighter shading indicates homology between two organisms.

corresponding complementary) bases in their stems. *S. pombe* and mouse have differences in the first, second, third and fifth (and the corresponding complementary) bases in their stems. Each difference is highlighted as indicated in Fig. 2.

Ribosomal frameshifting (tRNA^{Phe})

To assess whether *Xenopus* oocytes may serve as a model system to resolve the question of whether tRNA$^{Phe}_{-Y}$ is required for frameshifting or only enhances this event in mammalian retroviruses utilizing XXXUUUU at the frameshift site, oocytes were incubated with [^{35}S]Met following injection with GST-AAAUUUU mRNA and with or without tRNA$^{Phe}_{+Y}$ or tRNA$^{Phe}_{-Y}$ (Fig. 3). Control oocytes that were microinjected with only mRNA contained a highly expressed GST band at ~25 kDa and a second, poorly expressed GST-MMTV *gag-pro* band at ~37 kDa (Fig. 3A, lane 2) that were not present in non-injected oocytes (compare lanes 1 and 2). Oocytes also microinjected with tRNA$^{Phe}_{-Y}$ contained a more intense GST-MMTV *gag-pro* band relative to the GST band than found in control oocytes (compare lanes 2 and 3), while oocytes microinjected with tRNA$^{Phe}_{+Y}$ appeared to have a less intense GST-MMTV *gag-pro* band relative to that observed in the control system or in oocytes also containing tRNA$^{Phe}_{-Y}$ (compare lanes 2–4). Purification of each of these GST-tagged proteins and their electrophoresis on gels (Fig. 3B) confirmed the observations found in total protein extracts (Fig. 3A). The level of frameshifting increased from about 4% in control oocytes to greater than 10% in oocytes containing tRNA$^{Phe}_{-Y}$ (lanes 1 and 2, respectively), while the level of frameshifting was reduced in oocytes that contained tRNA$^{Phe}_{+Y}$ to less than 3% compared to the 4% observed in controls (lanes 1 and 3, respectively).

Oocytes microinjected with GST-AAAUUUC mRNA with or without tRNA$^{Phe}_{-Y}$ or tRNA$^{Phe}_{+Y}$ yielded no detectable GST-MMTV *gag-pro* product (data not shown).

Ribosomal frameshifting (tRNA^{Asn})

To assess whether *Xenopus* oocytes may serve as a model for examining and/or mimicking ribosomal frameshifting in mammalian retroviruses utilizing XXXAAAC at the frameshift site, oocytes were incubated with [^{35}S]Met following injection with GST-AAAAAAC and with or without tRNA$^{Asn}_{+Q(Sp)}$, tRNA$^{Asn}_{-Q(Sp)}$, or tRNA$^{Asn}_{-Q(Sc)}$ (Fig. 4A and B). All oocytes containing mRNA with or without tRNA^{Asn} had a highly expressed GST band and a less, but similarly expressed, GST-MMTV *gag-pro* band (Fig. 4A, lanes 2–5). Purification of the GST-tagged proteins and their electrophoresis showed that indeed the level of frameshifting was similar whether oocytes contained tRNA^{Asn} with or without Q base or no supplemental tRNA. Interestingly, tRNA$^{Asn}_{-Q(Sc)}$ appeared to stimulate frameshifting slightly (compare lane 4 with lanes 1–3 in Fig. 4B). No detectable frameshifting occurred in oocytes microinjected with GST-AAAAAAU mRNA with or without tRNA^{Asn} (data not shown).

To further assess the ability of tRNA$^{Asn}_{+Q(Sp)}$ and tRNA$^{Asn}_{-Q(Sp)}$ to support ribosomal frameshifting, we examined the ability of these two isoforms to enhance frameshifting in rabbit reticulocyte

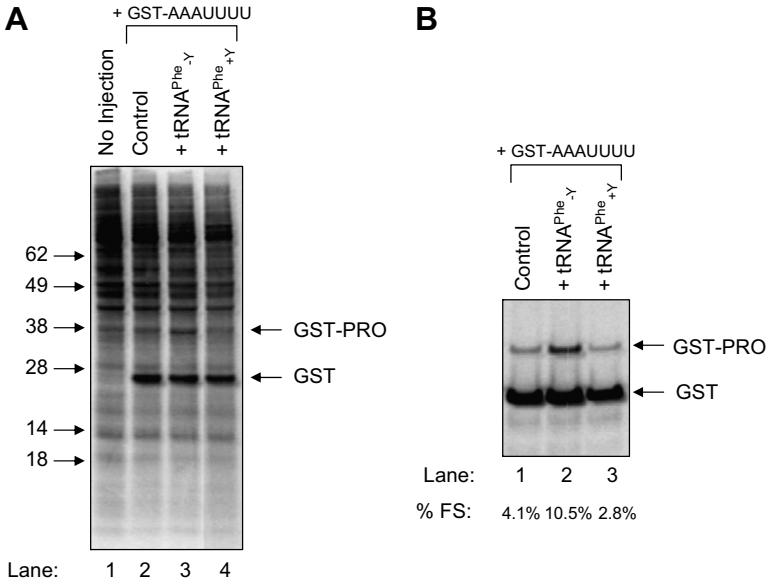

Fig. 3. Ribosomal frameshifting in *Xenopus* oocytes supplemented with tRNA$^{Phe}_{-Y}$ or tRNA$^{Phe}_{+Y}$. In A, all oocytes were incubated with [^{35}S]Met and in lane 1, no other addition, in lane 2, microinjected with GST-AAAUUUU, in lane 3, microinjected with GST-AAAUUUU and tRNA$^{Phe}_{-Y}$ and in lane 4, microinjected with GST-AAAUUUU and tRNA$^{Phe}_{+Y}$, extracts prepared, electrophoresed and an autoradiogram prepared as given in Materials and methods. In B, GST-tagged proteins were isolated from the extracts shown in A, lanes 2–4, electrophoresed and an autoradiogram prepared as given in Materials and methods. Microinjections and assays were carried out a total of three times with virtually identical results as those shown in the figure.

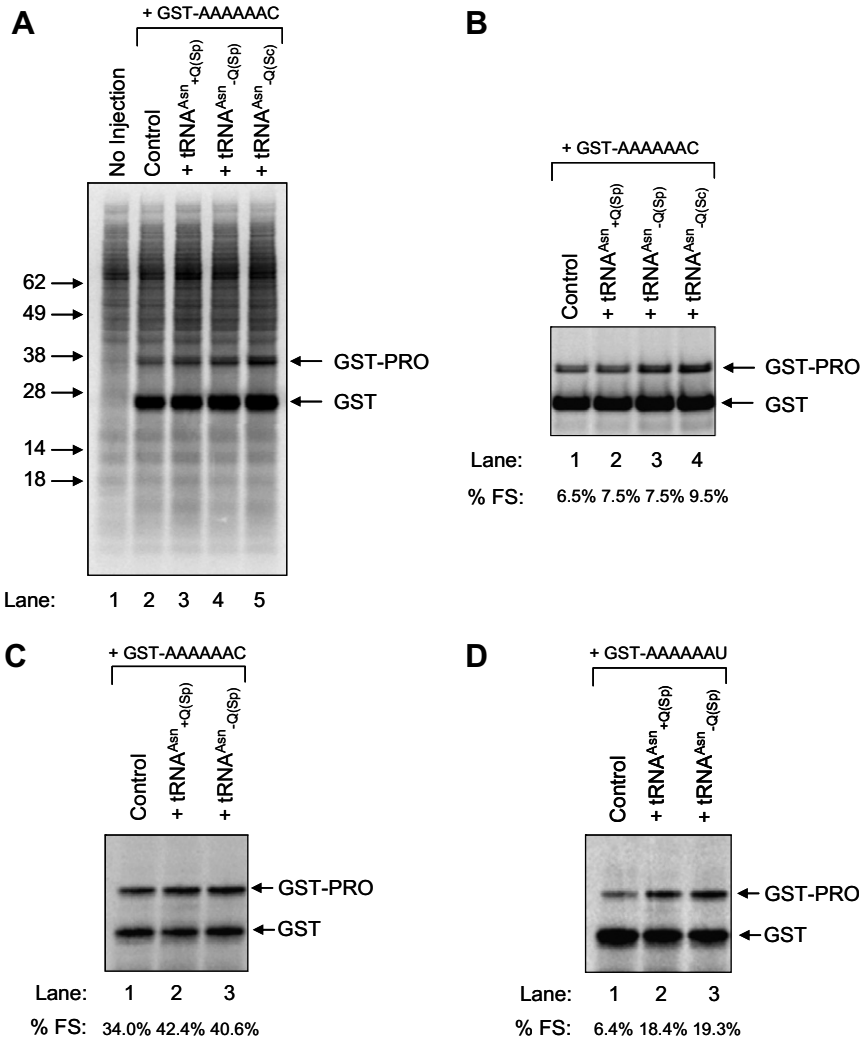

Fig. 4. Ribosomal frameshifting in *Xenopus* oocytes and rabbit reticulocyte lysates supplemented with $tRNA^{Asn}$ containing or lacking Q base. In (A) oocytes were incubated with [^{35}S]Met and in lane 1, no other addition, in lane 2, microinjected with GST-AAAAAAC, in lane 3, microinjected with GST-AAAAAAC and $tRNA^{Asn}_{+Q(Sp)}$, in lane 4, microinjected with GST-AAAAAAC and $tRNA^{Asn}_{-Q(Sp)}$, and in lane 5, microinjected with GST-AAAAAAC and $tRNA^{Asn}_{-Q(Sc)}$. In (B) GST-tagged proteins were isolated from the extracts shown in A, lanes 2–5, and the resulting gels prepared as given in the legend of Fig. 3B. In (C,D) rabbit reticulocyte lysates were supplemented without (lane 1) or with $tRNA^{Asn}_{+Q(Sp)}$ (lane 2) or $tRNA^{Asn}_{-Q(Sp)}$ (lane 3) and programmed with (C) GST-AAAAAAC or with (D) GST-AAAAAAU. Samples in (A–D) were prepared, electrophoresed and autoradiograms prepared as given in Materials and methods. Microinjections and assays were carried out a total of three times with virtually identical results as those shown in the figure.

lysates which is a loosely programmed system for carrying out protein synthesis [11,12,18,24,25]. The level of frameshifting with the wild-type AAC codon at the frameshift site was enhanced only slightly by supplementing the lysate system with either $tRNA^{Asn}_{+Q(Sp)}$ or $tRNA^{Asn}_{-Q(Sp)}$ (Fig. 4C). Interestingly, programming rabbit reticulocyte lysates with GST-AAAAAAU reduced the level of frameshifting dramatically [1–3], but supplementing this protein synthesis system with either $tRNA^{Asn}_{+Q(Sp)}$ or $tRNA^{Asn}_{-Q(Sp)}$ stimulated frameshifting about threefold (Fig. 4D). These data further suggest that frameshifting of XXXAAAC signals, or for that matter XXXAAAU signals, can utilize $tRNA^{Asn}_{+Q}$ or $tRNA^{Asn}_{-Q}$ indiscriminately to promote the frameshift event and that Q base modification status does not play a role in decoding one synonymous Asn codon better than the other.

Discussion

The present study employing *Xenopus* oocytes as an "*in vivo*" system to examine ribosomal frameshifting was prompted in part because of the high levels of frameshifting observed in cell free extracts and yeast cells which often exceeded that present in retroviruses within host cells (e.g., see [12,25] and references therein). The levels of frameshifting found in *Xenopus* oocytes following microinjection of a construct encoding a retroviral frameshift signal with either UUU or AAC at the frameshift site was between about 4% and 10.5%. These levels are lower than, but more closely approximate, that observed in retroviruses in infected cells. This observation, coupled with the finding of low levels of potential shifty (hypomodified) isoforms within the endogenous tRNA population, suggest that *Xenopus* oocytes indeed serve as an excellent model system for elucidating mechanisms of retroviral frameshifting. The high levels of frameshifting observed in widely used rabbit reticulocyte lysates and yeast cells, even in the absence of exogenous tRNAs, make it difficult to assess, for example, whether hypomodified $tRNA^{Phe}$ is required for frameshifting to occur or the hypomodified form merely stimulates the frameshift event wherein the shift in reading frames can still occur in the presence of fully modified $tRNA^{Phe}$, albeit not as efficiently. In fact, the use of *Xenopus* oocytes as a model system to elucidate the mechanism of frameshifting involving slippery tRNAs appears to have answered

the question of whether $tRNA^{Phe}$ is required or merely enhances the frameshift event. The low level of frameshifting observed in oocytes microinjected with only GST-AAAUUUU (i.e., in the absence of supplemental tRNA) is enhanced more than twofold by enriching the endogenous $tRNA^{Phe}_{-Y}$ population (Fig. 3) further demonstrating that this isoform is a slippery tRNA. Furthermore, the fact that microinjection of $tRNA^{Phe}_{+Y}$ which enriched the endogenous $tRNA^{Phe}$ population with the fully modified isoform reduced the level of frameshifting from about 4% in oocytes containing only GST-AAAUUUU mRNA to less than 3% demonstrates that the fully modified isoform inhibits the frameshift event (see also [12]). Thus, the approximately 4% level of frameshifting observed in the presence of only GST-AAAUUUU is apparently due to the small amount of $tRNA^{Phe}_{-Y}$ present intracellularly (Fig. 1A). We therefore propose that $tRNA^{Phe}_{-Y}$ is the slippery tRNA, while the frameshifting event is inhibited in the presence of $tRNA^{Phe}_{+Y}$, and the hypomodified isoform is essential for the frameshift to take place.

The question of the requirement of hypomodified tRNAs in promoting ribosomal frameshifting has been of considerable interest as their restriction may provide an alternative avenue for inhibiting retroviral expression [2,11,26]. In the case of $tRNA^{Asn}$, wherein Q base is obtained from the diet in mammals, and in the event Q base restricted the frameshift event, a diet rich in this base should theoretically reduce the endogenous population of $tRNA^{Asn}_{-Q}$. Unfortunately, reducing the level of endogenous $tRNA^{Asn}_{-Q}$ does not appear to offer a means of restricting retroviral ribosomal frameshifting since either $tRNA^{Asn}$ isoform seems to be used indiscriminately at the frameshift site [18,19]. However, it would be of interest to incubate oocytes in the presence of Q base to initially assess whether the endogenous $tRNA^{Asn}_{-Q}$ population (see Fig. 1B) could be reduced prior to injecting oocytes with GST-AAAAAAC mRNA. In the event the endogenous $tRNA^{Asn}_{-Q}$ population can be reduced, then the level of frameshifting can be further assessed which should unequivocally determine whether Q base is involved in reducing ribosomal frameshifting. As long as there is any endogenous level of $tRNA^{Asn}_{-Q}$ (see Fig. 1B), then the question of an involvement of this isoform in frameshifting cannot be completely resolved [18]. Interestingly, the reason that the occurrence of AAU at the frameshift site is inhibitory (e.g., see [1–4,24,27]) cannot be due to $tRNA^{Asn}$ modification as both isoforms also translate this codon indiscriminately ([18], and see Fig. 4D).

Furthermore, we have shown that $tRNA^{Asn}_{-Q(Sc)}$ enhances frameshifting [11] and that the base or bases responsible for the increase in frameshifting is not in the anticodon loop, but reside(s) elsewhere in the tRNA [18]. *Xenopus* oocytes provide a model system for assessing the location of the base(s) responsible for this enhancement by cloning the corresponding $tRNA^{Asn}$ gene from yeast and generating the appropriate mutations in the tRNA. Injection of the resulting mutant tRNAs into oocytes should determine which base(s) is (are) involved in promoting frameshifting shedding more light on the mechanism of this process.

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References

[1] T. Jacks, Translational suppression in gene expression in retroviruses and retrotransposons, Curr. Top. Microbiol. Immunol. 157 (1990) 93–124.
[2] D.L. Hatfield, J.G. Levin, A. Rein, S. Oroszlan, Translational suppression in retroviral gene expression, Adv. Virus Res. 41 (1992) 193–239.
[3] P.J. Farabaugh, Translational frameshifting: implications for the mechanism of translational frame maintenance, Prog. Nucleic Acid Res. Mol. Biol. 64 (2000) 131–170.
[4] T. Jacks, H.D. Madhani, F.R. Masairz, H.E. Varmus, Signals for ribosomal frameshifting in the Rous sarcoma virus gag-pol region, Cell 55 (1988) 447–458.
[5] D. Hatfield, Y.-X. Feng, B.J. Lee, A. Rein, J.G. Levin, S. Oroszlan, Chromatographic analysis of aminoacyl-tRNAs which are required for translation of codons at and around the ribosomal frameshift sites of HIV HTLV-1 and BLV, Virology 173 (1989) 736–742.
[6] H. Beier, M. Barciszewska, H.D. Sickinger, The molecular basis for the differential translation of TMV RNA in tobacco protoplasts and wheat germ extracts, EMBO J. 3 (1984) 1091–1096.
[7] F. Meier, B. Suter, H. Grosjean, G. Keith, E. Kubli, Queosine modification of the wobble base in tRNAHis influences 'in vivo' decoding properties, EMBO J. 4 (1985) 823–827.
[8] D.W.E. Smith, D.L. Hatfield, Effects of post-transcriptional base modifications on the site-specific function of transfer RNA in eukaryote translation, J. Mol. Biol. 189 (1986) 663–671.
[9] R.K. Wilson, B.A. Roe, Presence of the hypermodified nucleotide N6-((-2-isopentenyl)-2-methylthioadenosine prevents codon misreading by *Escherichia coli* phenylalanyl-transfer RNA, Proc. Natl. Acad. Sci. USA 86 (1989) 409–413.
[10] G.R. Bjork, The role of modified nucleosides in tRNA interactions, in: D.L. Hatfield, B.J. Lee, R.M. Pirtle (Eds.), Transfer RNA in Protein Synthesis, CRC Press, Boca Raton, FL, 1992, pp. 23–85.
[11] B.A. Carlson, S.Y. Kwon, M. Chamorro, S. Oroszlan, D.L. Hatfield, B.J. Lee, Transfer RNA modification status influences retroviral ribosomal frameshifting, Virology 255 (1999) 2–8.
[12] B.A. Carlson, J.F. Mushinski, D.W. Henderson, S.Y. Kwon, P.F. Crain, B.J. Lee, D.L. Hatfield, 1-Methylguanosine in place of Y base at position 37 in phenylalanine tRNA is responsible for its shiftiness in retroviral ribosomal frameshifting, Virology 279 (2001) 130–135.
[13] W.F. Waas, Z. Druzina, M. Hanan, P. Schimmel, Role of a tRNA base modification and its precursors in frameshifting in eukaryotes, J. Biol. Chem. 282 (2007) 26026–26034.
[14] I. Brierley, A.J. Jenner, S.C. Inglis, Mutational analysis of the "slippery-sequence" component of a coronavirus ribosomal frameshifting signal, J. Mol. Biol. 227 (1992) 463–479.
[15] Z. Tsuchihashi, P.O. Brown, Sequence requirements for efficient translational frameshifting in the *Escherichia coli* dnaX gene and the role of an unstable interaction between tRNA(Lys) and an AAG lysine codon, Genes Dev. 6 (1992) 511–519.
[16] I. Brierley, M.R. Meredith, A.J. Bloys, T.G. Hagervall, Expression of a coronavirus ribosomal frameshift signal in *Escherichia coli: influence of tRNA anticodon modification on frameshifting, J. Mol. Biol. 270 (1997) 360–373.*
[17] S. Napthine, M. Vidakovic, R. Girnary, O. Namy, I. Brierley, Prokaryotic-style frameshifting in a plant translation system: conservation of an unusual single-tRNA slippage event, EMBO J. 22 (2003) 3941–3950.
[18] B.A. Carlson, S.Y. Kwon, B.J. Lee, D.L. Hatfield, Yeast asparagine (Asn) tRNA without Q base promotes eukaryotic frameshifting more efficiently than mammalian Asn tRNAs with or without Q base, Mol. Cell 10 (2000) 113–118.
[19] B. Marczinke, T. Hagervall, T.I. Brierley, The Q-base of asparaginyl-tRNA is dispensable for efficient −1 ribosomal frameshifting in eukaryotes, J. Mol. Biol. 295 (2000) 179–191.
[20] B.J. Lee, S.K. Kang, D. Hatfield, Transcription of *Xenopus* seleno–cysteine tRNASer (formerly designated opal suppressor phosphoserine tRNA) is directed by multiple 5′ extragenic regulatory elements, J. Biol. Chem. 264 (1989) 9696–9702.
[21] I.S. Choi, A.M. Diamond, P.F. Crain, J.D. Kolker, J.A. McCloskey, D.L. Hatfield, Reconstitution of the biosynthetic pathway of selenocysteine tRNAs in *Xenopus* oocytes, Biochemistry 33 (1994) 601–605.
[22] D. Hatfield, C.R. Matthews, M. Rice, Aminoacyl-transfer RNA populations in mammalian cells chromatographic profiles and patterns of codon recognition, Biochim. Biophys. Acta 564 (1979) 414–423.
[23] A.D. Kelmers, D.E. Heatherly, Columns for rapid chromatographic separation of small amounts of tracer-labeled transfer ribonucleic acids, Anal. Biochem. 44 (1971) 486–495.
[24] T. Jacks, M.D. Power, F.R. Masiarz, P.A. Luciw, P.J. Barr, H.E. Varmus, Characterization of ribosomal frameshifting in HIV-1 gag-pol expression, Nature 331 (1988) 280–283.
[25] E.P. Plant, J.D. Dinman, Comparative study of the effects of heptameric slippery site composition on -1 frameshifting among different eukaryotic systems, RNA 12 (2006) 666–673.
[26] A. Meskauskas, J.W. Harger, K.L. Jacobs, J.D. Dinman, Decreased peptidyltransferase activity correlates with increased programmed -1 ribosomal frameshifting and viral maintenance defects in the yeast *Saccharomyces cerevisiae*, RNA 9 (2003) 982–992.
[27] T. Jacks, H.E. Varmus, Expression of the Rous sarcoma virus pol gene by ribosomal frameshifting, Science 230 (1985) 1237–1242.