

Antisense-induced ribosomal frameshifting

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Received February 9, 2006; Revised June 7, 2006; Accepted July 7, 2006

ABSTRACT

Programmed ribosomal frameshifting provides a mechanism to decode information located in two overlapping reading frames by diverting a proportion of translating ribosomes into a second open reading frame (ORF). The result is the production of two proteins: the product of standard translation from ORF1 and an ORF1–ORF2 fusion protein. Such programmed frameshifting is commonly utilized as a gene expression mechanism in viruses that infect eukaryotic cells and in a subset of cellular genes. RNA secondary structures, consisting of pseudoknots or stem–loops, located downstream of the shift site often act as *cis*-stimulators of frameshifting. Here, we demonstrate for the first time that antisense oligonucleotides can functionally mimic these RNA structures to induce +1 ribosomal frameshifting when annealed downstream of the frameshift site, UCC UGA. Antisense-induced shifting of the ribosome into the +1 reading frame is highly efficient in both rabbit reticulocyte lysate translation reactions and in cultured mammalian cells. The efficiency of antisense-induced frameshifting at this site is responsive to the sequence context 5' of the shift site and to polyamine levels.

INTRODUCTION

The standard triplet readout of the genetic code can be reprogrammed by signals in the mRNA to induce ribosomal frameshifting [reviewed in (1–3)]. Generally, the resulting *trans*-frame protein product is functional and may in some cases be expressed in equal amounts to the product of standard translation. This elaboration of the genetic code (4,5) demonstrates versatility in decoding.

Requirements for eukaryotic ribosomal frameshifting include a shift-prone sequence at the decoding site and often a downstream secondary structure in mRNA. The majority of –1 programmed frameshift sites consist of a heptanucleotide sequence X XXY YYZ [where X can be A, G, C or U; Y can be A or U; and Z can be any nucleotide (6)].

In this configuration, the P- and A-site tRNAs can re-pair with at least 2 out of 3 nt when shifted 1 nt towards the 5' end of the mRNA. Similarly, for +1 frameshift sites, the identity of the codons in the P- and A-sites of the ribosome is critical for efficient frameshifting. One factor affecting +1 frameshift efficiency is the initial stability of the P-site tRNA–mRNA interaction in the 0 frame (7). High-efficiency frameshifting occurs when the P-site tRNA does not form standard codon–anticodon interactions (8). In some studies, a correlation between +1 frameshift efficiency and the final stability of the P-site tRNA–mRNA interaction in the +1 frame has been shown previously (9,10). However, in other systems there appears to be little correlation (11). In addition, competition between decoding of the 0 frame and +1 frame codons in the A-site may affect frameshifting efficiency (7). Slow to decode 0 frame codons such as stop codons or those decoded by low abundance tRNAs favor frameshifting, as do +1 frame codons with high levels of corresponding cognate tRNAs (12–16).

High levels of frameshifting are often achieved by the stimulatory action of a *cis*-acting element located downstream of the shift site. A wide variety of structures, most commonly H-type pseudoknots (17), have been identified which stimulate –1 frameshifting in eukaryotes [for reviews see (18,19)]. Mutagenic and structural data for several of the frameshift stimulators have demonstrated that each pseudoknot has key structural features required for frameshift stimulation (20–28). However, unifying structural feature essential for frameshifting has not yet been identified. This observation combined with recent reports that simple antisense oligonucleotides can functionally mimic *cis*-acting 3' stimulators of –1 frameshifting (29,30) demonstrates that many different structures can stimulate frameshifting. Although it should be noted that not all structures of equal thermodynamic stability can stimulate frameshifting (Discussion).

RNA pseudoknots have also been shown to stimulate programmed +1 frameshifting in many eukaryotic antizyme genes (31,32). Antizyme is a negative regulator of cellular polyamine levels through its ability to target ornithine decarboxylase (the rate-limiting enzyme in polyamine biosynthesis) for degradation (33–35), inhibits polyamine import (36,37) and stimulates export (38). Antizyme expression is induced by high-intracellular polyamine levels, and decreased with lowered levels. The polyamine sensor is a programmed +1 frameshift event that is required for antizyme synthesis.

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At low polyamine levels, termination at the end of open reading frame 1 (ORF1) is efficient, whereas at high levels of polyamines, a substantial proportion of ribosomes shift to the +1 reading frame and then resume standard decoding to synthesize the full-length and active antizyme protein. Frameshifting at the mammalian antizyme mRNA shift site, UCC UGA, is stimulated by two *cis*-acting signals (39,40). One of these, the 5' element, encompasses ~50 bases upstream of shift site and is important for the polyamine effect (39–41). The other *cis*-acting element is a pseudoknot located 3' of the shift site. The mammalian antizyme pseudoknot and a structurally distinct counterpart in a subset of invertebrate antizyme mRNAs (31) are the only pseudoknots known to act as stimulators for +1 frameshifting in eukaryotes.

Although it is unknown if pseudoknots stimulate –1 frameshifting and +1 frameshifting by different mechanisms, one notable difference is found in positioning of the downstream structure relative to the shift site. Naturally occurring pseudoknots or stem-loop stimulators of –1 frameshifting typically begin ~6–9 nt downstream of the A-site codon of the shift site (18), whereas +1 frameshift pseudoknots are located closer with only a 2–3 nt separation from the A-site codon (31). Mutagenic studies have revealed that altering the size of the spacer affects frameshifting and, in general, reduces efficiency (27,31,42–44).

Here we have tested the ability of antisense oligonucleotides, annealed downstream of the shift-prone site, UCC UGA, to induce shifting of the ribosome to the +1 reading frame. The directionality of frameshifting (either into the +1 or –1 reading frame) is shown to be dependent upon the position of the duplex region relative to the shift site, and the efficiency of frameshifting is responsive to polyamine levels and enhanced by the inclusion of stimulatory sequences found upstream of the human antizyme +1 programmed frameshift site.

MATERIALS AND METHODS

Frameshift reporter constructs and 2'-O-Methyl oligonucleotides

Complementary oligonucleotides, to construct the sequences described in this paper, were synthesized at the University of Utah DNA/Peptide Core Facility such that when annealed they would have appropriate ends to ligate into the Sall/BamHI sites of the dual luciferase vector, p2luc (45). Dual luciferase constructs were prepared and their sequence was verified as described previously (46).

Insert sequences with shift site in boldface is given as follows:

P2lucAZ1wt: TCGACGGTCTCCCTCCACTGCTGTAG-TAACCCGGGTCCGGGGCCTCGGTGGTGCTCCTGATG-CCCCTACCCACCCCTGAAGATCCCAGGTGGGCGAG-GGAATAGTCAGAGGGATCACAAACGGATC;

P2lucAZ10sp: TCGACGGTCTCCCTCCACTGCTGTAG-TAACCCGGGTCCGGGGCCTCGGTGGTGCTCCTGAC-CCTACCCACCCCTGAAGATCCCAGGTGGGCGAGG-GAATAGTCAGAGGGATCACAAACGGATC;

P2lucAZ1hp: TCGACGGTCTCCCTCCACTGCTGTAG-TAACCCGGGTCCGGGGCCTCGGTGGTGCTCCTGATG-

CCCCTACCCACCCCTGAAGATCCCAGGTGGGCGAG-GGAATGGATC;

P2lucAZ1PKdel: TCGACGGTCTCCCTCCACTGCTGTAGTAACCCGGGTCCGGGGCCTCGGTGGTGCTCCTGATGCCCCCTGGATC;

P2lucAZ1PKm1: TCGACGGTCTCCCTCCACTGCTGTAGTAACCCGGGTCCGGGGCCTCGGTGGTGCTCCTGATGCCCCCTACCCACCCGGATCACAAAGGATC;

P2lucAZ1sl: TCGACGGTCTCCCTCCACTGCTGTAGTAACCCGGGTCCGGGGCCTCGGTGGTGCTCCTGATG-CCCCTACCCACCCGGATC;

P2lucAZ1FS: TCGACGTGCTCCTGATGCCCCCTGATC;

P2lucAZ1FSUGG: TCGACGTGCTCCTGGTGCCCCCTGATC.

2'-O-Methyl antisense oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA).

AZ1A: AGUUGAAGGAUCCAGGGGCA; AZ1B: GGAAGUUGAAGGAUCCAGGG; AZ1C: CAGGGAAGUUGAAGGAUCCA; PKm1: GAUCCCGGUGGGUGAGGG; PKm2: GAUCCAGGUGGGCGAGGG; SL1: GGUGGGUGAGGG; and SL2: GGAUCCGGGUGGGUGAGGG.

In vitro transcription and translation

The dual luciferase constructs (0.1 µg) described above were added directly to TNT coupled reticulocyte lysate reactions (Promega) with ³⁵S-labeled methionine in a volume of 10 µl. Reactions were incubated at 30°C for 1 h. Radiolabeled proteins were separated by SDS-PAGE and the gels were fixed with 7.5% acetic acid and methanol for 20 min. After drying under vacuum, the gels were visualized using a Storm 860 PhosphorImager (Molecular Dynamics) and radioactive bands quantified using ImageQuant software. Percent frameshifting was calculated as the percentage of full-length (frameshift) product relative to the termination product and the full-length product combined. The value of each product was corrected for the number of methionine codons present in the coding sequence. The reported values are the average and standard deviations obtained from at least three independent measurements. Tables showing percent frameshifting and standard deviations can be found in Supplementary Data.

Analysis of antisense-induced frameshifting in mammalian cultured cells

Plasmid p2lucAZ1PKdel was co-transfected into CV-1 cells with varying concentrations of AZ1B 2'-O-Methyl antisense oligonucleotides under the following conditions. CV-1 cells (1.5×10^4) in 50 µl of DMEM + 5% fetal bovine serum were added to wells (1/2 area 96-well tissue culture treated plates) containing 25 ng of DNA, varying amounts of AZ1B antisense oligonucleotides and 0.4 µl Lipofectamine 2000 (Invitrogen) in 25 µl of Optimem. Cells were incubated at 37°C (5% CO₂) for 20 h. Media were then removed from the cells and the transfected cells were lysed in 12.5 µl lysis buffer and luciferase activity determined by measuring light emission following injection of 25 µl of luminescence reagent (Promega). Percent frameshifting was calculated by comparing firefly/*Renilla* luciferase ratios of experimental constructs with those of control constructs: (firefly

experimental RLU/*Renilla* experimental RLU)/(firefly control RLU/*Renilla* control RLU) × 100.

RESULTS

The ability of *cis*-acting RNA structures or *trans*-acting 2'-*O*-Methyl antisense oligonucleotides to induce ribosomal frameshifting was determined by *in vitro* transcription and

translation of a dual luciferase reporter vector, p2Luc. p2Luc contains the *Renilla* and firefly luciferase genes on either side of a multiple cloning site, and can be transcribed using the T7 promoter located upstream of the *Renilla* luciferase gene (45). Sequences containing shift-prone sites were cloned between the two reporter genes such that the downstream firefly luciferase gene is in the +1 reading frame. The resulting constructs were then transcribed and translated *in vitro* with or without complementary

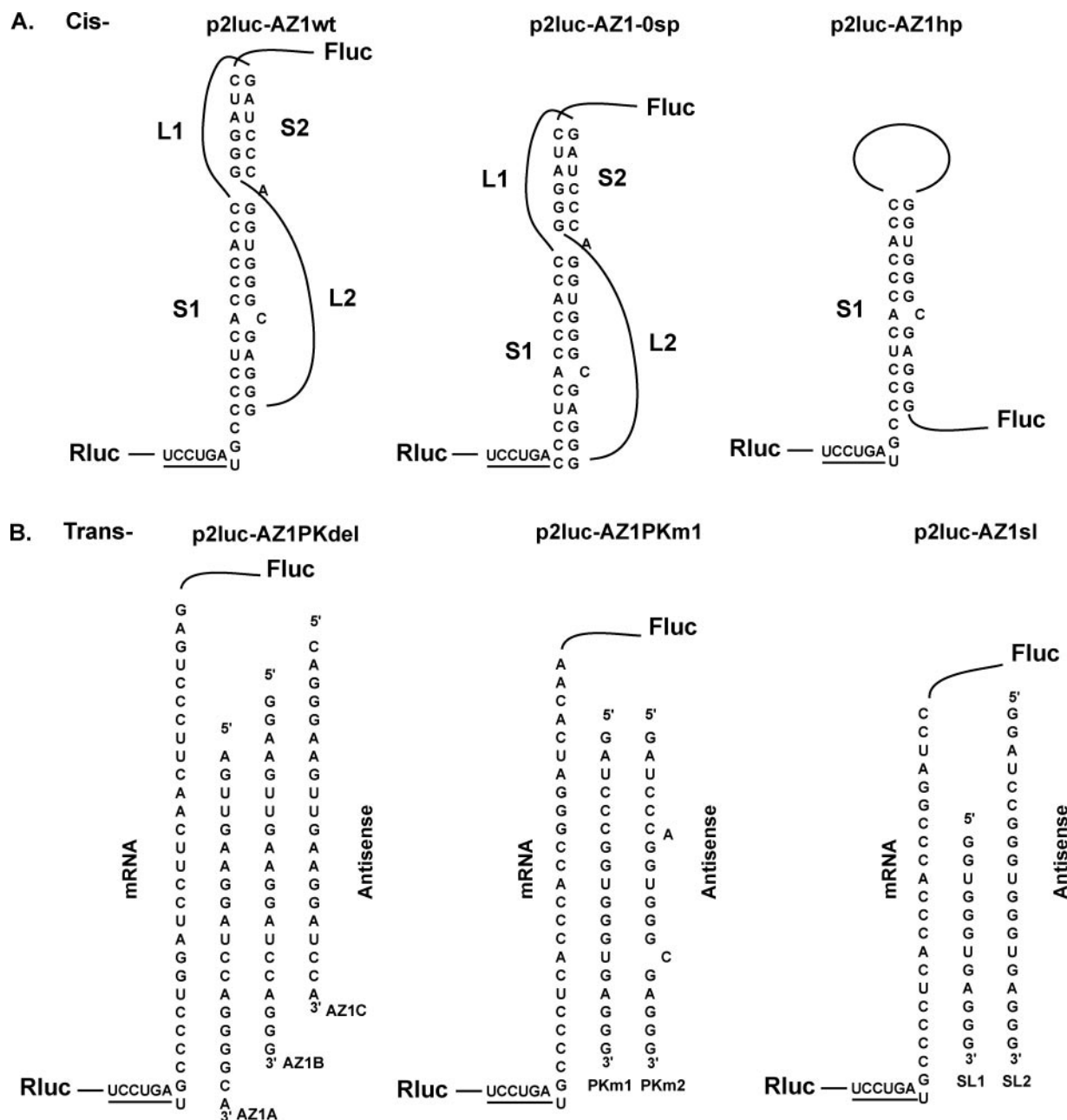


Figure 1. (A) Reporter construct design: *cis*- and *trans*-acting stimulators of frameshifting. Sequence of the shift site and downstream sequences for dual luciferase constructs containing *cis*-acting structures used in this paper. P2luc-AZ1wt contains the wild-type antizyme frameshift cassette, p2luc-AZ1-0sp has a 3 bp deletion of the spacer sequences separating the shift site from the pseudoknot and p2luc-AZ1hp contains a hairpin replacement of the pseudoknot structure. S1 and S2 refer to stem 1 and stem 2 of the RNA pseudoknot. L1 and L2 refer to loops 1 and 2 of the pseudoknot. Fluc and Rluc represent Firefly and *Renilla* luciferase genes, respectively. (B) Sequence of the shift site and downstream sequences for dual luciferase constructs and their complementary antisense oligonucleotide partners. Fluc and Rluc represent Firefly and *Renilla* luciferase genes, respectively.

2'-*O*-Methyl oligonucleotides, using rabbit reticulocyte lysate in the presence of ^{35}S -labeled methionine, and the products analyzed by electrophoresis on SDS-polyacrylamide gels as described in Materials and Methods.

cis-Acting stimulators of frameshifting at the antizyme shift site

Initially, three dual luciferase reporter vectors were generated containing the human antizyme 1 frameshift cassette (p2luc-AZ1wt) with the 5' and 3' stimulators of frameshifting, with the pseudoknot deleted (p2luc-AZ1PKdel), or replaced with a stem-loop (p2luc-AZ1hp) (Figure 1). Each constructs was then subjected to coupled transcription and translation reactions in the presence of increasing amounts of spermidine, and the ^{35}S -labeled products separated by SDS-PAGE. Frameshifting efficiency was measured by comparing the amount of full-length frameshift product (+1 FS) to the product of termination (Term) at the shift site stop codon (Figure 2 and Supplementary Table 1). Maximum levels of frameshifting (AZ1wt 5.6%, AZ1PKdel 2.1% and AZ1hp 1.5%) were observed in the presence of 0.4 mM spermidine. Low-level frameshifting, 0.1% or less, was observed in the absence of exogenous spermidine.

Trans-acting stimulators of frameshifting at the antizyme shift site

2'-*O*-Methyl antisense oligonucleotides were designed to anneal downstream of the UCC UGA shift site of RNA produced from p2luc-AZ1PKdel (AZ1PKdel) such that the 3' ends were located 0 (AZ1A), 3 (AZ1B) or 6 (AZ1C) nt downstream of the UGA codon of the shift site (Figure 1B). Frameshift efficiency was measured following transcription/translation reactions of p2luc-AZ1PKdel in the presence of 2 μM of each antisense oligonucleotide and increasing amounts of spermidine (Figure 3A–C and Supplementary Table 2). Maximal levels of frameshifting were found to occur when 2–4 μM of antisense oligonucleotide was added to the transcription/translation reactions (Supplementary Table 3). In the presence of 0.4 mM exogenous spermidine, highly efficient shifting of ribosomes into the +1 reading frame (higher than that observed in the wild-type antizyme frameshift cassette) was observed with the addition of AZ1A (26.1%), AZ1B (51.8%) and AZ1C (31.8%) (Supplementary Table 2). The most efficient frameshifting is observed with the antisense oligonucleotide AZ1B which anneals such that spacing between the shift site and the beginning of the duplex region is the same as that observed between the shift site and the beginning of stem 1 of the natural antizyme 3' pseudoknot structure (i.e. each has a 3 nt spacer). To verify that the antisense oligonucleotide was activating ribosomal frameshifting and not transcription slippage, RNA was transcribed from p2luc-AZ1PKdel in the absence of oligonucleotide and added to reticulocyte lysate translations in the presence of increasing amounts of 2'-*O*-Methyl AZ1B oligonucleotide. Frameshifting levels were increased to the same level as that observed in coupled transcription and translation reactions demonstrating that the oligonucleotide acts to induce frameshifting during translation (Supplementary Figure).

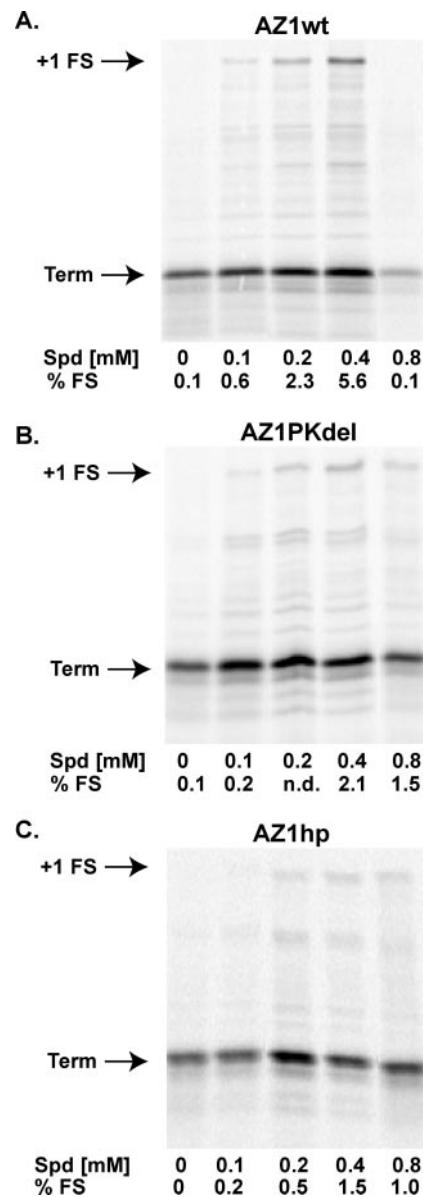


Figure 2. *Cis*-acting 3' stimulators of frameshifting. Plasmids p2Luc-AZ1wt (AZ1wt), p2luc-AZ1PKdel (AZ1PKdel) and p2luc-AZ1hp (AZ1hp) were transcribed and translated in rabbit reticulocyte lysate in the absence or presence of increasing amounts of spermidine (final concentration indicated in mM). SDS-PAGE of ^{35}S -methionine-labeled protein products from transcription and translation reactions is shown. The location of the full-length frameshift product (+1 FS) and non-frameshift termination product (Term) are indicated. The efficiency of frameshifting (% FS) is indicated.

Surprisingly, the addition of AZ1A (0 spacer) also induced high-level frameshifting into the -1 reading frame in a manner which was modestly inhibited by the addition of spermidine (19% in the absence and 10% in the presence of 0.4 mM exogenous spermidine) (Figure 3A and Supplementary Table 2). No -1 frameshift product was observed when the wild-type antizyme cassette was examined in the absence of antisense oligonucleotide addition (Figure 2; AZwt). As the AZ1A antisense oligonucleotide was designed to anneal directly adjacent to the UGA codon of the shift site, it was of interest to determine whether the wild-type antizyme

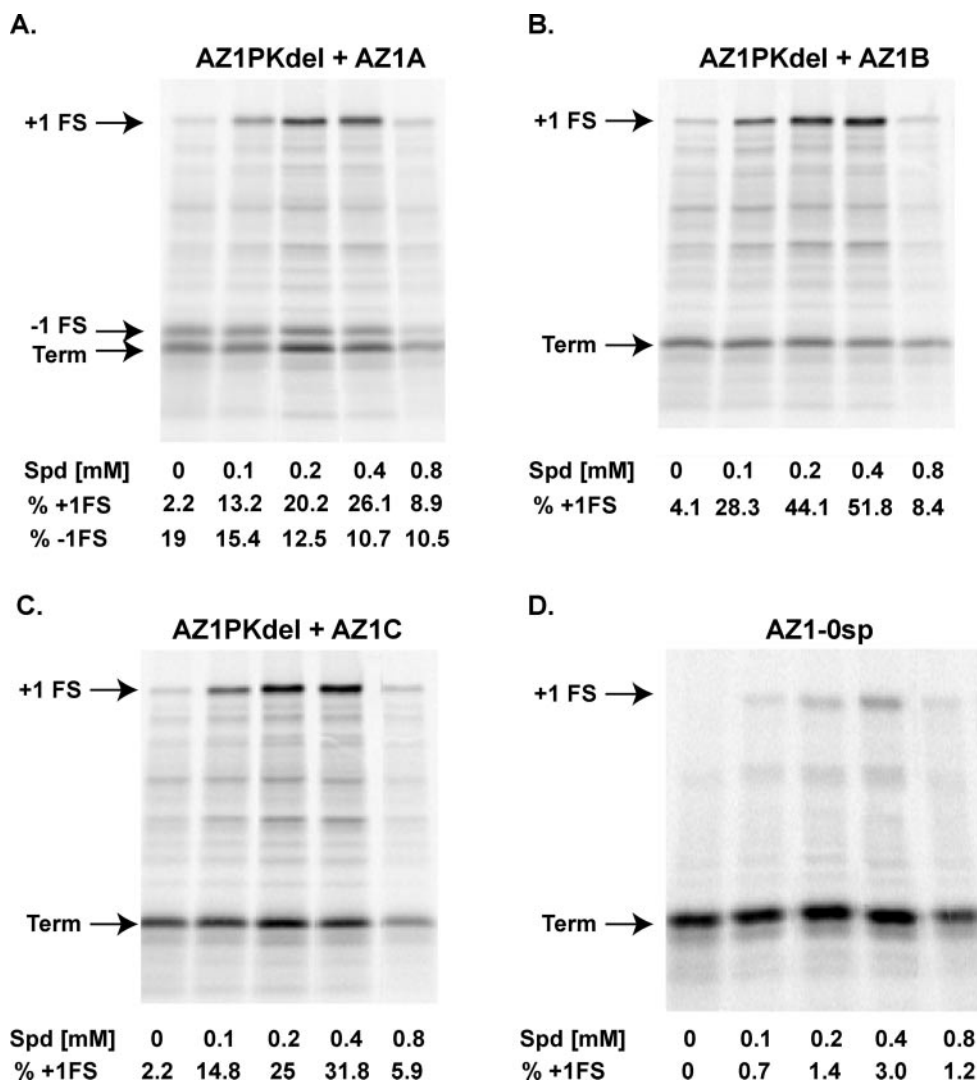


Figure 3. Antisense oligonucleotide stimulators of frameshifting. Plasmids p2Luc-AZ1PKdel (AZ1PKdel) or p2luc-AZ1-0sp (AZ1-0sp) were transcribed and translated in rabbit reticulocyte lysate in the absence or presence of increasing amounts of spermidine (final concentration indicated in mM). Either 2'-O-Methyl antisense oligonucleotide AZ1A (A), AZ1B (B) or AZ1C (C) was added to the transcription and translation reactions at 2 μ M final concentration. No antisense oligonucleotide was added to reactions with AZ1-0sp (D). SDS-PAGE of 35 S-methionine-labeled protein products from transcription and translation reactions is shown. The efficiency of frameshifting (% FS) is indicated.

pseudoknot could induce -1 frameshifting when located in the equivalent position. To address this, a new construct p2luc-AZ1-0sp (Figure 1A) was made by deleting the 3 nt spacer between the pseudoknot and the shift site of p2luc-AZ1wt. In this case, the wild-type pseudoknot is directly 3' adjacent to the shift site. The products of *in vitro* transcription and translation were separated by SDS-PAGE. No -1 frameshift product was observed and levels of the +1 frameshift product were significantly reduced to $\sim 3\%$ (Figure 3D and Supplementary Table 2).

AZ1A, AZ1B and AZ1C were designed to complement RNA sequences encoded by the originating vector. To determine if duplexes formed between the antisense oligonucleotide and 3' adjacent antizyme sequences would result in more efficient frameshift stimulation, reporter vectors were designed to contain a portion of the antizyme 3' stimulator. Construct p2luc-AZ1PKm1 contains sequences from the 5' half of the axis formed by the stacking of stem 1 and stem

2 of the pseudoknot (Figure 1A). Two complementary 2'-O-Methyl antisense oligonucleotides were designed. First, PKm1 has perfect complementarity to the region starting 3 nt and ending 21 nt downstream of the UGA shift site codon. Second, PKm2 is the same except that a mispaired C and bulged A were located at positions 9 and 16, respectively. These two alterations were included to more closely mimic the natural pseudoknot which also contains a mispaired C and bulged A at equivalent positions along the extended stem formed by the stacking of pseudoknot stems 1 and 2 (Figure 1; compare p2luc-AZ1wt with the duplex formed between p2luc-PKm1 and antisense oligonucleotide PKm2). PKm1 and PKm2 induced 30 and 22% frameshifting, respectively, when added to coupled transcription and translation reactions of p2luc-AZ1PKm1 in the presence spermidine (Figure 4A and B, and Supplementary Table 4). Neither PKm1 nor Pkm2 induced frameshifting to the same levels seen with AZ1B, suggesting that the sequence content

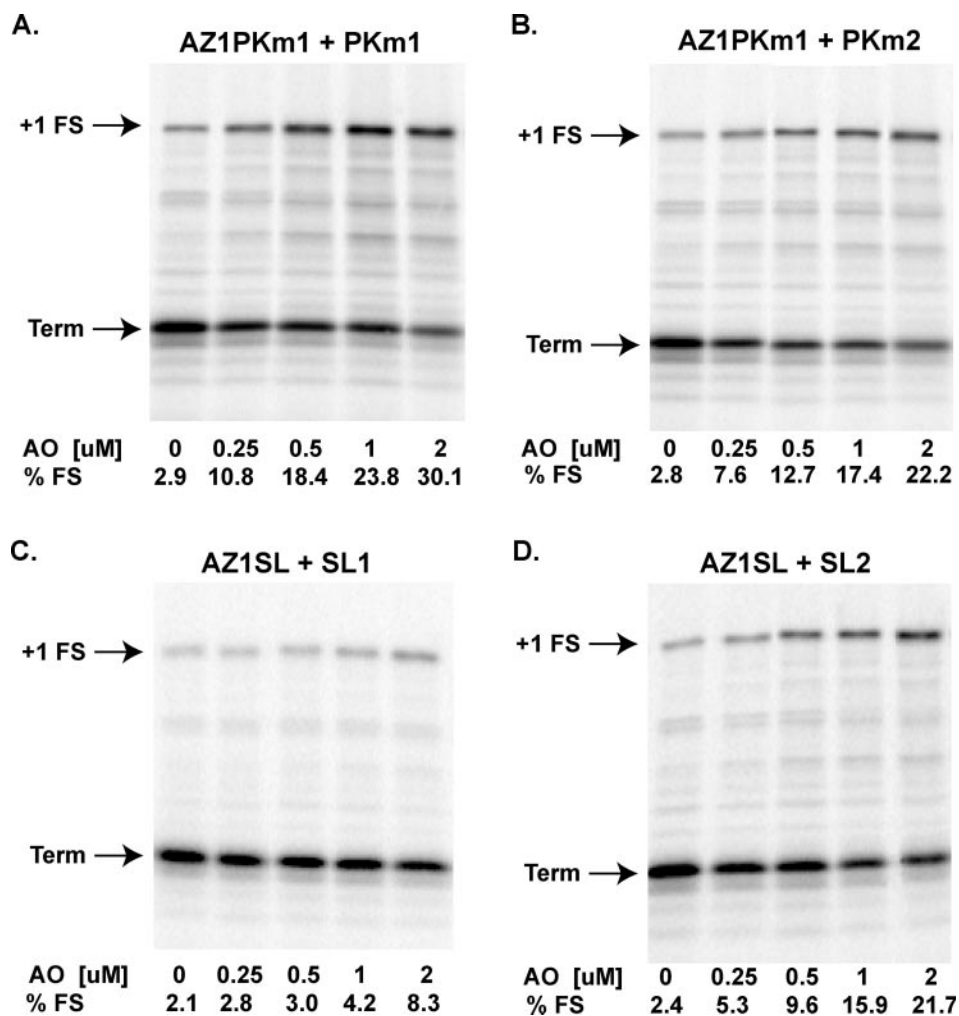


Figure 4. Sequence effect 3' of the shift site on 2'-O-Methyl induced frameshifting. Plasmids p2Luc-AZ1PKm1 (AZ1PKm1) (A and B) or p2Luc-AZ1sl (AZ1SL) (C and D) were transcribed and translated in rabbit reticulocyte lysate in the presence of 0.4 mM spermidine. Either 2'-O-Methyl antisense oligonucleotide PKm1 (A), PKm2 (B), SL1 (C) or SL2 (D) was added to the transcription and translation reactions at increasing concentrations. Final concentration of the antisense oligonucleotide (AO) is indicated in μM. SDS-PAGE of ³⁵S-methionine-labeled protein products from transcription and translation reactions is shown. The efficiency of frameshifting (% FS) is indicated.

of the duplex region can affect the efficiency of frameshift stimulation and that native antizyme sequences are not required.

A second construct, p2Luc-AZ1sl, was designed to contain only the 5' half of stem 1 of the antizyme pseudoknot downstream from the UCC UGA shift site (Figure 1A). 2'-O-Methyl antisense oligonucleotides were designed to anneal between 3 and 15 nt (SL1) or 3 and 22 nt (SL2) downstream from the UGA codon of the shift site. Frameshift efficiency induced by these two antisense oligonucleotides, 8 and 22% respectively, was somewhat lower than that observed with PKm1 and PKm2 (Figure 4C and D and Supplementary Table 4). In these cases frameshift efficiency was higher for the longer antisense oligonucleotide (SL2), suggesting that frameshift efficiency most probably correlates with stability of the duplex. As was seen with AZ1A, AZ1B and AZ1C, frameshifting efficiency stimulated by antisense oligonucleotides PKm1, PKm2, SL1 and SL2 was also strongly correlated with the concentration of exogenously added spermidine (Supplementary Table 5).

Trans-acting stimulators of frameshifting at a simple sequence, UCC UGA

The importance of the antizyme 5' sequence context to antisense oligonucleotide induced ribosome frameshifting was examined by testing the frameshift site, UCC UGA, without the 5' and 3' stimulatory antizyme sequences. To this end, the 5' antizyme stimulatory sequences were deleted from p2Luc-AZ1PKdel to make p2Luc-AZ1FS. Each of the antisense oligonucleotides AZ1A, AZ1B or AZ1C was added to coupled transcription and translation reactions with p2Luc-AZ1FS in the presence or absence of spermidine. Frameshift efficiency was measured at 11, 8 and 4%, in the presence of spermidine and 3, 0.4 and 0.2% in its absence for AZ1A, AZ1B and AZ1C, respectively (Figure 5A and B).

To determine whether the stop codon of the shift site is essential for frameshifting, the UGA codon of p2Luc-AZ1FS was altered to UGG such that the shift site was UCC UGG (p2Luc-AZ1-UGG). Frameshift efficiency was significant, but reduced, compared to the shift site UCC UGA, and

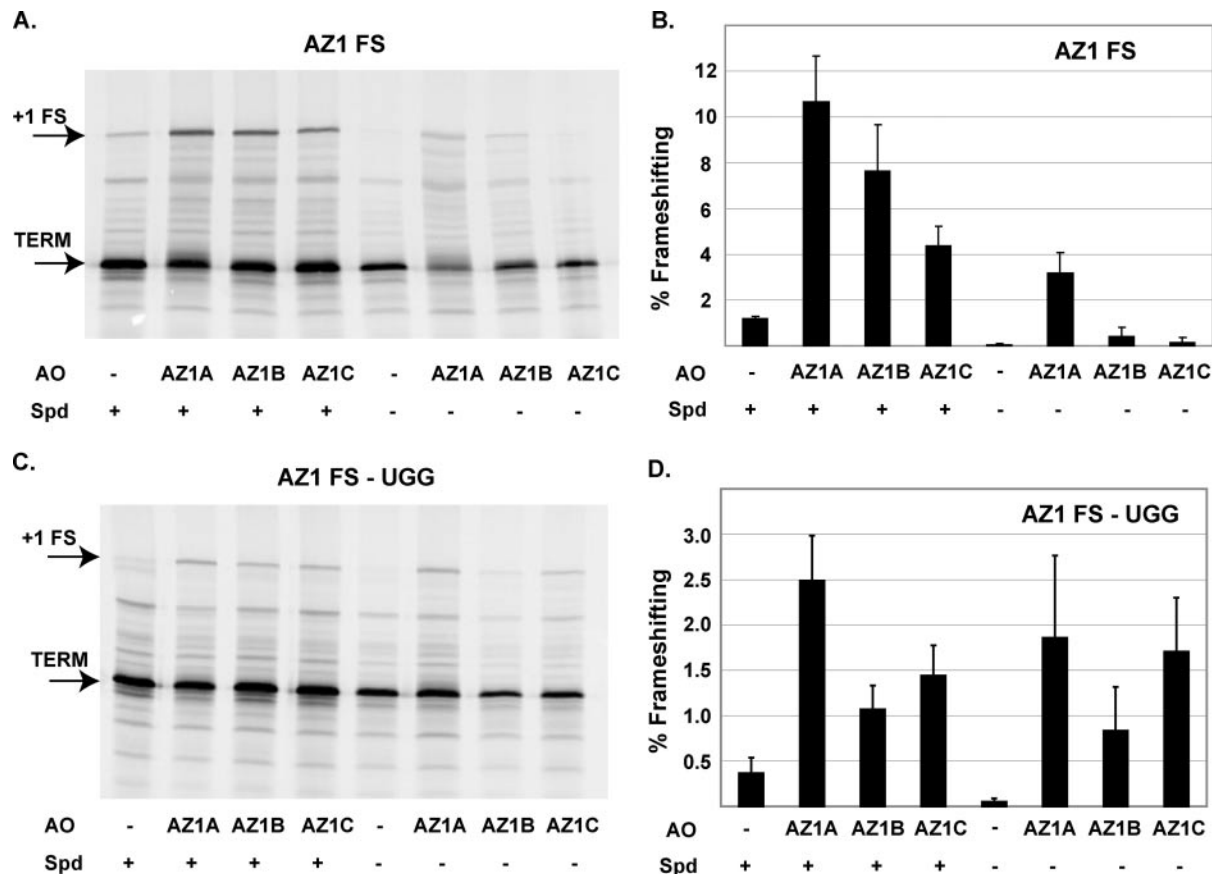


Figure 5. Sequence effect 5' of the shift site on 2'-O-Methyl induced frameshifting. Plasmids p2Luc-AZ1FS (AZ1 FS) (A and B) or p2Luc-AZ1FS-UGG (AZ1FS-UGG) (C and D) were transcribed and translated in rabbit reticulocyte lysate in the presence or absence of 0.4 mM spermidine. Either 2'-O-Methyl antisense oligonucleotide AZ1A, AZ1B or AZ1C were present. SDS-PAGE of ³⁵S-methionine-labeled protein products from transcription and translation reactions is shown for AZ1FS (A) and AZ1FS-UGG (C). The efficiency of frameshifting (% FS) is indicated.

shows little stimulation by the addition of spermidine; AZ1A, AZ1B and AZ1C induced 3, 1 and 1.4% frameshifting in the presence of spermidine, and 1.9, 0.8 and 1.7% frameshifting in its absence, respectively (Figure 5C and D).

Trans-acting stimulators of frameshifting in cultured mammalian cells

The ability of antisense oligonucleotides to induce frameshifting in cultured mammalian cells was examined by co-transfection of CV-1 cells with p2LucAZ1PKdel and increasing amounts of 2'-O-Methyl antisense oligonucleotides AZ1B as described in Materials and Methods. In the absence of antisense oligonucleotide frameshifting levels were determined to be 1.1%, whereas a graded increase in frameshift levels was observed upon the addition of AZ1B (Figure 6). Maximal frameshifting levels were 13% in the presence of 2 μ M AZ1B in the transfection media.

DISCUSSION

Several models attempting to explain pseudoknot stimulation of programmed -1 frameshifting have been proposed [for reviews see (18,19)]. Most models invoke a pausing mechanism whereby the ribosome is paused over the shift site such that time is allowed for the tRNAs to reposition in the new

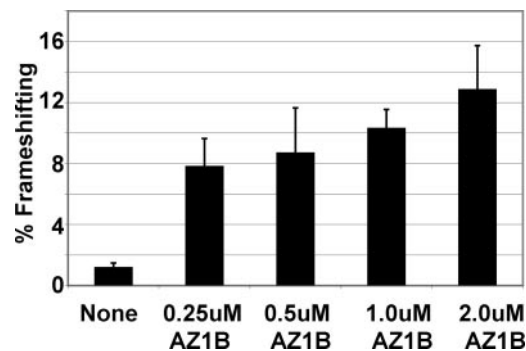


Figure 6. Antisense induced frameshifting in cultured mammalian cells. Plasmid p2LucAZ1PKdel was transfected into cultured CV-1 cells along with increasing amounts of 2'-O-Methyl antisense oligonucleotide AZ1B. The cells were incubated for 20 h and the percent frameshifting was determined by assaying firefly and *Renilla* luciferase activity in cell lysates as described in Materials and Methods.

reading frame. This explanation is clearly too simplistic as stem-loops and pseudoknots of similar thermodynamic stability that cause ribosome pausing are not necessarily effective frameshift stimulators (47-49). In addition, variations of the IBV pseudoknot have demonstrated a lack of correlation between the extent of pausing and the efficiency of frameshifting (47). A recent publication by Brierley

and co-workers (50) presents structural data demonstrating that the IBV frameshift stimulating pseudoknot blocks the mRNA entrance tunnel and leads to a structural deformation of the P-site tRNA. The resulting movement of the tRNA displaces the anticodon loop towards the 3' end of the mRNA. A model is presented in which this movement results in disruption of the codon–anticodon interactions, thus allowing for tRNA slippage relative to the mRNA. Similar tRNA movements were not observed with non-frameshift stimulating stem–loop structures. This model provides a feasible mechanistic explanation for the ability of some downstream structures to induce frameshifting.

The ability of antisense oligonucleotides to induce high-level –1 frameshifting (29,30) demonstrates that elaborate tertiary structures are not required, and that a duplex formed by complementary antisense oligonucleotides (with a variety of chemistries, including RNA, 2'-O-Methyl, morpholino) is sufficient to induce high-level frameshifting. Here we demonstrate for the first time that *trans*-acting antisense oligonucleotides may stimulate ribosome shifting to the +1 reading frame at surprisingly high levels, levels which are greater than those achieved by natural 3' *cis*-acting mRNA pseudoknot structures in programmed +1 frameshifting.

Structural studies indicating that the mRNA begins to enter the ribosome 7–9 nt downstream from the A-site codon is of direct relevance to this study (50,51). Our results indicate that maximal frameshifting is induced when the antisense–mRNA duplex begins 3 nt downstream of the UGA of the shift site, in agreement with the distance found between the UGA of the shift site and the beginning of stem 1 of the pseudoknot stimulator found in antizyme genes. Given this distance, the implication is that the stimulatory secondary structure would be encountered by the ribosome when the UCC codon enters the A-site of the ribosome. Perhaps as suggested by the structural studies of the IBV-1 frameshift inducing pseudoknot, the codon–anticodon interactions between the UCC codon and Ser-tRNA^{Ser} are disrupted during translocation to the P-site. Given the importance of the UGA codon during frameshifting at the UCC UGA shift site, subsequent events following translocation of the UCC codon to the P-site and UGA to the A-site must influence frameshifting efficiency. This latter event most probably involves competition between termination and +1 frame decoding when the UGA codon is in the A-site. Various discussions have been presented for the importance of A-site and P-site events during ribosomal frameshifting (7,52) and clearly, further investigations of this topic are warranted.

The observation presented here that the antisense oligonucleotide, AZ1A, which anneals directly adjacent to the UGA stop codon can induce ribosome frameshifts to either the +1 or –1 reading frame is surprising. In light of the above discussion of spacing for naturally occurring *cis*-acting frameshift stimulators, it is possible that frameshifting may occur at codons upstream of the known UCC UGA shift site. However, visual examination of upstream codons does not reveal an obvious –1 or +1 frameshift site.

The ability of spermidine to stimulate antisense oligonucleotide induced ribosome frameshifting to the +1 reading frame at the UCC UGA shift site in the absence of the natural 3' stimulator demonstrates that this *cis*-acting element is not required for polyamine responsiveness. Similarly, spermidine

stimulation was observed in the absence of the 5' element but virtually eliminated by altering the UGA codon of the shift site to UGG. These observations are in agreement with previous studies examining the importance of *cis*-acting elements for polyamine induced frameshifting during expression of antizyme genes (39–41).

Finally, the ability to direct ribosomes to the +1 reading frame in living cells (Figure 6) suggests a potential therapeutic application for antisense oligonucleotides. Directed frameshifting to the +1 reading frame near a disease causing –1 frameshift mutation would cause some ribosomes to resume decoding in the wild-type ORF, thus restoring partial production of full-length protein from mutant alleles. The importance of the stop codon for efficient frameshifting suggests that the stop codon following the frameshift mutation presents a promising target for antisense induced phenotypic suppression, and that modulation of intracellular polyamine levels, although not essential, may increase the effectiveness of this approach. Further experiments are required to determine the therapeutic potential of this approach *in vivo* including the generality and efficiency of frameshift induction at non-programmed frameshift sites.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

The authors would like to thank Drs Pasha Baranov, John Atkins and Lorin Petros for critical reading of the manuscript. This project was funded by an MDA Development grant and NIH R21NS051792 to M.T.H. Funding to pay the Open Access publication charges for this article was provided by NIH R21NS051792.

Conflict of interest statement. None declared.

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