Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 3959–3963, April 1994
Biochemistry

Polyamines regulate the expression of ornithine decarboxylase antizyme *in vitro* by inducing ribosomal frame-shifting

ERAN ROM AND CHAIM KAHANA*

Department of Molecular Genetics and Virology, Weizmann Institute of Science, Rehovot 76100, Israel

Communicated by Daniel Nathans, December 29, 1993

ABSTRACT **We provide here an example of a mammalian cellular gene expressed by frame-shifting. Conventional reading of the sequence of ornithine decarboxylase-antizyme mRNA (a protein that modulates the rate of ornithine decarboxylase degradation) results in premature termination at an in-frame termination codon (stop-1), located shortly after the initiation codon. By translating, *in vitro* in reticulocyte lysate, antizyme mRNA with a full coding capacity and various mutants derived from it, we demonstrate that antizyme expression requires that ribosomes shift from the first open reading frame (termed ORF_0) to a second +1 open reading frame (ORF_1). Our studies show that this frame-shifting, which occurs at maximal efficiency of ≈20%, is stimulated by polyamines and requires the functional integrity of the stop codon (stop-1) of ORF_0. By introducing in-frame deletions, we have shown that an 87-nt segment surrounding stop-1 enhances frame-shifting efficiency, whereas the 6 nt located just upstream to stop-1 are absolutely essential for this process. Because this segment does not contain sequences that were previously characterized as shifty segments, our results suggest that another mechanism of frame-shifting is involved in mediating antizyme expression.**

Gene expression is regulated at several control levels, including transcription initiation and elongation, RNA processing and modification, and translation. In the past several years, another regulatory mechanism has been demonstrated that involves the synthesis of a single protein from two different reading frames on an mRNA template. The combining of the two reading frames requires that the ribosome will shift from one reading frame to another (1–4). This event, the spontaneous occurrence of which is extremely low (5), is termed programmed frame-shift because it is programmed by a specific primary sequence of the mRNA and sometimes also by secondary or tertiary structure (3, 4). Such sequences of the mRNA can program translational shifts with variable efficiencies (for review, see refs. 4–7). Ribosomal frame-shifting is governed by two elements. A recording site (6), in which the reading frame is shifted upstream or downstream, and a stimulator site (4), which increases the efficiency of recoding. Most frame-shifting events are the result of a −1 or +1 shift relative to the original reading frame (3, 4). However, a similar event in which the shift is of multiple nucleotides (translational hop) was also described (4, 8, 9). With one recent exception (10), in all described frame-shifts and translational hops, the recoding segment is composed of alternative cognate or near-cognate codons, thus allowing the ribosome-bound tRNA to slip from the first open reading frame (ORF) to the second.

Interestingly, most of the documented frame-shifts and translational hops have been demonstrated in viral genes (2, 3). Presently, only four cellular genes expressed by frame-shifting (11–16) or hopping (9, 17, 18) have been characterized, all of them in *Escherichia coli*.

Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the pathway of polyamine biosynthesis in mammalian cells (19). ODC, which has the shortest intracellular half-life monitored in mammalian cells (20), is negatively regulated by its products, the polyamines, via translational (21–23) and posttranslational (24, 25) mechanisms. Polyamines have been demonstrated to induce a 24-kDa protein, termed antizyme, which binds to ODC and inhibits its activity in a stoichiometric manner (26–29). More recent studies have demonstrated that antizyme is actually involved in mediating the degradation of ODC (30–32). Because induction of antizyme by polyamines is sensitive to cycloheximide, but not to actinomycin D, it was suggested that antizyme expression is controlled posttranscriptionally (29, 33).

While inspecting the recently published sequence of the rat antizyme gene (34), we noted that translation initiating at the first ATG codon should terminate 68 amino acids thereafter at an in-frame TGA termination codon (stop-1) (Fig. 1). The resulting ORF, termed ORF_0, encodes a polypeptide completely different from the functional antizyme encoded by the previously isolated Z1Az cDNA clone (29). This Z1Az clone, which lacks a 5′ segment encoding the 15 amino-terminal amino acids, encodes a protein that is recognized by antiserum directed against antizyme (29) and stimulates ODC degradation *in vitro* (31, 35) and *in vivo* (30). Expression from the Z1Az cDNA was enabled by an initiator codon that was appended (using synthetic oligonucleotide linkers) in a +1 frame compared with that of the first ATG of the genomic clone (refs. 29 and 34; Fig. 1). A shift to a +1 frame should, therefore, occur before stop-1 to produce a full-length antizyme protein ($FLAz^{FS}$ in Fig. 1). Several cellular regulatory mechanisms could account for the production of a functional antizyme from the native antizyme gene. These include the following: transcriptional or posttranscriptional RNA editing and processing, translational initiation at an alternative initiation codon, or a ribosomal frame-shift at or before stop-1.

Here, by using an *in vitro* reticulocyte lysate-based translation system, we show that: (*i*) Translation of antizyme mRNA starts at the second ATG and not at the first one, as inferred previously (34). (*ii*) Expression of mammalian antizyme requires a +1 ribosomal frame-shift. (*iii*) Frame-shifting efficiency is regulated by the concentration of polyamines, the end products of a chain of reactions initiated by ODC.

MATERIALS AND METHODS

Construction of Antizyme cDNA with Full Coding Capacity. The available Z1Az cDNA clone lacks 15 amino-terminal amino acids and the entire 5′ noncoding region (29). Expression from Z1Az is enabled via an initiator ATG that was appended to it in a +1 frame compared with the initiation codon of wild-type antizyme mRNA (Fig. 1). We have used

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: ODC, ornithine decarboxylase; ORF, open reading frame.

*To whom reprint requests should be addressed.

```
  1 cgcgggaagtgcagcggaggtttttgctggtttcggaccccagcggccgg ATG GTG
  1                                                    M   V
                                        (Z1Az) ATG GCC CGG CCA►
 57 AAA TCC TCC CTG CAG CGG ATC CTC AAC AGC CAC TGC TTC GCC AGA
  3 K   S   S   L   Q   R   I   L   N   S   H   C   F   A   R
102 GAG AAA GAA GGG GAC AAA CGC AGC GCC ACG CTT CAC GCC AGC CGC
 18 E   K   E   G   D   K   R   S   A   T   L   H   A   S   R
147 ACC ATG CCG CTT CTT AGT CAG CAC AGC CGC GGC GGC TGC AGC AGC
 33 T   M   P   L   L   S   Q   H   S   R   G   G   C   S   S
192 GAG AGT TCT AGG GTT GCC CTT CAT TGC TGT AGT AAC CTG GGT CCG
 48 E   S   S   R   V   A   L   H   C   C   S   N   L   G   P
237 GGG CCT CGG TGG TGC TCC TGA TGTCCCTCACCCACCCCTGAAGATCCCAGGTG
 63 G   P   R   W   C   S   *
290 GGCGAGGGAACAGTCAGCGGGATCACAGTCTTTCAGCTAGCATCCTGTACTCCGACGAGC
350 GGCTGA ATG TGA CAGAGGAGCCGACGTCTAACGACAAGACCAGGGTCCTGAGCATCC
  1       M   *
407 AGTGCACGCTCACGGAGGCCAAGCAGGTCACCTGGAGGGCGGTGTGGA ATG GTG GTG
  1                                                   M   V   V
464 GCC TCT ACA TCG AGC TCC CAG CTG GGC CTC TGC CAG AAG GCA GCA
  4 A   S   T   S   S   S   Q   L   G   L   C   Q   K   A   A
509 AGG ACA GTT TTG CAG CTC TAC TAG AGTTCGCAGAGGAGCAGCTCCGGGCCGA
 19 R   T   V   L   Q   L   Y   *
561 CCACGTTTTCATCTGCTTCCCCAAGAACCGTGAGGACAGAGCCGCCCTACTCCGAACCTT
621 CAGCTTTCTTGGCTTTGAGATTGTGAGACCCGGGCATCCCCTCGTCCCCAAGAGACCCGA
681 CGCTTGCTTC ATG GTC TAC ACG CTG GAG AGA GAG GAC CCG GGT GAG
  1         M   V   Y   T   L   E   R   E   D   P   G   E
727 GAG GAC TAG gtgccagccctgcccagtgtccctgcaccctctcccgggtttgtccac
 13 E   D   *
784 atgtcatgattgtgcagaataaacgctcactccattagcggggtgcttcttcgagctgaa
844 tgctgtgtttgtcacactcaagtggtggctttaattctaaataaaggtttctattttact
904 tttttattgctgtttaagatggtcaggtgacctgttttagagcagcctcctttgaagtct
964 ggaaaaatggtgtcacctcccctggctcaaacctaataaataaagtgatctcgttc
```

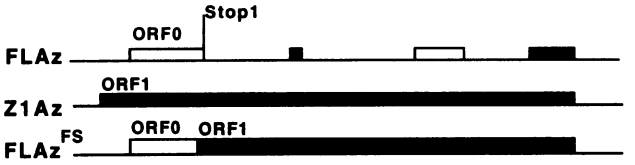

FIG. 1. Schematic representation of the ORFs of antizyme mRNA. The sequence of the five exons of the antizyme gene (34) was combined and is presented at top. The resulting ORFs are presented by amino acid sequence (*Upper*) and schematically (FLAz, *Lower*). The sequence of the 5′ end of the Z1Az clone is denoted by italic letters. Sequences of the two oligonucleotides used as primers in the PCR are underlined, the initiator ATG codon is boxed, and stop-1 is thickly underlined. For demonstration purpose, cytidine 240 (denoted by a black dot) was removed, and the resulting ORF is presented (FLAz^{FS}, *Lower*), as is the ORF of Z1Az.

the PCR to construct a wild-type antizyme cDNA with a full coding capacity (FLAz). Two synthetic oligonucleotides complementary to nt 1215–1234 and 1391–1408 of the antizyme gene (34) (or nt 1–20 and 177–194, according to the nucleotide numbering used in Fig. 1) were used as opposing primers in a PCR, using first-strand rat cDNA as a template. A *Sac* II site was appended to the 5′ end of the upstream oligonucleotide, whereas the downstream oligonucleotide primer overlapped an internal *Sac* II site. The PCR-generated 180-bp fragment was digested with *Sac* II and cloned between the *Sac* II site of Bluescript (5′) and the internal *Sac* II site of the Z1Az cDNA clone (29, 34) (3′). Several of the resulting clones in which the insert was in the correct orientation were sequenced and were identical. One of the resulting clones, denoted FLAz, was used in the studies presented.

Construction of Mutants. Oligonucleotide-directed point mutations and deletions were introduced into FLAz cDNA cloned in the Bluescript plasmid (Stratagene), using the uracil incorporation method of site-directed mutagenesis (36). Mutations were confirmed by sequencing.

Synthesis of Antizyme and ODC in Reticulocyte Lysate. Wild-type and mutant antizyme and ODC were produced *in vitro* in a reticulocyte lysate-based translation mixture (Promega) by using *in vitro*-transcribed RNA. Synthesis of antizyme was monitored by fractionating equal-volume samples by SDS/PAGE. The relative translation efficiency of various mutant antizyme RNAs was determined by monitoring radioactivity in the individual antizyme bands with a Fujix Bas-1000 Bioimager. The ODC degradation assay was done as described (37).

RESULTS

A Polyamine-Stimulated Nonconventional Mechanism Is Required for Antizyme Expression. The PCR was used to generate an antizyme cDNA with full coding capacity. The sequence of the resulting clones (Fig. 1) demonstrated the presence of a stop codon shortly after the inferred site of translational initiation. This analysis clearly shows that a nonconservative mechanism is required for antizyme expression. Translation of RNA representing one of these clones (denoted FLAz, see Fig. 1), in reticulocyte lysate, yielded a protein that is efficiently immunoprecipitated by anti-rat antizyme antiserum (Fig. 2*A*) and which accelerates the degradation of ODC in a reticulocyte lysate-based degradation mixture (Fig. 2*B*). Interestingly, under standard translation conditions FLAz RNA is poorly translated compared with RNA representing the previously isolated truncated Z1Az cDNA clone (ref. 29 and Fig. 1), for which the appended initiation codon is in a +1 frame compared with that of FLAz (Fig. 2*C*). However, addition of spermidine (0.4 mM) to the translation mixture stimulated the production of FLAz, whereas the production of the Z1Az protein remained unchanged or even slightly reduced (Fig. 2*C*). This result shows that, as in intact cells, spermidine stimulates antizyme production in reticulocyte lysate and excludes transcriptional editing as the underlying mechanism.

Translation of Antizyme mRNA Starts at the Second ATG Codon. To identify the actual initiation codon, the first ATG of FLAz RNA was converted to AAG by using site-directed mutagenesis. The resulting mutant RNA directed efficient, but somewhat reduced, production of antizyme in spermidine-supplemented translation mixture (Fig. 3*A*, lane 2). This result suggests that translation of FLAz RNA is initiated at an alternative initiation codon. A second ATG codon is located in the same ORF, 102 nt downstream to the first one. When this ATG was converted to AAG, the resulting mutant RNA completely failed to program the production of antizyme in the reticulocyte lysate (Fig. 3*A*, lane 3). We

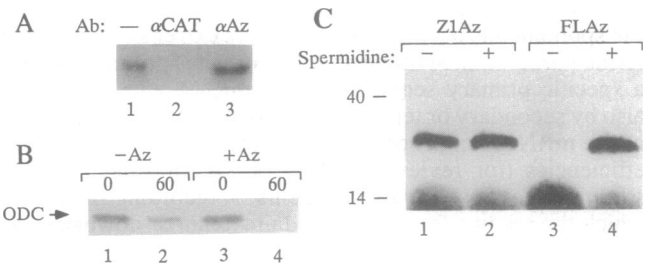

FIG. 2. Polyamines stimulate the production of antizyme *in vitro* in a reticulocyte lysate. (*A*) Reticulocyte lysate (Promega) was programmed in the presence of [^{35}S]cysteine with FLAz RNA under optimal translation conditions (see *C*). The resulting protein was fractionated by SDS/PAGE either directly (lane 1) or after immunoprecipitation with control [anti-chloramphenicol acetyltransferase (αCAT)] (lane 2) or anti-rat antizyme (αAz) (lane 3) antiserum. Ab, antibody. (*B*) [^{35}S]ODC was subjected to degradation assay in a reticulocyte-lysate-based degradation mixture, without (lanes 1 and 2) or with (lanes 3 and 4) added antizyme. Aliquots were removed after zero (lanes 1 and 3) or after 60 (lanes 2 and 4) min and fractionated by SDS/PAGE. (*C*) Reticulocyte lysate was programmed with equal amounts of Z1Az RNA (lanes 1 and 2) or FLAz RNA (lanes 3 and 4), in the presence of [^{35}S]cysteine and in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of added spermidine (0.4 mM, Sigma, molecular biology grade). Synthesized antizyme was resolved by electrophoresis in a SDS/20% polyacrylamide gel. Positions of two molecular weight markers are indicated at left.

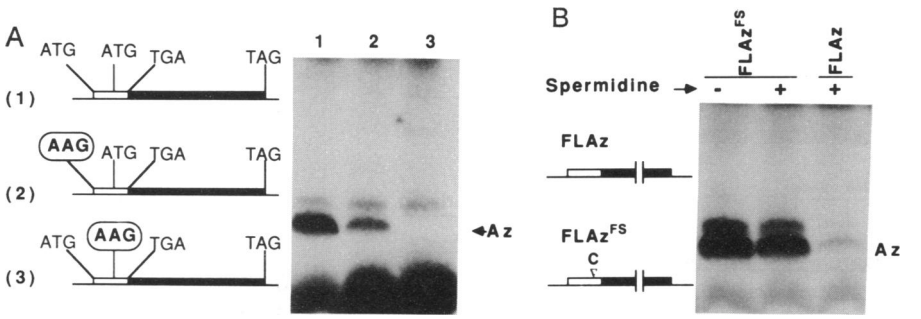

FIG. 3. Antizyme expression is initiated at the second ATG codon and requires bypassing of a nucleotide before stop-1. Wild-type and mutant FLAz RNAs were transcribed *in vitro* and translated in reticulocyte lysate in the presence of added spermidine (see the legend to Fig. 2). (*A*) The first and second ATG codons of FLAz RNA were converted to AAG. Numbers of the constructs at left correspond to the lane numbers at right. (*B*) Cytidine 241 of FLAz was deleted to yield the $FLAz^{FS}$ mutant. The ability of $FLAz^{FS}$ RNA to program antizyme production in the presence and absence of added spermidine was compared with the production of antizyme in a lysate programmed with wild-type FLAz RNA in the presence of added spermidine.

therefore conclude that translation of antizyme mRNA initiates at this second ATG codon (boxed in Fig. 1) and not at the first one, as inferred previously (34). Moreover, because this initiator ATG is located in the same ORF, a +1 shift in the translation frame should occur between this initiator ATG and the stop codon of ORF_0 (stop-1) so that the full-length antizyme protein will be produced. Indeed, deletion of a single nucleotide (cytidine 240, denoted by a black dot in Fig. 1) yielded $FLAz^{FS}$ RNA (see Fig. 1) that was efficiently translated, both under standard translation conditions and in the presence of added spermidine ($FLAz^{FS}$ in Fig. 3*B*). These results indicate that spermidine promotes editing or frameshifting during translation of wild-type FLAz mRNA. By comparing the amount of antizyme synthesized in reticulocyte lysate programmed with wild-type FLAz mRNA and its deregulated $FLAz^{FS}$ derivative (in the presence of optimal concentration of spermidine) (Fig. 3*B*), we have estimated that bypassing of stop-1 occurs at an efficiency of ≈20%.

The Functional Integrity of the ORF_0 Stop Codon Is Required for Antizyme Expression. Because in the best-studied cases frameshifting has been shown to occur at or near a stop codon (3), we set out to determine whether stop-1 is required for antizyme expression. To do so, stop-1 was converted into four different sense codons, TCA, TGC, TGG, and TTC, and into the two other stop codons, TAA and TAG. Converting stop-1 to the serine-encoding TCA codon severely inhibited antizyme production, whereas its conversion to the three other sense codons, TGC, TGG, and TTC, completely abolished expression (Fig. 4). In contrast, converting stop-1 to the other two stop codons did not affect antizyme production

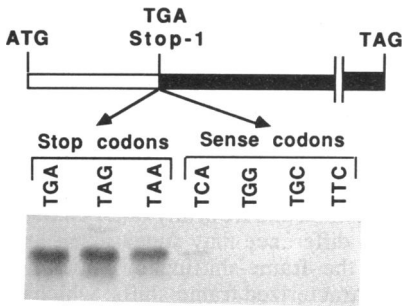

FIG. 4. Translation termination at stop-1 is required for frameshifting. The TGA stop codon of ORF_0 (stop-1) was converted to the four sense codons TCA, TGG, TGC, and TTC and to the two other stop codons TAA and TAG. Wild-type and mutant RNAs were transcribed *in vitro* and translated in reticulocyte lysate in the presence of [^{35}S]cysteine and added spermidine (0.4 mM). After 60 min of incubation the material was fractionated in a SDS/20% polyacrylamide gel and visualized by autoradiography.

(Fig. 4). We therefore suspect that antizyme expression is mediated by frame-shifting and that ribosomal pausing at the stop codon of ORF_0, but not the specific type of stop codon, is required for frame-shifting.

Expression of Antizyme Is Mediated by Ribosomal Frameshifting. Although, the requirement for a functional stop codon argues in favor of frame-shifting as the mechanism that enables antizyme expression, it does not completely exclude editing as the underlying mechanism. To distinguish between these possibilities, two cytidine residues (333 and 334) were deleted from the deregulated $FLAz^{FS}$ RNA. The resulting RNA, denoted $FLAz^{FS}\Delta CC$ (Fig. 5*A*), could produce antizyme only if an additional nucleotide is removed due to editing activity before stop-1. Frame-shifting cannot occur in this deregulated RNA, as the ribosomes do not encounter stop-1. Without editing, a 7.7-kDa polypeptide will be produced due to termination at position 378. However, deletion of a nucleotide before stop-1 by editing activity will lead to premature termination at position 275, yielding a 4.1-kDa polypeptide that is undetectable under our assay condition. Therefore, thymidine 275 was changed to adenine to enable the production of a full-length antizyme upon possible editing of the mRNA. Fig. 5*B* shows that only the 7.7-kDa polypeptide was programmed by $FLAz^{FS}\Delta CC$ RNA, demonstrating that the RNA remained unedited. To exclude the possibility that changing thymidine 275 to adenine interfered with such potential editing, the same alteration was introduced also into wild-type FLAz RNA. The resulting FLAz T276A RNA was as efficient as wild-type FLAz RNA in programming the production of antizyme in the lysate (Fig. 5*B*). On the basis of these observations, we conclude that antizyme expression is regulated by frame-shifting and is not regulated by editing.

Identification of Sequences Required for Efficient Frameshifting. A set of deletions were introduced into FLAz, covering the region located between the initiator ATG up to 96 nt downstream to stop-1, and the corresponding RNAs were translated in spermidine-supplemented reticulocyte lysate (Fig. 6). This analysis revealed that a 87-nt segment encompassing nt 237–324 is important for antizyme expression; nt 209–214, located just upstream to stop-1, were absolutely essential (Fig. 6). These results suggest that frameshifting occurs just upstream to stop-1. However, determination of the precise site of frame-shifting must await sequencing of the antizyme protein. Our preliminary experiments also suggest that the 87-nt segment suffices to subvert the ribosomal reading frame during translation of heterologous mRNA (data not shown).

DISCUSSION

Here we demonstrate that the mammalian cellular gene encoding ODC antizyme, a protein that mediates the degra-

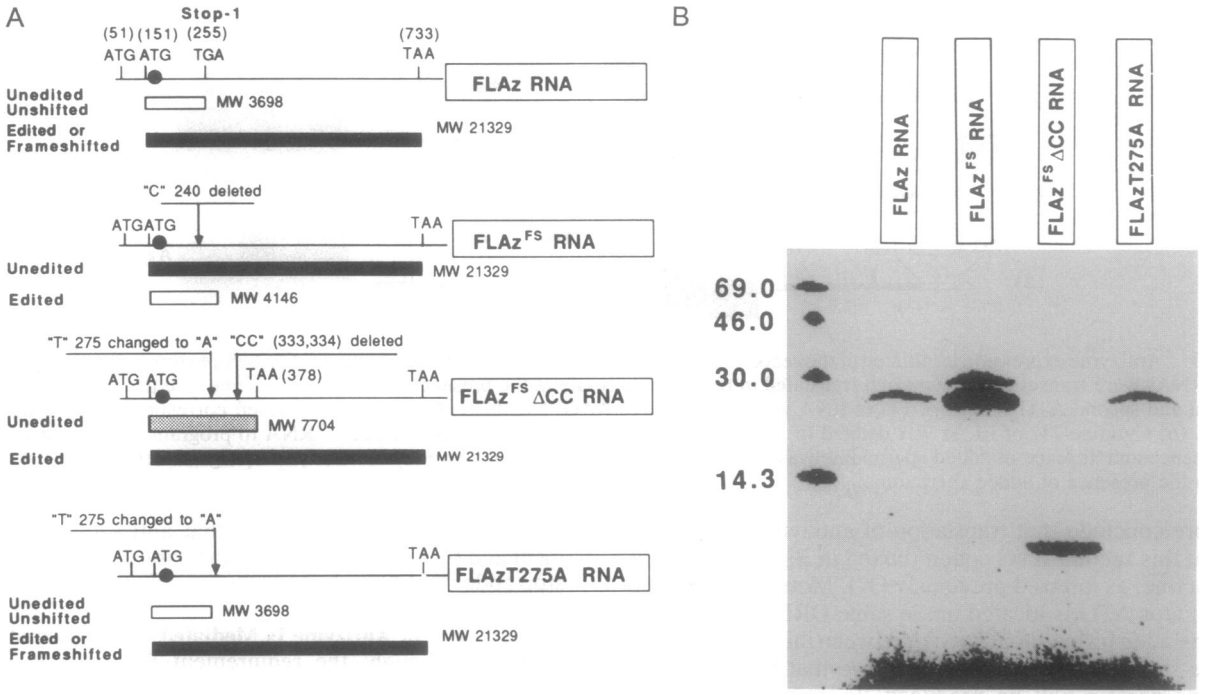

FIG. 5. Antizyme is expressed by frame-shifting but is not expressed by editing. (*A*) Two cytidine residues (333 and 334) were deleted from FLAz^{FS}, generating FLAz^{FS}ΔCC RNA. Without editing before stop-1, translation of this RNA will yield a 7.7-kDa polypeptide. Because editing will lead to the production of an undetectable 4.1-kDa polypeptide due to a stop codon present at position 275, thymidine 275 was altered to adenine, thus enabling the production of full-length antizyme. To control for possible negative effect of the change of thymidine 275 to adenine, the same change was introduced also into wild-type FLAz RNA, giving rise to FLAzT276A RNA. To improve detection of the resulting proteins, four methionine codons were implanted into each of these four antizyme RNAs between amino acids 8 and 9 (denoted by black dot). Molecular weight markers are on left. (*B*) The four antizyme RNAs described in *A* were translated in reticulocyte lysate supplemented with 0.4 mM spermidine and [^{35}S]methionine. After translation, the material was fractionated electrophoretically in an SDS/20% polyacrylamide gel.

dation of ODC (30, 32), is expressed by frame-shifting. Moreover, the efficiency at which this frame-shifting occurs is regulated by polyamines. Inspection of the primary sequence of the antizyme gene (34) revealed that conventional decoding cannot yield a full-length antizyme protein because of an in-frame stop codon located 35 amino acids after the initiation codon. As we demonstrate here, polyamines induce antizyme expression by enabling the ribosomes to shift from one ORF (ORF_0) to another (ORF_1). On the basis of our results, we conclude that at low polyamine concentration, translation of antizyme mRNA stops predominantly at codon 36. At high concentration of polyamines, a higher proportion of ribosomes shift into a +1 reading frame, resulting in the production of a complete functional 194-residue antizyme protein. Although the evidence provided here was obtained *in vitro* in a reticulocyte lysate-based translation mixture, it agrees with previous studies showing that polyamines regulate antizyme production in cells by a posttranscriptional mechanism (29, 33).

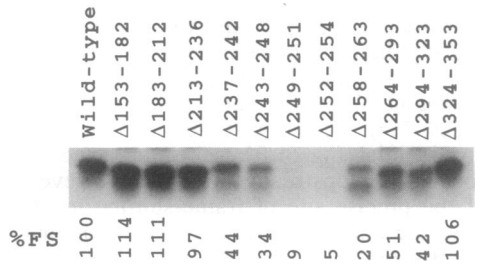

FIG. 6. Characterization of mRNA sequences required for efficient frame-shifting. Oligonucleotide-directed deletions were introduced into FLAz cDNA (see top and Fig. 1 for numbers of deleted nucleotides). The corresponding *in vitro* transcribed RNAs were translated in reticulocyte lysate supplemented with spermidine. Frame-shifting efficiency (FS) as percentage of wild-type (100%) is presented.

Most of the characterized frame-shifts have been found in prokaryotic and eukaryotic viral genes (2, 3). Only four cellular genes have been demonstrated to be expressed by frame-shifting, all of them in *E. coli* (9, 11–18). As shown here, the mammalian cellular ODC-antizyme gene is expressed by polyamine-regulated frame-shifting. In this respect, it resembles the only other described case of regulated frame-shifting that occurs during the expression of the *E. coli* gene encoding the peptide-chain release factor 2 (12, 38). Although, as for the +1 frame-shift of release factor 2, our results suggest that the frame-shift of antizyme may also occur at or just upstream to the stop codon of ORF_0, determination of the precise site at which frame-shifting occurs must await sequencing of the antizyme protein. Such sequence analysis could also discriminate between the related processes of frame-shifting and translational hopping (9, 39).

Our results suggest that the shifty segment of antizyme mRNA is located within the 9-nt segment TGC-TCC-TGA encoding amino acids 34, 35, and stop-1. If this is the case, the shifty segment of antizyme differs from that of peptide-chain release factor 2 and from any other characterized shifty sequence. This difference may suggest that another mechanism controls the frame-shifting of antizyme. Moreover, unlike other characterized frame-shifts, which involve tRNA slippage between cognate or near-cognate codons in the mRNA (for review, see ref. 40), the sequence of the putative shifty segment of antizyme mRNA does not seem to allow tRNA slippage. There is presently only one documentation of a nonslippery frame-shifting between the *GAG3* and *POL3* genes of the retrotransposon Ty*3* of yeast (10). This recent study showed the importance of the last decoding tRNA of the first ORF for frame-shifting, suggesting that interaction

between this tRNA and rRNA, mRNA, or the first incoming tRNA of the downstream ORF may mediate this frame-shifting event (10). Determination of the precise site of frame-shifting within the antizyme protein will reveal whether such interactions are also important for the occurrence of the ribosomal frame-shifting that permits antizyme expression.

The ability of polyamines, the end product of the chain of reactions initiated by ODC, to induce the production of antizyme, a mediator of ODC degradation, is an unusual and interesting regulatory loop that controls gene expression. Presently, how polyamines subvert the ribosomal mechanism for maintaining the reading frame is unclear. Based on the ability of polyamines to interact with nucleic acids and our present results, it is possible that polyamines are involved in remodeling the extensive secondary structures that are noted [by computerized modeling (41)] around the site of frame-shifting into an alternative structure that permits the ribosomes to shift into the +1 frame. Alternatively, polyamines may alter interactions between mRNA sequences and the two tRNAs involved in frame-shifting.

We thank S.-I. Hayashi for the Z1Az clone and for anti-rat antizyme antiserum. This study was supported by the Israel Academy of Science and Humanities and by the Minerva Foundation. C.K. is a recipient of a Career Development Award from the Israel Cancer Research Fund.

1. Craigen, W. J. & Caskey, C. T. (1987) *Cell* **50**, 1–2.
2. Varmus, H. (1988) *Science* **240**, 1427–1435.
3. Parker, J. (1989) *Microbiol. Rev.* **53**, 273–298.
4. Atkins, J. F., Weiss, R. B. & Gesteland, R. F. (1990) *Cell* **62**, 413–423.
5. Kurland, C. G. (1992) *Annu. Rev. Genet.* **26**, 29–50.
6. Gesteland, R. F., Weiss, R. B. & Atkins, J. F. (1992) *Science* **257**, 1640–1641.
7. Jacks, T. (1990) *Curr. Top. Microbiol. Immunol.* **157**, 93–124.
8. Huang, W. M., Ao, S.-Z., Casjens, S., Orlandi, R., Zeikus, R., Weiss, R., Winge, D. & Fang, M. (1988) *Science* **239**, 1005–1012.
9. Benhar, I. & Engelberg, K. H. (1993) *Cell* **72**, 121–130.
10. Farabaugh, P. J., Zhao, H. & Vimaladithan, A. (1993) *Cell* **74**, 93–103.
11. Craigen, W. J., Cook, R. G., Tate, W. P. & Caskey, C. T. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 3616–3620.
12. Craigen, W. J. & Caskey, C. T. (1986) *Nature (London)* **322**, 273–275.
13. Blinkowa, A. L. & Walker, J. R. (1990) *Nucleic Acids Res.* **18**, 1725–1729.
14. Flower, A. M. & McHenry, C. S. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 3713–3717.
15. Sekine, Y., Nagasawa, H. & Ohtsubo, E. (1992) *Mol. Gen. Genet.* **235**, 317–324.
16. Sekine, Y. & Ohtsubo, E. (1992) *Mol. Gen. Genet.* **235**, 325–332.
17. Benhar, I. & Engelberg, K. H. (1991) *Gene* **103**, 79–82.
18. Benhar, I., Miller, C. & Engelberg, K. H. (1992) *Mol. Microbiol.* **6**, 2777–2784.
19. Heby, O. & Persson, L. (1990) *Trends Biochem. Sci.* **15**, 153–158.
20. Dice, F. J. (1990) *Semin. Cell Biol.* **1**, 411–413.
21. Kahana, C. & Nathans, D. (1985) *J. Biol. Chem.* **260**, 15390–15393.
22. Persson, L., Holm, I. & Heby, O. (1986) *FEBS Lett.* **205**, 175–178.
23. Holm, I., Persson, L., Stjernborg, L., Thorsson, L. & Heby, O. (1989) *Biochem. J.* **258**, 343–350.
24. Ghoda, L., Sidney, D., Macrae, M. & Coffino, P. (1992) *Mol. Cell. Biol.* **12**, 2178–2185.
25. Li, X. & Coffino, P. (1992) *Mol. Cell. Biol.* **12**, 3556–3562.
26. Fong, w. F., Heller, J. S. & Canellakis, E. S. (1976) *Biochim. Biophys. Acta.* **428**, 456–465.
27. Heller, J. S., Fong, W. F. & Canellakis, E. S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1858–1862.
28. Matsufuji, S., Kanamoto, R., Murakami, Y. & Hayashi, S.-I. (1990) *J. Biochem.* **107**, 87–91.
29. Matsufuji, S., Miyazaki, Y., Kanamoto, R., Kameji, T., Murakami, Y., Baby, T. G., Fujita, K., Ohno, T. & Hayashi, S. (1990) *J. Biochem. (Tokyo)* **108**, 365–371.
30. Murakami, Y., Matsufuji, S., Miyazaki, Y. & Hayashi, S. (1992) *J. Biol. Chem.* **267**, 13138–13141.
31. Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., Tanaka, K. & Ichihara, A. (1992) *Nature (London)* **360**, 597–599.
32. Li, X. & Coffino, P. (1993) *Mol. Cell. Biol.* **13**, 2377–2383.
33. Murakami, Y. & Hayashi, S. (1985) *Biochem. J.* **226**, 893–896.
34. Miyazaki, Y., Matsufuji, S. & Hayashi, S. (1992) *Gene* **113**, 191–197.
35. Murakami, Y., Tanaka, K., Matsufuji, S., Miyazaki, Y. & Hayashi, S. (1992) *Biochem. J.* **283**, 661–664.
36. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 488–492.
37. Bercovich, Z., Rosenberg, H. Y., Ciechanover, A. & Kahana, C. (1989) *J. Biol. Chem.* **264**, 15949–15952.
38. Garcia, A., van, D. J. & Pleij, C. W. (1993) *Nucleic Acids Res.* **21**, 401–406.
39. Weiss, R. B., Huang, W. M. & Dunn, D. D. (1990) *Cell* **62**, 117–126.
40. Ten Dam, E., Pleij, C. & Bosch, L. (1990) *Virus Genes* **4**, 121–136.
41. Zuker, M. & Stiegler, P. (1981) *Nucleic Acids Res.* **9**, 133–148.