The Stability of Human β-Globin mRNA Is Dependent on Structural Determinants Positioned Within Its 3’ Untranslated Region

By J. Eric Russell and Stephen A. Liebhaber

Controls that act at both transcriptional and posttranscriptional levels assure that globin genes are highly expressed in developing erythroid cells. The extraordinary stabilities of α- and β-globin mRNAs permit globin proteins to accumulate to substantial levels in these cells, even in the face of physiologic transcriptional silencing. Structural features that determine α-globin mRNA stability have recently been identified within its 3’UTR; in contrast, the structural features that determine β-globin mRNA stability remain obscure. The current study begins to define the structural basis for β-globin mRNA stability. Two tandem antitermination mutations are introduced into the wild-type human β-globin gene that permit ribosomes to read into the 3’UTR of the encoded β-globin mRNA. The readthrough β-globin mRNA is destabilized in cultured erythroid cells, indicating that, as in human α-globin mRNA, an unperturbed 3’UTR is crucial to maintaining mRNA stability. Additional experiments show that the β-globin and α-globin mRNA 3’UTRs provide equivalent levels of stability to a linked β-globin mRNA coding region, suggesting a parallel in their function(s). However, destabilization of the antiterminated β-globin mRNA is independent of active translation into the 3’UTR, whereas translation into the α-globin mRNA 3’UTR destabilizes a linked β-globin coding region in a translationally dependent manner. This indicates that the α- and β-globin 3’UTRs may stabilize linked mRNAs through distinct mechanisms. Finally, it is shown that neither of the two mutations that, in combination, destabilize the β-globin mRNA have any effect on β-globin mRNA stability when present singly, suggesting potential redundancy of stabilizing elements. In sum, the current study shows that a functionally intact β-globin mRNA 3’UTR is crucial to maintaining β-globin mRNA stability and provides a level of stability that is functionally equivalent to, although potentially mechanistically distinct from, the previously characterized α-globin mRNA 3’UTR stability element.

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tein observed in affected α<sup>+</sup> heterozygotes (typically <2% of normal α-globin expression) contrasts sharply with the approximately 40% level of expression of readthrough β-globin proteins in individuals heterozygous for β<sup>0</sup>, β<sup>0</sup>β<sup>0</sup>, or β<sup>0</sup>α<sup>+</sup>. This observation implies normal or near-normal stability of the mutant (antiterminated) β-globin mRNAs. The distinction between the α- and β-globin mRNA 3'UTRs is further supported by the observation that the α-globin mRNA is fully destabilized when ribosomes read as few as 4 codons into the 3'UTR. Stability control elements, if present in the β-globin mRNA 3'UTR, must therefore be positioned further 3' relative to the termination codon than they are situated within the α-globin mRNA 3'UTR. In parallel with this apparent difference in positioning, the sequence-specific mRNP complex that assembles in vitro on the α-globin mRNA 3'UTR does not assemble on the 3'UTR of β-globin mRNA. These contrasting features of the human α- and β-globin mRNA 3'UTRs suggest that their stabilities may be determined by distinct structures and/or mechanisms.

In the current study, we begin to define the mechanism(s) through which human β-globin mRNA is stabilized. The results show that the native structure of the β-globin mRNA 3'UTR is crucial to the stability of β-globin mRNA and has a stabilizing effect equivalent to that of the α-globin mRNA 3'UTR. Additional data are presented that support the hypothesis that the functional similarities of the α- and β-globin mRNA stability elements may arise through distinct structures and/or independent mechanisms.

**MATERIALS AND METHODS**

**Plasmids.** Plasmids containing the human β-globin gene were derived from p158.2 (kind gift of S. Orkin, Boston, MA), which contains the 4.1-kb Hpa I/Hpa I genomic fragment coding the entire 1.6-kb gene along with 0.8 kb of 3' flanking region and 1.7 kb of 5' flanking sequence, adjacent to a 1.9-kb Kpn I/Pvu II DNA fragment containing the human β-globin locus control region DNase hypersensitive site 2. Plasmid pSV2Aneoa2 contains the 1.5-kb Pst I human α2-globin gene fragment previously described. To facilitate construction of β-globin gene variants, the polylinker EcoRI site in p158.2 was eliminated by partial EcoRI digestion and religation of the 1.4-kb Kpn I/EcoRI site of the β-globin exon III (p158.2K13). Variant β-globin genes were synthesized by creating point mutations within the 904-bp EcoRI-EcoNI fragment by splice-overlap-extension (SOE; see below) and ligating the mutant sequences into the prepared EcoRUEcuNI site of pCranston. Variant α-globin genes were created that contained one, two, or three mutations. Mutation Sm is an AU-CG substitution at mRNA positions +51/52 (numbered from the cap site) that destroys the translation initiation codon; mutation B is a UG insertion following position 486; and mutation Af is a U<sup>+</sup>A-W substitution of normal α-globin expression) contrasts sharply with the 40% level of expression of readthrough β-globin proteins in individuals heterozygous for β<sup>0</sup>, β<sup>0</sup>β<sup>0</sup>, or β<sup>0</sup>α<sup>+</sup>. This observation implies normal or near-normal stability of the mutant (antiterminated) β-globin mRNAs. The distinction between the α- and β-globin mRNA 3'UTRs is further supported by the observation that the α-globin mRNA is fully destabilized when ribosomes read as few as 4 codons into the 3'UTR. Stability control elements, if present in the β-globin mRNA 3'UTR, must therefore be positioned further 3' relative to the termination codon than they are situated within the α-globin mRNA 3'UTR. In parallel with this apparent difference in positioning, the sequence-specific mRNP complex that assembles in vitro on the α-globin mRNA 3'UTR does not assemble on the 3'UTR of β-globin mRNA. These contrasting features of the human α- and β-globin mRNA 3'UTRs suggest that their stabilities may be determined by distinct structures and/or mechanisms.

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DNA ligase according to the manufacturer’s recommended protocol. Transfection-competent component origin (coding region, 5’UTR, or 3’ flanking region) to indicate sequence origin. Oligomers that contain mutations that encode gel-purified, and ligated into the EcoRI/EcoNI site of pPa" using T4 polymerases (kind gift of D. Portnoy, Philadelphia, PA) were electroporated with the ligation mix between the EcoRI site and the 3’ flanking region. Other variant mutant plasmids were identified by 95°C for 1 minute, 45°C for 15 seconds, and 72°C for 30 seconds (30 cycles); and 72°C for 5 minutes.

Transcription/translation

For 1.5 minutes (4 cycles); 95°C for 1 minute, 56°C for 15 seconds, and 72°C for 30 seconds. This approach of selectively adding the '*P-labeled primer for the final extension reaction was used.

Reverse transcription-PCR (RT-PCR) assay. A 10-μL reaction mixture containing 50 pmol oligomer β1541/1520 and 4 μL of sample RNA was heated to 95°C for 3 minutes and then chilled on ice. Ten microliters of RT reaction master mix was then added (2 μL 10× PCR salts, 18 U avian myeloblastosis virus (AMV) reverse transcriptase, and 500 μmol of each dNTP), and tubes were incubated for 45 minutes at 45°C. A 30-μL PCR reaction master mix containing 100 pmol oligo β347/456 was then added to a final 50-μL reaction mixture containing 50 pmol oligo β347/456 was then added to a final 50-μL reaction mixture containing 50 pmol oligo β347/456. The samples were amplified for a single cycle of 95°C for 9 minutes, 58°C for 15 seconds, and 72°C for 30 seconds (30 cycles); and 72°C for 1 minute (1 cycle).

Ten microliters of RT reaction master mix was then added (2 μL 10× PCR salts, 18 U avian myeloblastosis virus (AMV) reverse transcriptase, and 500 μmol of each dNTP), and tubes were incubated for 45 minutes at 45°C. A 30-μL PCR reaction master mix containing 100 pmol oligo β347/456 was then added to a final 50-μL reaction mixture containing 50 pmol oligo β347/456. The samples were amplified for a single cycle of 95°C for 9 minutes, 58°C for 15 seconds, and 72°C for 30 seconds (30 cycles); and 72°C for 1 minute (1 cycle).

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**β-GLOBIN mRNA STABILITY**

Assay of β-globin mRNA stability in transiently transfected MEL cells. To compare the relative stabilities of two highly similar β-globin mRNAs transiently expressed in MEL cells, an RT-PCR assay was developed. This assay permits the relative quantitation of the two mRNAs when present at low concentrations in the same sample (Fig 1A and Materials and Methods). The wild-type (βWT) and variant (βVT) β-globin mRNAs present in a typical sample are reverse-transcribed and PCR-amplified. A final extension reaction is performed with a nested 32P-labeled primer, so that all end-labeled cDNAs are perfect duplexes that are fully susceptible to restriction enzyme analysis. The cDNA products are then digested with a restriction enzyme that recognizes the mutation that defines each specific βVT. The uncut (βWT) and cut (βVT) fragments are resolved on a denaturing acrylamide gel and their relative concentrations are determined. The capacity of this assay to linearly amplify input DNAs was confirmed using mixtures containing known ratios (ranging from 1:8 to 8:1) of wild-type human β-globin gene (pβWT) and a β-globin gene variant containing a mutation that encodes a Bcl I restriction site immediately 5' of the termination codon (pβVT; Fig 1B). Additional experiments (data not shown) confirm that the ratio of the coamplified products is not influenced by the number of PCR cycles, even when an amplification plateau is reached.

Using the RT-PCR assay, the relative stability of two mRNAs can be determined by measuring the time-dependent change in their relative levels in transcriptionally silenced cells. To determine the stability of a variant β-globin mRNA (βVT) relative to a parental wild-type β-globin mRNA (βWT), equal amounts of the corresponding genes are mixed and cotransfected into MEL cells (Fig 2A). After a 24-hour recovery period, the cells are treated with a transcriptional inhibitor (Act D or DRB) and purified cytoplasmic RNA prepared from aliquots harvested after an additional 0, 3, 6, and 9 hours. The level of βVT mRNA is determined relative to parental βWT mRNA in each aliquot by the RT-PCR assay (see above), and the normalized ratio was plotted as a function of the duration of time posttranscriptional inhibition. Transcriptional arrest of the Act D-treated MEL cells is confirmed by Northern analysis of aliquots for short-lived (T1/2 = 15 minutes) c-myc mRNA (Fig 2B). As expected, the high-intensity signal observed at T = 0 is lost at subsequent time points, indicating rapid and complete transcriptional inhibition.

The 3'UTR of human β-globin mRNA is involved in mRNA stabilization. Structural features of the α-globin mRNA 3'UTR that determine its stability have recently been described. Similar stability elements might be positioned within the 3'UTR of the wild-type β-globin mRNA, in which event they would be protected from disruption by translating ribosomes. To test this hypothesis, two tandem mutations were introduced into the wild-type β-globin gene that, in combination, permit ribosomes to read into the β-globin mRNA 3'UTR for a total of 37 codons, until the next in-frame UAA translation stop codon is encountered (22 nt upstream of the poly-adenylation site). ββWT mRNA is a full-length human β-globin mRNA with two sets of substitutions: a 2-nt insertion 5' upstream from the native termination codon (mutation B; directs a shift to the +1 translational reading frame) and an in-frame antitermination mutation 10 codons into the 3'UTR (mutation A). The mRNA containing
these two mutations, \( \beta^{\text{B,SM}} \) mRNA (Fig 3A, left), is the \( \beta \)-globin mRNA analog of the antiterminated \( \alpha^{\text{CS}} \) mRNA. The stability of the \( \beta^{\text{B,SM}} \) mRNA relative to the \( \beta^{\text{wt}} \) mRNA was determined in Act D-treated MEL cells using the RT-PCR assay (Fig 3B, left) and was confirmed in parallel experiments using DRB, a mechanistically distinct transcriptional inhibitor\(^{11} \) (Fig 3C). Both studies indicate an approximate 35% decrease in the level of \( \beta^{\text{B,SM}} \) mRNA relative to \( \beta^{\text{wt}} \) mRNA during a 9-hour transcriptional chase. These results suggest that a structurally intact 3'UTR is critical to maintaining full \( \beta \)-globin mRNA stability.

The \( \alpha \) - and \( \beta \)-globin mRNA 3'UTRs provide an equivalent degree of stability to a linked \( \beta \)-globin mRNA coding region.

The long half-lives of human \( \alpha \)- and \( \beta \)-globin mRNAs and the balanced synthesis of their encoded globin chains in differentiating erythroid cells suggests that the two mRNAs may be stabilized by related mechanisms. To investigate the possibility that the \( \alpha \)- and \( \beta \)-globin 3'UTRs provide functionally equivalent degrees of stability, the \( \beta \)-globin 3'UTR was substituted by the \( \alpha \)-globin 3'UTR to create the chimeric gene \( p\beta\alpha \) (Fig 3A, right). A readthrough variant of this \( \beta \)-chimeric gene (\( p\beta\alpha^{-} \)) was also constructed by mutating the termination codon TAA\( \rightarrow \)TCA (creating a \( \psi\nu\) II site at this position). This antitermination mutation permits ribosomes to read into the \( \alpha \)-globin 3'UTR for an additional 31 codons until the next in-frame codon is encountered 19 nt upstream of the polyadenylation site, exactly as occurs in \( \alpha^{\text{CS}} \) mRNA. Relative to \( \beta\alpha \) mRNA, the level of readthrough \( \beta\alpha^{-} \) mRNA decreased by approximately 35% over 9 hours in Act D-treated MEL cells (Fig 3B, right). This result, when compared with the effect of translational readthrough into the \( \beta \)-globin mRNA 3'UTR in the previous experiment (~35% decrease per 9 hours; Fig 3B, left), suggests that the \( \beta \)- and \( \alpha \)-globin mRNA 3'UTRs provide an equivalent degree of stability to a linked \( \beta \)-globin mRNA.

The \( \alpha \) - and \( \beta \)-globin mRNA 3'UTRs may stabilize a linked \( \beta \)-globin mRNA coding region through distinct mechanisms.

Although the \( \alpha \)- and \( \beta \)-globin mRNA 3'UTRs stabilize a linked \( \beta \)-globin mRNA coding region to the same degree, computer-assisted comparison of the \( \alpha \)- and \( \beta \)-globin mRNA 3'UTRs fails to show significant primary or secondary structural similarities that might constitute a shared stability-determining element (data not shown). Additional indirect evidence indicates that the two regions may act in mechanistically distinct fashions (see Introduction and Discussion). We therefore tested whether conditions known to affect the function of the \( \alpha \)-globin mRNA stability element also affect the function of the \( \beta \)-globin mRNA stability element. Instability of \( \alpha^{\text{CS}} \) mRNA is known to be translationally dependent.\(^{28} \) We asked whether destabilization caused by readthrough mutations within the \( \beta^{\text{B,A}} \) and \( \beta\alpha^{\text{P}} \) mRNAs was also dependent on active translation. To do this, the initiation codons of the \( \beta^{\text{B,A}} \) and \( \beta\alpha^{\text{P}} \) mRNAs were destroyed by an AUG\( \rightarrow \)CCG mutation (\( \beta^{\text{B,A}} \) and \( \beta\alpha^{\text{P}} \)). This initiation codon mutation (mutation Sm) has no demonstrable effect on the stability of the \( \beta^{\text{wt}} \) or \( \beta\alpha \) mRNA when present alone (\( \beta^{\text{wt}} \) and \( \beta\alpha^{\text{sm}} \); Fig 4A and B). When the Sm mutation is introduced into the antiterminated \( \beta\alpha \)-chimeric mRNA (Fig 4A, \( \beta\alpha^{\text{sm,sm}} \)), normal stability is restored (Fig 4B, right). This is consistent with the restoration of stability to \( \alpha^{\text{CS}} \) mRNA by any of several

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Fig 1. The relative concentrations of wild-type and variant \( \beta \)-globin mRNAs quantitated by RT-PCR. (A) Assay. Cytoplasmic RNA is isolated from transfected MEL cells. In this example, two mRNAs, wild-type (\( \beta^{\text{wt}} \)) and variant (\( \beta^{\text{Var}} \)), are expressed from the transfected genes (depicted as gray and dark lines, respectively). The two mRNAs are identical except for the presence of a mutation (\( \star \)) in \( \beta^{\text{Var}} \). The mixture of mRNAs is reverse-transcribed and PCR-amplified with a set of unlabeled primers (\( \star \) and \( \star \)) that bracket the divergent site. A final elongation cycle is then performed with a nested \( ^{32}\text{P} \)-labeled oligonucleotide (\( \star \)). The \( ^{32}\text{P} \)-labeled cDNAs generated during this final cycle are obligate cDNA homodimers that contain the wt or mutant sequence. The two cDNAs are distinguished by digestion with a restriction endonuclease specific for the \( \beta^{\text{Var}} \) mutant site, yielding uncut and cut \( ^{32}\text{P} \)-labeled fragments corresponding to \( \beta^{\text{wt}} \) and \( \beta^{\text{reverse}} \) amplified product, respectively. The two different-sized fragments are resolved on a denaturing acrylamide/urea gel and their relative intensities determined. (B) Quantitation control for the RT-PCR assay. Mixtures containing known ratios of supercoiled wild-type (\( \beta^{\text{wt}} \)) and variant DNA (the example \( \beta^{\text{wt}} \) contains a \( BcI1 \) site) were PCR-amplified, extended a final cycle with a \( ^{32}\text{P} \)-labeled nested primer, digested with \( BcI1 \), and resolved on a denaturing acrylamide/urea gel as described in (A). The positions of \( \beta^{\text{wt}} \) and \( \beta^{\text{reverse}} \) restriction fragments are identified to the right of the autoradiograph. The ratio of \( \beta^{\text{wt}} \) to \( \beta^{\text{reverse}} \) PCR products (\( C \)) was determined by Phospholmager and plotted below each lane along with the original ratio of input \( \beta^{\text{wt}} \) to \( \beta^{\text{reverse}} \) DNAs (\( C \)) to show the accuracy of the PCR assay.
methods that arrest its translation. Surprisingly, when the same translation-arresting Sm mutation was substituted into the $\beta^{A,F}$ mRNA (Fig 4A, $\beta^{B,B,A,F}$), the mRNA remained unstable (Fig 4B, left). This observation was confirmed using DRB as transcriptional inhibitor (data not shown). These results suggest that, although the 3'UTRs of the $\alpha$- and $\beta$-globin mRNAs provide similar levels of stability to a linked globin coding region, the mechanisms through which they work may in fact be distinct.

The inability of the translation initiation (Sm) mutation to stabilize $\beta^{B,A,F}$ mRNA might reflect ongoing translation via initiation at a cryptic translation initiation site. This formal possibility was unlikely, because the same sites would be available for cryptic translation initiation and subsequent destabilization of $\beta^{A,F}$, which does not appear to occur. However, to confirm that the introduced Sm mutation effectively blocks translation in the $\beta^{B,B,A,F}$ mRNA, a linked RT-PCR/translation assay was used (see Materials and Methods). The $\beta^{B,A,F}$ mRNA translated an elongated globin chain, as predicted, whereas the $\beta^{B,B,A,F}$ mRNA failed to express a protein product (Fig 4C). $\beta^{A,F}$ mRNA present in the $\beta^{B,A,F}$ and $\beta^{B,B,A,F}$ samples functions as an internal control for each step, yielding $\beta^{A,F}$ protein in both cases. The predicted sizes of the $\beta^{A,F}$ and $\beta^{B,A,F}$ proteins were verified by SDS-PAGE (data not shown). These data confirm that the Sm mutation effectively blocks translation of the $\beta^{B,B,A,F}$ mRNA. Thus, the tandem B + Af mutations destabilize $\beta$-globin mRNA by a translationally independent mechanism.

Mutations that destabilize the $\beta$-globin mRNA in tandem have no effect when introduced singly. The translational independence of $\beta^{B,A,F}$ instability suggests that mutation B or mutation Af may destabilize $\beta$-globin mRNA directly rather than via their permissive effects on ribosomal entry into the 3'UTR. This direct destabilization mechanism would parallel the direct destabilizing effect that results from introduction of site-specific mutations into any one of three segments of the $\alpha$-globin mRNA 3'UTR stability determinant. The destabilizing effects of mutations B and Af were therefore tested individually (Fig 5A; $\beta^{B}$ and $\beta^{A,F}$). Surprisingly, both the $\beta^{B}$ and $\beta^{A,F}$ mRNA ratios remained constant during a 9-hour transcriptional chase, indicating that the $\beta^{B}$ and $\beta^{A,F}$ mRNAs are both equally stable to $\beta^{A,F}$ mRNA (Fig 5B). To confirm that mutation B had the anticipated permissive effect on ribosomal entry into the 3'UTR, RNA from the transfected cells was assayed by the linked RT-PCR/translation/transcription assay as described above (Fig 5C). The observed elongated $\beta$-globin chain indicates that mutation B permits ribosomal translation into the 3'UTR as far as codon 10, where an in-frame TAA terminates chain elongation. These results suggest that neither the B nor the Af mutation alone is responsible for $\beta^{B,A,F}$ instability. Instead, mRNA sequences that are interrupted by the two mutations must both be altered for the mRNA to be destabilized.

DISCUSSION

The overall expression of a gene reflects the level of its encoded mRNA. Consequently, profound differences in gene expression can result from moderate changes in mRNA synthesis and/or decay rates. The impact of mRNA stability on overall gene expression is most pronounced in mRNAs with exceptionally long or short $t_{1/2}$. For example, the cytoplasmic levels of highly unstable mRNAs such as c-myc, c-fos, interleukin-2 (IL-2), and granulocyte-macrophage colony-stimulating factor (GM-CSF) rapidly reflect even small changes in gene transcription, providing the cell with rapidly responsive and precise control over their expression. In contrast, the stable nature of globin mRNAs permit them to accumulate to high levels in maturing erythroid cells. Their long $t_{1/2}$ also insure that globin mRNAs remain translationally active for several days after extrusion of the erythroid nucleus. Using an estimate of 24 to 48 hours for the $t_{1/2}$ of $\beta$ mRNA, the unstable $\beta$-globin mRNAs assayed in the current study would be expected to have $t_{1/2}$s of 8 to 12 hours (see Materials and Methods). The consequences of such a decrease in the $t_{1/2}$ of a globin mRNA is quite dramatic when the physiologic transcriptional silencing of maturing
erythroid cells is considered. These transcriptionally silent cells remain translationally active for up to 3 days in the bone marrow and for an additional 1 to 2 days (as reticulocytes) in the peripheral blood. Relative to a stable (βm) mRNA, the concentration of an unstable β-globin mRNA decreases exponentially during this period. Consequently, the 3:1 or 4:1 ratio of their τ1/2 significantly underestimates the ratio of globin protein expressed by the stable and unstable β-globin mRNAs, respectively. Hence, even a modest change in the τ1/2 of a globin mRNA can have a major impact on the accumulation of its encoded protein in maturing erythroid cells, showing the critical importance of globin mRNA stability to normal erythroid development and function.

The present work begins to identify structural features that contribute to the stability of the human β-globin mRNA. A transient transfection system and assay was developed that accurately and reproducibly determines the relative stabilities of variant β-globin mRNAs (Figs 1 and 2). This approach was then applied to the specific question of whether the 3'UTR of the β-globin mRNA enhances its stability by determining whether antitermination mutations that permit ribosomal readthrough into the 3'UTR disrupt β-globin mRNA stability. To establish a β-globin mRNA comparable to αm mRNA, two tandem mutations were introduced into the wild-type human β-globin gene that permit ribosomes to readthrough 109 nt of the 132 nt β-globin mRNA 3'UTR (Fig 3A). Destabilization of βm mRNA relative to parental βm mRNA suggests that one or more structures within the 3'UTR are crucial to β-globin mRNA stability (Fig 3B, left, and C). In a parallel set of experiments, we determined that the β-globin mRNA coding region can be stabilized to a similar degree by the α-globin mRNA 3'UTR (βα; Fig 3A, right, and B, right). These observations confirm the importance of globin mRNA 3'UTRs to globin mRNA

![Diagram](https://example.com/diagram.png)
stability and show functional similarity between the α- and β-globin mRNA 3'UTR stability elements.

Several key characteristics appear to distinguish the mechanisms through which human α- and β-globin mRNAs are stabilized. These differences are especially surprising considering that the two mRNAs are evolutionarily related and appear to be equally stable in adult erythroid cells.1,46 Previous studies from this lab show that, under in vitro conditions that favor assembly of a ribonucleoprotein complex within the 3'UTR of α-globin mRNAs, no equivalent complex is assembled on the β-globin 3'UTR.31 The current study supports this apparent difference by showing that the α- and β-globin mRNAs are stabilized through potentially distinct mechanisms. Although translational arrest restores baseline stability to antiterminated α-globin mRNA28 and to chimeric βα-globin mRNA (Fig 4B, right), the instability of translationally arrested readthrough βα-globin mRNA is unaffected (Fig 4B, left). These results were duplicated using a second, mechanistically distinct transcriptional inhibitor (DRB; data not shown) and were further supported by direct verification of translational arrest (Fig 4C). The hypothesis that α- and β-globin mRNAs may have evolved distinct stabilizing components is supported by other experimental observations. Whereas the α-globin mRNA is destabilized by ribosomes that read as few as 4 codons into the 3'UTR,28 the β-globin mRNA retains full stability despite readthrough as far as 10 codons into the 3'UTR (Fig 5),32-34 suggesting different positioning of the respective stability elements. Furthermore,
α-globin mRNA can be destabilized by single-site mutations at any of several disparate positions within its 3′UTR, indicating that α-globin mRNA stability is maintained by interacting, nonredundant elements. In contrast, the β-globin mRNA may be stabilized by multiple elements positioned within the terminal coding region and 3′UTR. Neither of two mutations present in the unstable readthrough βB,Af mRNA can individually destabilize β-globin mRNA (Fig 5); destabilization requires presence of the two mutations in tandem. The need for both mutations to be in place for this destabilization does not reflect their permissive effect on ribosomal entry into the 3′UTR, because specific inhibition of mRNA translation does not relieve this destabilization. Thus, the two mutations appear to act directly on a determinant or determinants in the mRNA sequence. This observation would account for the fact that, among the several hundred distinct nondeletional β-thalassemic mutations described, no naturally occurring unstable readthrough β-globin mRNA has been observed. To destabilize β-globin mRNA, simple antitermination is not sufficient, and a highly unlikely double mutation would be required.

Different stability mechanisms for α- and β-globin mRNAs may be necessary to insure developmental stage-specific expression of different globin genes. For example, separate systems may permit β-globin mRNA to be stabilized exclusively in adult erythroid cells, whereas α-globin mRNA is stabilized in both fetal and adult erythroid cells. Despite these potential mechanistic differences, the α-globin mRNA stability element is capable of stabilizing the β-globin coding region, suggesting that functional similarity of the two stability elements is preserved to insure balanced α- and β-globin protein synthesis that is crucial to normal erythrocyte function.

The positioning of stability-determining elements in the 3′UTR appears to be a common molecular strategy; unique positive stability-determining elements have been mapped to the 3′UTRs of histone and transferrin receptor (TIR) mRNAs, as well as the human α-globin mRNA. The stability elements in α-globin, TIR, and histone mRNAs are single (perhaps multicomponent) determinants that can be disrupted by appropriately positioned mutations. By comparison, the linear separation of mutations B and Af in the β-globin mRNA 3′UTR and the requirement that both be present to destabilize the mRNA suggest that the stability determinant present in this region may comprise a complex and potentially redundant structure. Studies that show distinct high-order RNA structures or identify interaction with discrete trans-acting factors at the B and Af sites would be of value in both validating and refining the proposed model.

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REFERENCES


The stability of human beta-globin mRNA is dependent on structural determinants positioned within its 3’ untranslated region

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