Destabilization of Human α-Globin mRNA by Translation Anti-termination Is Controlled during Erythroid Differentiation and Is Paralleled by Phased Shortening of the Poly(A) Tail*

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The extraordinary stability of globin mRNAs permits their accumulation to over 95% of total cellular mRNA during erythroid differentiation. The stability of human α-globin mRNA correlates with assembly of a sequence-specific ribonucleoprotein complex at its 3′-untranslated region. A naturally occurring anti-termination mutation, Constant Spring (CS), which permits ribosomes to enter the 3′-untranslated region of the α-globin mRNA, results in accelerated mRNA decay. To study the mechanism of this destabilization in vivo, we established transgenic mouse lines carrying the human αCS gene. Relative to wild-type human α-globin mRNA (αwt), αCS mRNA is destabilized in marrow erythroblasts. The poly(A) tails of both the αCS and αwt mRNAs show a periodicity of 20–25 nucleotides consistent with phased binding of poly(A) binding proteins. However, the mean size of poly(A) tails of the unstable αCS mRNA is significantly shorter than that of the αwt mRNA. Unexpectedly, the αwt and αCS mRNAs are of equal stability in peripheral reticulocytes, where their respective poly(A) tails shorten coordinately. These findings demonstrate a characteristic organization of the poly(A) tail on α-globin mRNA which is maintained during normal and accelerated decay, a correlation between poly(A) metabolism and anti-termination-mediated accelerated mRNA turnover, and a switch in the mechanism of mRNA decay during erythroid terminal differentiation.

The phenotype and function of a cell are defined by the spectrum of its cytoplasmic mRNAs. The level of a specific mRNA is determined by the balance between its rates of synthesis and degradation. Recent studies in a variety of experimental systems highlight the central role of mRNA stability in the control of gene expression. The mechanisms that control this property appear complex and encompass distinct pathways for different subgroups of mRNAs (reviews in Refs. 1–3). The half-lives of mRNAs in higher eukaryotes can differ by over 1000-fold, with a range of minutes to days (3). For example, the half-lives (t1/2) of c-myc and c-fos mRNAs are approximately 10–15 min (4) while the t1/2 of globin mRNA is more than 24 h (5). In some instances, mRNA stability may be a dynamic property that changes dramatically in response to development, environmental, or metabolic signals.

The stability of an mRNA is a function of its sequence and structure and may reflect interaction(s) of cis elements with trans-acting factors. For example, the stability of the transferrin receptor mRNA is controlled by intracellular concentration of iron; at low iron concentrations the transferrin receptor mRNA is quite stable, while a rise in cytosolic iron triggers its accelerated decay (6). This control is mediated by interaction of a specific series of secondary structures in the transferrin receptor mRNA 3′-untranslated region (3′-UTR)1 with an iron binding protein (6). In contrast to the transferrin receptor mRNA, the structural determinants that underlie the stability of most mRNAs are poorly defined.

mRNAs are degraded via a number of interrelated pathways, several of which have been defined in yeast (2). A common degradation pathway is initiated by shortening of the poly(A) tail, followed by removal of the 7-methylguanosine cap structure. The deadenylated and decapped mRNA is then degraded by a 5′ → 3′ exonuclease (7, 8). This pathway implies that there is communication between the 5′- and 3′-termini of the mRNA; the mechanism(s) involved in this interaction is not known (9). In higher eukaryotes, deadenylation also frequently accompanies degradation of mRNA, although the mechanism of degradation subsequent to deadenylation remains to be established (10). Two other less common degradation pathways are independent of prior deadenylation: direct 5′-decapping triggered by nonsense mutations (11) and site-specific endonuclease cleavage in the 3′-UTR (12, 13). The general importance of these pathways, how each is controlled, and how they might be interrelated are questions that are presently under study.

The globin genes provide a unique model system with which to study mechanisms responsible for mRNA stability. During terminal erythroid differentiation, globin mRNAs accumulate to over 95% of total cellular mRNA (14). This remarkable enrichment of a single group of mRNAs relies on their unusually long half-lives as well as selective degradation of non-globin mRNAs (5, 15–17). The importance of mRNA stability to globin gene expression is clearly demonstrated by the phenotype resulting from the loss of this property. For example, a UAA → CAA anti-termination mutation in the α-globin gene (Constant Spring, aCS), results in mRNA destabilization and consequent loss of over 95% expression from the affected locus (18).

Recent studies have demonstrated that αCS mRNA is destabilized by the physical entry of ribosomes into its 3′-UTR.

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1 The abbreviations used are: UTR, untranslated region; CS, Constant Spring; bp, base pair(s); kb, kilobase pair(s); PBS, phosphate-buffered saline; PIPES, 1,4-piperazinediethanesulfonic acid; wt, wild type; h, human; m, mouse; RNP, ribonucleoprotein; PABP, poly(A) binding protein.
which is permitted by the CS anti-termination mutation. This finding suggested that α-globin mRNA contains a stability determinant in this region (19). Subsequent studies confirmed this hypothesis and identified the critical cis-determinant as a pyrimidine-rich motif in the 3'-UTR that functions in an erythroid-specific manner (20). The structure motif serves as an assembly site for a sequence-specific mRNA-protein (mRNP) complex (α-complex, 21). The presence of this complex correlates with α-globin mRNA stability; mutations that block assembly of the α-complex in vitro also destabilize the mRNA in vivo (20, 21). Although structural studies have identified one component of the α-complex (22), its overall composition remains to be established.

Although the importance of the α-complex to α-globin mRNA stability is apparent, the mechanism(s) of its stabilizing function remains to be defined. One approach to this problem is to follow the pathway of globin mRNA degradation triggered by a mutation that interferes with normal stabilizing functions. In the present report we describe a fully physiologic in vitro model system to facilitate such an approach. Transgenic mice that express readily detectable levels of αCS mRNA were established. The stability and structure of αCS mRNA in these mice can be characterized and compared with wild-type human α-globin mRNA (ho or αES) expressed in a parallel set of transgenic mice. Using this model, we have focused our attention on the structure and dynamics of the poly(A) tail of hα-globin mRNA in early erythroblasts in the bone marrow and in mature reticulocytes in the peripheral blood. Our findings demonstrate a phased organization of the poly(A) tail, which is independent of mRNA stability and maintained during differentiation. The data also reveal a substantial effect of the αCS mutation on its poly(A) tail size. Finally, we present evidence for a switch in the mechanism of ho-globin mRNA decay during erythroid differentiation.

**EXPERIMENTAL PROCEDURES**

**αCS Gene Construction**—The αCS mutation was introduced by splice-overlap extension/polymerase chain reaction (Ref. 20 and included reference to the cloned-gene plasmid) by adjusting the amount of vector DNA. PCR amplifications were carried out using Vent polymerase (New England Biolabs) under the following conditions: 1 cycle (95°C for 5 min, 57°C for 15 s, and 73°C for 25 s), 28 cycles (92°C for 1 min, 57°C for 15 s, and 73°C for 25 s), 1 cycle (73°C for 3 min). Primer names indicate its position relative to the transcriptional start site of the human α2-globin gene. Primer SP72U hybridizes in the vector immediately 3' to the α-globin gene. Reaction 1 contained 10 ng of pSP72H2 template, 100 pmol each of primers 589 (CTCGCAGACCTCCTAAAGCCA) and 717 (a reverse primer: GAGGCTTCCAGCTTGAGCTTA) and yielded the expected 146-bp product. Reaction 2 contained 10 ng of template and 100 pmol each of primers 717 (TACCTGCAAGCTGAGGCTC) and SP72U (TAACTAGCTCCTATAGGA) and yielded the expected 240-bp product. 1 μl of each reactions and 2 were combined, heat-denatured, reannealed, and amplified with primers 589 and SP72U as above except that the extension time was increased to 30 s. The expected 368-bp splice-overlap extension product was digested with SpIh and BstEII and ligated into the SpIhBstEII site of pSP72H2 and cloned into the pBluescript II vector. This composite plasmid was isolated after digestion with EcoRI and used as a probe to identify the αCS α-globin transgene in mouse DNA. Candidate clones were purified and sequenced to ensure sequence fidelity.

**Generation of Transgenic Mice Expressing the αCS Gene**—All transgenic mice were generated by the Transgenic Mouse Core Facility at the University of Pennsylvania School of Medicine. The αCS gene was released from its host plasmid vector by EcoRI digestion and ligated into the EcoRI site of the mouse CS mRNA in cultured reticulocytes, cell number for each aliquot was determined, and RNA was isolated in the presence of a trace amount of [32P]UTP-labeled glyceroldehyde-3-phosphate dehydrogenase mRNA (specific activity, 700,000 cpm/μg) to control for RNA extraction efficiency. The amount of mα-globin mRNA was determined per 5 x 10⁶ cells by RNase protection assay.

**RNase Protection Assay**—Antisense RNA probes complementary to the first exon-encoded region of the human α-globin mRNA and the second exon-encoded region of the mouse α-globin mRNA were synthesized from plasmids pGEM3H and pGEM3M (23). Human α-globin (ho-globin) probe was synthesized at 10 times the specific activity of the mouse cDNA. The exact amount of cDNA was adjusted to 240 bp in the transcribed mix (Maxicrypt transcript kit, Ambion). Total RNA isolated from mouse bone marrow (500 ng of total RNA) or reticulocytes (100 ng of total RNA) was mixed with an excess of ho- and mα-globin RNA probes in 20 μl of hybridization buffer (80% formamide, 40 mM PIPES, pH 6.4, 400 mM NaCl, 1 mM EDTA) denaturated at 75°C for 15 min, and then hybridized at 52°C for 16 h. Excess probe and unhybridized RNA were then digested by addition of 4 μg of RNase A and 200 μl of RNase T1 in a total of 200 μl of buffer (300 mM NaCl, 10 mM Tris, pH 7.4, 5 μl EDTA) and incubated for an additional 20 min at room temperature. The reaction was terminated by the addition of 17 μl of 1:4 mix of proteinase K (10 mg/ml) and SDS (10%), phenol-extracted, ethanol-precipitated, and resolved on an 8% acrylamide, 8 μl urea gel. Signal intensity was quantified on a PhosphorImager with ImageQuant software (Molecular Dynamics).

**Poly(A) Tail Analysis**—Poly(A) tail length was determined by a site-specific RNase H cleavage assay (7). Total RNA (0.1–1 μg) was hybridized to oligonucleotide 662 (5′AATCAGTTCAGGGGAGGCTG′) and digested with 0.05 units/μl RNase H in 20 μl of 20 μM Tris, pH 7.5, 50 mM NaCl, 10 mM MgCl2, 1 mM dithiothreitol, 30 μg/ml bovine serum albumin at 30°C for 1 h. The reaction was terminated by phenol extraction and ethanol precipitation and then electrophoresed on a 6% acrylamide, 8 μl urea gel. The RNA fragments were electrotransferred onto a nylon membrane (Nytran, Schleicher & Schuell). The membrane was hybridized 16 h at 42°C in 50% formamide, 5 x SSC, 0.1% SDS, and 5 x Denhardt’s reagent with 10–10⁵ cpm of probe/ml. Blots were then washed in 0.1 x SSC, 0.1% SDS at 65°C, exposed, and scanned on a PhosphorImager. The length of the α-globin 3'-UTR was determined by using primer HindIII/726 (5′TGAAGGTTGGTCCAGGGACGCTG′) and primer EcoRI/833 (5′CCGAATTCGGGAGGCTGACGTTA′) in a polymerase chain reaction containing 20 ng of pSP602FL template (α-globin cDNA; 25), 2.5 mM MgCl2, 0.2 mM each dATP, dCTP, and dGTP, 0.06 mM dTTP, 2 μCi of [α-32P]dCTP (Amersham Corp.), and 2.5 units of Taq polymerase.

α-globin transgene have been previously described (23); four additional lines constructed in the same manner were added for this study. Transgene copy numbers for αCS and αCS were in the same range.
The initiation site is marked by the three exons (Fig. 1a). The mutation in the gene is a single base substitution (UAA → CAA) at the translation termination codon in the third exon. The αCS transgene is linked to the β-globin μLCR to ensure high levels of erythroid cell-specific expression in transgenic mice. B, Southern blot analysis of five αCS transgenic lines. The transgene copy number, calculated from the relative signal intensities of the 1.5- and 4.5-kb bands (see “Experimental Procedures”), is shown below each respective lane. Normal mouse and human genomic DNA samples were run in parallel control lanes.

The first goal was to demonstrate that the CS mutation destabilizes the human αCS mRNA (Fig. 1a). The specific lines used in this representative study are shown above the respective lanes. B, normalized stabilities of the αwt and αCS mRNAs. Each symbol represents the mean value for an independent line as determined from analysis of two or more mice. Solid bars represent the average normalized stabilities of the transgenic mRNAs, and the dashed line indicates the stability of the endogenous ma-globin mRNA (defined as 1.0).

RESULTS

Generation of Mice Expressing the Human αCS Transgene—In humans, the presence of the CS anti-termination mutation in the α-globin mRNA results in accelerated message decay. To establish an in vivo model in which to study the mechanism(s) of this accelerated decay, transgenic mouse lines that expressed the human αCS gene were generated. An αCS gene was constructed by introducing the CS mutation into the αwt globin gene by splice-overlap extension (see “Experimental Procedures”). The αCS gene was then ligated to a DNA fragment containing all four hypersensitive sites of the human β-globin locus control region to ensure that the αCS transgene is highly linked to the endogenous ma-globin mRNA (defined as 1.0).

The Poly(A) Tail of αCS mRNA is Organized in Increments of 20–25 Nucleotides and Shortens during Erythroid Maturation—The αwt and αCS transgenic mRNAs were studied for the size and organization of their poly(A) tails. If accelerated decay was linked to deadenylation, we might be able to detect a difference in the poly(A) profile of the unstable αCS mRNA in transgenic mice. A, analysis of ho-globin mRNA stability. Total mRNA was isolated from bone marrow (BM) and peripheral blood (PB) of phenylhydrazine-treated mice transgenic for the human αwt or αCS genes, and the levels of ho- and ma-globin mRNA were determined by quantitative RNase protection assay. The position and identity of each protected fragment is indicated (ha and ma). The specific lines used in this representative study are shown above each respective lane. B, normalized stabilities of the αwt and αCS mRNAs. Each symbol represents the mean value for an independent line as determined from analysis of two or more mice. Solid bars represent the average normalized stabilities of the transgenic mRNAs, and the dashed line indicates the stability of the endogenous ma-globin mRNA (defined as 1.0).

\[ A \]
The poly(A) tails of \(\alpha\)-globin mRNA are present in incremental sizes and are abnormally short in \(\alpha^{CS}\) mRNA. The diagram indicates the positions of the antisense oligonucleotide used to target RNase H digestion and the position of the probe used for Northern hybridization. B, poly(A) tail analysis of \(\alpha^{wt}\) and \(\alpha^{CS}\) mRNAs in marrow and peripheral blood erythroid cells. Fragment lengths were calculated using a DNA ladder (not shown). Poly(A) tail length of a particular fragment was determined by subtracting the length of the fully deadenylated fragment (\(dT\) lane) from its total length. C, scanning densitometry of gel (B). The positions of each of the sized peaks is indicated, as is the position of the deadenylated fragment. The direction of electrophoretic migration is noted below the scan.

Analysis of \(\alpha^{wt}\) mRNA revealed that poly(A) tail lengths have a periodicity representing increments of 20–25 nucleotides (Fig. 3B). In the bone marrow, the \(\alpha^{wt}\) mRNA contains a dominant A60 peak flanked by peaks of lower intensity that correspond to A85 and A40 (Fig. 3B). By comparison, the poly(A) tail of the \(\alpha\)-globin mRNA in peripheral blood reticulocytes is partitioned into four peaks with an overall shift to a smaller mean size; although A60 is still the dominant peak, A85 decreases in intensity, and a significant A20 peak appears. Of note, we do not detect any evidence of fully deadenylated mRNA. These analyses of the \(\alpha^{wt}\) globin mRNA indicate that the poly(A) tail is organized in a modular fashion and that the mean poly(A) tail size decreases as bone marrow erythroid cells mature into peripheral reticulocytes. Therefore, \(\alpha\)-globin mRNA poly(A) tail phasing is maintained despite its age-related shortening in differentiating erythroid cells.

The CS Mutation Is Linked to an Accelerated and Phased Shortening of Poly(A) Tail—The size distribution of the \(\alpha^{CS}\) mRNA poly(A) tail was determined using the RNase H assay. The fully deadenylated \(\alpha^{CS}\) fragment migrated at the same position as the \(\alpha^{wt}\) fragment (data not shown), indicating that the 3' end of both mRNAs was processed identically. In bone marrow, the \(\alpha^{CS}\) mRNA poly(A) tail was significantly shorter than the \(\alpha^{wt}\) and was present in two major peaks of A85 and A40 (Fig. 3, B and C). In reticulocytes, the \(\alpha^{CS}\) A40 peak became more prominent. In contrast to the \(\alpha^{wt}\) globin mRNA, there was no significant A20 peak signal. Thus, like the \(\alpha^{wt}\) mRNA, the \(\alpha^{CS}\) mRNA shows age-related, phased shortening of its poly(A) tail. However, the distribution of the poly(A) tail size on the \(\alpha^{CS}\) mRNA is significantly different than on the \(\alpha^{wt}\) mRNA in both the bone marrow and in peripheral reticulocytes.

\(\alpha^{wt}\) and \(\alpha^{CS}\) mRNAs Are of Equal Stability in Late Stage Reticulocytes—Under normal conditions, all erythroid mRNAs undergo final degradation over a 2–3-day period in peripheral blood reticulocytes. This terminal event in erythroid cell maturation may involve stability mechanisms and determinants distinct from those that favor rapid accumulation of globin mRNAs during the earlier phases of erythroid maturation. To directly assess this possibility, we followed mRNA decay during a 48-h \(ex vivo\) incubation of peripheral blood reticulocytes. Reticulocyte viability appeared fully maintained during incubation. Endogenous \(\alpha\)-globin mRNA levels fell by 70% during the 48-h incubation (Fig. 4A), indicating that this \(ex vivo\) incubation reproduces the reticulocyte mRNA clearance observed \(in vivo\) (28).

The levels of transgenic \(\alpha^{wt}\) and \(\alpha^{CS}\) mRNAs were measured relative to endogenous mouse \(\alpha\)-globin mRNA \(ex vivo\) incubated reticulocytes over a 48-h period. A typical RNase protection assay is shown in Fig. 4B, and average stabilities from studies of four \(\alpha^{wt}\) and three \(\alpha^{CS}\) lines are plotted in Fig. 4C. Consistent with the accelerated decay of the \(\alpha^{CS}\) mRNA in bone marrow erythroid cells, the initial levels of \(\alpha^{CS}\) mRNA were considerably lower than \(\alpha^{wt}\) mRNA levels. Surprisingly, however, the stabilities of the \(\alpha^{CS}\) and \(\alpha^{wt}\) mRNAs were equivalent. Thus the \(\alpha^{CS}\) mRNA is unstable relative to the \(\alpha^{wt}\) mRNA in the bone marrow, but the \(\alpha^{wt}\) and \(\alpha^{CS}\) mRNAs decay at comparable rates in peripheral reticulocytes.

Poly(A) Tails of \(\alpha^{wt}\) and \(\alpha^{CS}\) Globin mRNAs Shorten Coordinate Cell lines are plotted in Fig. 4C. The \(\alpha^{wt}\) globin mRNA shortened over the 48-h incubation from an initial multipeak distribution to a single intense peak of A20. At the beginning of the incubation period, \(\alpha^{CS}\) mRNA was distributed into two peaks, A60 and A40. Like the \(\alpha^{wt}\) mRNA poly(A) tails, the \(\alpha^{CS}\) poly(A) tails that remain after an additional 48 h were shortened to a single A20 peak. Thus for both mRNAs there is a progressive shortening of the poly(A) tail to a minimal size of A20. Whether deadenylation precedes or parallels mRNA degradation during late reticulocyte differentiation is unknown. The appearance of the A20 peak in the \(\alpha^{CS}\) mRNA poly(A) tail, in coordination with a equivalent rate of decay for both \(\alpha^{wt}\) and \(\alpha^{CS}\) mRNA, suggests a switch in the degradation pathway of globin mRNA in the late stages of reticulocyte differentiation.

The Level of an Essential Subunit of the \(\alpha\)-Globin Stabilizing Complex Decreases During Erythroid Terminal Differentiation—\(\alpha^{CS}\) is degraded faster than \(\alpha^{wt}\) mRNA in marrow erythroid cells. Therefore, \(\alpha^{CS}\) mRNA poly(A) tail phasing is maintained during \(ex vivo\) incubation of peripheral blood reticulocytes.
α-Globin mRNA Stability

**DISCUSSION**

The α-globin gene encodes an mRNA whose extraordinary stability has been demonstrated in multiple experimental systems (5, 14, 15, 17, 20). This stability is dependent upon the assembly of an RNP complex on its 3′-UTR (21). Although the cis and trans elements of the α-complex are under study, the mechanisms by which the mRNA is stabilized in the developing erythroblast and subsequently cleared from the terminally differentiating peripheral reticulocyte are poorly understood. The generation of transgenic mice that express the hα-globin (α<sup>wt</sup>) mRNA or the mutant α<sup>CS</sup> permits detailed study of these pathways in vivo.

The α<sup>CS</sup> Transgenic Mouse Model—Of the one hundred or more mutations of the α-globin gene documented in <i>a</i>-thalassemic individuals, the CS defect is unique in its direct destabilization of the mRNA (29, 30). The accelerated decay of α<sup>CS</sup> mRNA in mice appears to recapitulate its observed instability.
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in humans. The two-point decay curve that was determined in vivo in transgenic mice demonstrated a 4–5-fold lower stability for αCS mRNA than for αwt-globin mRNA (Fig. 2). Cells from the bone marrow compartment, which include transcriptionally inactive normoblasts and reticulocytes, were compared in this analysis with a population of purely posttranscriptional, nonnucleated erythroblasts as well as transcriptionally inactive erythroblasts in the bone marrow compartment, which include transcription-

Poly(A) Tails of α-Globin mRNA Display a Discontinuous Size Distribution That May Reflect a Phased Array of Poly(A) Binding Proteins—Analysis of the αwt mRNA revealed that the size distribution of its poly(A) tails is not continuous but rather peaks with a periodicity of 20–25 nucleotides. This spacing suggests a role for the poly(A) binding protein (PABP) in the protection and/or organization of the poly(A) tail. Mammalian PABP is a 70-kDa protein that is involved in mRNA stability and translation (9, 32–34). PABP monomers bind poly(A) with a footprint of 20–25 residues (35, 36). The observed poly(A) tail profile for the α-globin mRNA (Fig. 3) indicates that the PABPs are “anchored” at fixed positions relative to the mRNA, i.e. they are phased with regard to the terminus of the 3′-UTR.

The demonstration of multiple peaks of poly(A) tail size is scarce in the literature. Previous studies on globin mRNA of other species (37, 38) have shown a similar pattern. One other report shows discrete poly(A) tail lengths for long-lived ribulose-bisphosphate carboxylase/oxygenase small subunit 2 mRNA in Chlamydomonas (34). Poly(A) tail phasing may be important for maintaining stability of long-lived mRNAs. Using an in vivo system, we have demonstrated that the poly(A) phasing pattern is dynamic. Specifically, we show that the 20–25 nucleotide spacing in poly(A) tail size was maintained during terminal erythroid differentiation and, its shortening by integral 20–25 nucleotide units may reflect rapid degradation of proteo-protected segments as PABP monomers are released from the 3′-terminus.

Despite the accelerated shortening of its poly(A) tail, phasing at intervals of 20–25 nucleotides is maintained for the αCS mRNA. Because accelerated decay of the αCS mRNA is likely to reflect the displacement of the α-complex by the translating ribosome (19), PABP phasing would not appear to be dependent on the presence of the α-complex. However, the α-complex might serve to stabilize the poly(A) tail, perhaps by direct physical interaction with PABP or by strengthening the interaction between PABP and the poly(A) tail.

Selective Degradation of αCS mRNA Occurs Early but Not Late in Erythrocyte Differentiation—An unexpected observation in the present study was that the αCS and αwt mRNAs were degraded at equivalent rates in peripheral reticulocytes. This contrasts with the accelerated decay of αCS mRNA compared with αwt mRNA in the less differentiated erythroid cells both in vivo (this report) and in cultured mouse erythroblasts (MEL, Ref. 20). The 60 h t1/2 of the α-globin mRNA in MEL cells (39) is shortened to 20 h by the CS mutation. Since the rate of decay of both αCS and αwt mRNAs paralleled that of the endogenous mouse α-globin mRNA (Fig. 4), we estimated their t1/2 in reticulocytes to be approximately 24 h. This change in the relative stability of the αCS and αwt mRNA may reflect a difference between the mechanism that determines αwt mRNA

3 J. Morales, J. E. Russell, and S. A. Liebhaber, unpublished results.
α-Globin mRNA Stability

**FIG. 7. Regulation of human α-globin stability during erythroid cell maturation: a model.** In early erythroblasts the globin genes are actively transcribed. At this stage the α-complex forms on the 3′-UTR of the α-globin mRNA, stabilizing the mRNA, and facilitating its accumulation to high concentrations. This stabilization may occur via an interaction between the α-complex and the poly(A)-PABP complex, protecting the poly(A) tail from degradation. Mutations (for example CS) that prevent formation or function of the α-complex reduce mRNA stability and expose the poly(A) tail to accelerated shortening. As the erythroblast differentiates and accumulates a critical level of globin mRNA, all transcription is silenced. At this point the levels of αCP fall and the α-complex dissociates from the 3′-UTR. Both the normal (wt) and mutant (CS) α-globin mRNAs now lack the α-complex and have equivalent stability. The poly(A) tails of both are now shortened at an equal rate with consequent clearance of the mRNA.

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