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AUF-1 and YB-1 independently regulate β-globin mRNA in developing erythroid cells through interactions with poly(A)-binding protein

Sebastiaan van Zalen [1], *Alyssa A. Lombardi*, *Grace R. Jeschke*, *Elizabeth O. Hexner*, *J. Eric Russell* *

Department of Medicine (Hematology-Oncology), Perelman School of Medicine, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, USA

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ABSTRACT

The normal expression of β-globin protein in mature erythrocytes is critically dependent on post-transcriptional events in erythroid progenitors that ensure the high stability of β-globin mRNA. Previous work has revealed that these regulatory processes require AUF-1 and YB-1, two RNA-binding proteins that assemble an mRNP *β-complex* on the β-globin 3′UTR. Here, we demonstrate that the β-complex organizes during the erythropoietic interval when both β-globin mRNA and protein accumulate rapidly, implicating the importance of this regulatory mRNP to normal erythroid differentiation. Subsequent functional analyses link β-complex assembly to the half-life of β-globin mRNA *in vivo*, providing a mechanistic basis for this regulatory activity. AUF-1 and YB-1 appear to serve a redundant post-transcriptional function, as both β-complex assembly and β-globin mRNA levels are reduced by coordinate depletion of the two factors, and can be restored by independent rescue with either factor alone. Additional studies demonstrate that the β-complex assembles more efficiently on polyadenylated transcripts, implicating a model in which the β-complex enhances the binding of PABPC1 to the poly(A) tail, inhibiting mRNA deadenylation and consequently effecting the high half-life of β-globin transcripts in erythroid progenitors. These data specify a post-transcriptional mechanism through which AUF1 and YB1 contribute to the normal development of erythropoietic cells, as well as to non-hematopoietic tissues in which AUF1- and YB1-based regulatory mRNPs have been observed to assemble on heterologous mRNAs.

1. Introduction

Post-transcriptional events that regulate the stabilities of individual mRNAs are increasingly recognized for their impact on cell development and differentiation: genome-wide analyses attribute ~50% of changes in gene expression to processes that alter mRNA half-lives (Cheadle et al., 2005). Events that regulate mRNA stability are particularly important in terminally differentiating erythroid progenitor cells that are transcriptionally silent, but remain translationally active (Greer et al., 2009). The critical impact of post-transcriptional process to normal erythropoiesis is illustrated by functional studies of mRNAs that encode human α and β globins, the principal

[1] Present address: The Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104, USA.
* Corresponding author. Department of Medicine (Hematology-Oncology), Perelman School of Medicine, University of Pennsylvania, Biomedical Research Building II/III, Room 808, 421 Curie Boulevard, Philadelphia, PA 19104, USA. Tel.: +1 (215) 898 4406; fax: 215-573-7039.
E-mail address: jeruss@mail.med.upenn.edu (J.E. Russell).
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proteins expressed in erythrocytes. The two globin mRNAs exhibit half-life values that exceed 20 hr *in vivo* (Ross and Sullivan, 1985; Volloch and Housman, 1981), which provide for their selective enrichment both in transcriptionally silent erythroid progenitors and in their anucleate progeny, and account for the consequent high-level expression of their encoded α- and β-globin proteins. Congenital molecular defects that compromise the stabilities of either mRNA effect significant decrements in globin production that can precipitate serious clinical conditions that are collectively termed *thalassemias* (Clegg et al., 1971; Peixeiro et al., 2011; Weiss and Liebhaber, 1994). The clear relevance of post-transcriptional processes to normal hemoglobinization has fostered intense interest in defining the *cis*-acting mRNA determinants and corresponding *trans*-acting factors that mediate this critical aspect of definitive erythropoiesis.

We recently identified two *trans*-acting factors that regulate the level of β-globin mRNA in erythroid cells through a mechanism that, while not yet fully elucidated, exhibits several novel features (van Zalen et al., 2012). The two RNA-binding proteins (RBPs), AUF-1 and YB-1, assemble an erythroid-specific messenger ribonucleoprotein (mRNP) on a region of 3′UTR that has previously been implicated as a determinant of β-globin mRNA stability (Jiang et al., 2006). This mRNP, which we term the 'β-complex', is cytoplasm-restricted and erythroid-specific, suggesting that it participates in regulating β-globin mRNA at the stages of erythroid differentiation when progenitor cells are transcriptionally silenced and, subsequently, extrude their nuclei. Several well-described properties of AUF-1 and YB-1 are consistent with this possibility: both proteins express at high levels in late erythropoiesis (van Zalen et al., 2012), display mRNA-stabilizing activities (Capowski et al., 2001; Fellows et al., 2012; McGray et al., 2011), and interact with poly(A) binding protein (PABP) (Higashi et al., 2011; Laroia et al., 1999), a factor that enhances the half-lives of heterologous mRNAs by maintaining the integrities of their polyadenylate tails (Bernstein et al., 1989; Wilusz et al., 2001).

Investigations from our laboratory and from others have provided insights into the specific mechanisms through which AUF-1 and YB-1 may effect the unusually high stability of β-globin mRNA. Functional analyses conducted *in vivo* suggest that AUF-1 and YB-1 act redundantly, as coordinate depletion of both factors is required to reduce levels of β-globin mRNA at steady state (van Zalen et al., 2012). Structural assays performed *in vitro* demonstrate that the two proteins bind independently to the β-globin 3′UTR, fully according with the proposed model for functional redundancy (van Zalen et al., 2012). While revealing, these data do not address more fundamental mechanistic questions, e.g., whether AUF-1 and YB-1 bind simultaneously to single β-globin transcripts or, alternatively, bind independently to different β-globin mRNAs. Both possibilities are consistent with previously described interactions between the two RBPs and several non-globin RNAs. For example, AUF-1 and YB-1 bind simultaneously to a synthetic AU-rich RNA (Moraes et al., 2003), consistent with their inclusion in a functional multiprotein mRNP (Skalweit et al., 2003). In contrast, AUF-1 and YB-1 bind independently to defined mRNA-stability motifs in the 3′UTRs of mRNAs that encode GM-CSF, VEGF, and TSP-1 (Capowski et al., 2001; Fellows et al., 2012; McGray et al., 2011), illustrating the capacities of these regulatory factors for structural and functional independence. A more comprehensive description of the mechanism(s) through which AUF-1 and YB-1 regulate β-globin mRNA, as either heteromeric or monomeric mRNPs, is fundamental to understanding the post-transcriptional processes that are required for normal erythropoiesis.

It is likely that the mechanism through which AUF-1 and YB-1 regulate levels of β-globin mRNA reflects a modification of molecular processes that contribute to the decay of heterologous transcripts. mRNA degradation in eukaryotes characteristically initiates with 3′→5′ exonucleolytic digestion of the poly(A) tail, proceeds with enzymatic removal of the 5′m^7GpppG cap structure, and is completed by endonucleolytic degradation of the deadenylated, decapped transcript (Parker and Song, 2004; Wahle and Winkler, 2013). This process is inhibited by the cytoplasmic form of PABP (PABPC1), which binds as a multimer to the mRNA poly(A) tail and protects it from decay-initiating deadenylation (Bernstein et al., 1989; Wilusz et al., 2001). A number of RBPs that stabilize heterologous mRNAs – including αCP, HuR, and TTP – appear to execute their effects by enhancing the interaction between PABPC1 and the poly(A) tail (Kedar et al., 2010; Nagaoka et al., 2006; Wang et al., 1999). As both AUF-1 and YB-1 have previously been shown to interact with PABPC1 bound to non-globin mRNAs (Higashi et al., 2011; Laroia et al., 1999), it is reasonable to speculate that these two regulatory factors stabilize β-globin mRNA through a related process.

The present manuscript defines key features of the mechanism through which AUF-1 and YB-1 regulate β-globin mRNA during normal erythropoiesis. We demonstrate that primary erythroid progenitor cells acquire the capacity to assemble an mRNP β-complex during the developmental interval when there is both an exponential increase in the level of β-globin mRNA and a corresponding accumulation of β-globin protein. Subsequent experiments demonstrate that the two components of the β-complex – AUF-1 and YB-1 – exhibit both structural and functional redundancy. Additional analyses consider the mechanism that underlies the mRNA-stabilizing properties of the β-complex, and establish causal relationships between β-complex assembly, β-globin mRNA polyadenylation, and β-globin mRNA stability. Our results are consistent with the unique process of global transcriptional silencing that characterizes terminal differentiation in erythroid cells, and demonstrate that the β-complex ensures the high half-life of β-globin mRNA by enhancing a protective interaction between PABPC1 and the mRNA poly(A) tail. The data additionally specify a post-transcriptional mechanism that may participate in the regulation of mRNAs that assemble AUF1- and YB1-based mRNPs in a variety of non-hematopoietic cells and tissues.

2. Results

2.1. *Concurrent induction of β-globin expression and β-complex assembly during erythropoiesis*

The putative regulatory properties of the mRNP β-complex predict its assembly in erythroid progenitor cells during the interval when β-globin mRNA begins to accumulate rapidly. We