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Translational regulation and deregulation in erythropoiesis

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Translational regulation plays a critical role in erythropoiesis, as it reflects the translational needs of enucleated mature erythroid cells in the absence of transcription and the large translational demands of balanced globin chain synthesis during erythroid maturation. In addition, red blood cells need to respond quickly to changes in their environment and the demands of the organism. Translational regulation occurs at several levels in erythroid cells, including the differential utilization of upstream open reading frames during differentiation and in response to signaling and the employment of RNA-binding proteins in an erythroid cell-specific fashion. Translation initiation is a critical juncture for translational regulation in response to environmental signals such as heme and iron availability, whereas regulatory mechanisms for ribosome recycling are consistent with recent observations highlighting the importance of maintaining adequate ribosome levels in differentiating erythroid cells. Translational deregulation in erythroid cells leads to disease associated with ineffective erythropoiesis, further highlighting the pivotal role translational regulation in erythropoiesis plays in human physiology and homeostasis. Overall, erythropoiesis has served as a unique model that has provided invaluable insight into translational regulation.

Protein synthesis is a well-orchestrated fundamental process that is tightly regulated in every organism. All cells share the basic machinery and mechanisms for translating mRNA into proteins; however, different cell types have specialized translational needs. Protein synthesis plays a key role in maintaining cellular homeostasis and is carefully regulated at both the transcription and translation levels. Although regulation of protein synthesis can occur at any one of the various stages of the translation pathway, it is translation initiation that is the key regulatory stage [1]. Moreover, translational control plays an important role in regulating the expression of many genes in response to exogenous or endogenous signaling [2].

Erythroid progenitors (colony forming units–erythroid or CFU-Es) divide three to five times over 2 or 3 days as they differentiate and undergo dramatic morphological and molecular changes, such as a decrease in cell and nuclear size, chromatin condensation, hemoglobinization, and eventually loss of the nuclei, resulting in enucleated reticulocytes that are released into the circulation [3]. Reticulocytes are highly translationally active before losing their ribosomes, organelles, and mRNAs as they transition to mature erythrocytes in the circulation [4]. Because of the lack of active transcription in reticulocytes and lack of translation in mature cells, the fine tuning of translational control is essential for ensuring that protein synthesis provides for the necessary protein complement for physiological erythroid cell functions. Disturbances in the translational machinery can lead to anemia and bone marrow failure syndromes [5]. As such, erythropoiesis has served as a very good model for studying translational control in cell differentiation and physiology. For instance, iron-regulated protein translation in erythroid cells was one of the first examples of translational regulation to be described.

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Here, IRP1 and IRP2, as two RNA-binding proteins (RBPs) that recognize a conserved iron response element (IRE) close to the cap region, interact with the translational machinery to regulate gene expression depending on iron availability in erythroid cells [6]. This mechanism is essential for the homeostatic regulation of many mRNAs, such as those associated with iron storage (e.g., ferritin) [7].

Another example of translational control is that of microRNAs (miRNAs), which were reported to play a role in erythropoiesis by regulating the translation of erythroid genes or of transcription factors involved in erythropoiesis [8]. Examples include miR-144 and miR-451, which are the highest-expressing miRNAs in erythroid cells, activated by GATA1 in late erythroid maturation and fulfilling key roles in erythroid differentiation [9–12]. Another GATA1-induced miRNA is miR-23a, which regulates SHP2, a protein tyrosine phosphatase that is essential for normal erythropoiesis [13]. Several other miRNAs regulating translation during erythropoiesis are extensively described in Bianchi et al. [14].

Despite the utility of the erythroid cell in studying basic translation, the control mechanisms regulating translation in erythropoiesis have not been fully clarified. In this review, we provide an overview of translational regulation in red blood cell differentiation by focusing on translation initiation as a major regulatory juncture and on ribosome recycling as an emerging principle in translational regulation.

Regulation of translational initiation in erythropoiesis

Translational initiation represents a major stage for translational regulation in all cells. In eukaryotes, it requires a large set of eukaryotic translation initiation factors (eIFs) that coordinate several phases of the process. Several eIFs are involved in the formation and the activation of various complexes that facilitate the identification and the correct binding of the start codon (AUG) and the formation of different preinitiation and elongation-competent initiation ribosomal complexes. In brief, during the early stages of translation initiation, a ternary complex (TC) composed of transcription factor 2 (eIF2), GTP, and methionyl-tRNA (Met-tRNAi) binds to the small ribosomal subunit (40S) [15]. The binding of TC to 40S is thought to be stimulated by eIF1A and leads to the formation of the 43S preinitiation complex (43S PIC). eIF1 also participates in the assembly and activation of the 43S PIC by supporting the formation of the codon–anticodon interaction [16,17]. The 43S PIC binds to messenger RNA (mRNA), with the contribution of eIF4B. eIF3:eIF4B is responsible for the recruitment of mRNA to the initiation complex, whereas eIF3 is involved in the attachment of PIC to the mRNA [16,18]. eIF3 binds the 40S subunit, eIF1, and eIF4G and interacts with the mRNA cap binding complex eIF4F. EIF4F proteins mediate the binding of PABP, eIF4E, eIF4A, and eIF3, leading to the formation of a loop structure which represents the activated form of mRNA. After binding, the 43S PIC scans the mRNA to find an AUG start codon to base-pair with the anticodon of methionine tRNA. This triggers the transformation of eIF2–GTP to eIF2–GDP activated by eIF5, which, in turn, interacts with the 40S initiation complex [19]. Finally, the large ribosomal subunit (60S) binds the 43S complex, with the participation of eIfF5B, forming the complete and fully functional 80S ribosome [15].

Enhanced translational initiation of erythroid-specific proteins

Recent work in erythroid cells revealed that translation initiation factors eIF2B, eIF4H, and eIF4G and cofactors eIF5A and RBM42 are upregulated with terminal erythroid differentiation, thus reflecting an upregulation of initiation complexes and enhanced translation initiation [20]. This was reflected by an upregulation in the translation of proteins with more specific erythroid functions in the final stages of differentiation, despite the fact that mRNA levels decrease globally with differentiation. Interestingly, deep sequencing of total RNA (RNAseq) and RNA in complex with ribosomes (Ribo-seq) for the parallel profiling of gene expression and mRNA translation in murine fetal liver erythropoiesis revealed extensive translation of upstream open reading frames (ORFs) in mRNAs coding for proteins with important erythroid functions [20]. Upstream ORFs occur in about 50% of mammalian mRNAs, and a significant proportion of them are translated, suggesting broad regulatory functions [21–23]. Examples of out-of-frame uORF translation identified in erythroid cells include the transcription factors BCL11A, a key regulator of hemoglobin switching [24], and TAL-1, a key activator of the erythroid transcription program [25]. Interestingly, translation of the TAL-1 uORF results in the expression of truncated TAL-1 isoforms previously associated with erythroid lineage choice [26]. There was also evidence of alternative in-frame 5′ UTR start-codon utilization, resulting in N-terminal protein extensions, and of stop-codon bypass, resulting in C-terminal extensions in proteins enriched for transcriptional regulation and cell homeostasis functions. Taken together, these observations provide compelling evidence for alternative translation initiation in erythropoiesis that is very likely of functional significance.

Translational regulation in response to SCF signaling

Regulation at the level of translational initiation appears to be an important regulatory aspect also in the expansion of erythroid progenitors in response to stem cell factor (SCF)

signaling. Specifically, it was previously reported that SCF signaling influences the availability of the limiting eIF4E translation initiation factor [27]. Availability of eIF4E is determined by its interaction with the inhibitory 4E-binding proteins (4E-BPs) which, when unphosphorylated, bind to eIF4E, thus preventing its participation in the initiation complex [28]. SCF signaling results in the PI3K-mediated, rapid 4E-BP phosphorylation and its disassociation from eIF4E, which is now available to carry out its translation initiation functions (Fig. 1). In addition, SCF further influences eIF4E availability through an increase in eIF4E expression levels [27]. Interestingly, overexpression of eIF4E in murine pro-erythroblasts blocks terminal erythroid differentiation, suggesting that eIF4E levels represent a critical juncture in the translational regulatory mechanism that controls expansion of erythroid progenitors versus terminal differentiation [27]. Further work indicated that the SCF-mediated increase in eIF4E levels results in the selective recruitment to polysomes of distinct subsets of mRNAs, which presumably fulfill important functions in promoting erythroid progenitor expansion [29].

Balanced globin chain synthesis

Another critical aspect of translational control in erythropoiesis is the massive synthesis of the components of hemoglobin (α-globin, β-globin, and heme). This needs to be balanced as an imbalance is cytotoxic to erythrocytes [30]. Besides its role in hemoglobin synthesis, heme also functions as a signaling molecule as it can recognize and bind to two specific binding sites of the heme-regulated inhibitor (HRI) protein [30]. HRI is an eIF2α kinase that, depending on the availability of heme, regulates the synthesis of globin chains in erythroid cells [1,31,32]. eIF2α forms part of a translation initiation ternary complex (TC) with eIF2β and eIF2γ that binds to GTP and recruits the first methionine tRNA to the 40S ribosomal subunit, thus initiating scanning along the 5′ UTR for the detection of a start codon (Fig. 2A). Recognition of a start codon results in GTP hydrolysis to GDP and release of the TC, which becomes available for recycling in further rounds of translational initiation [1]. In heme deficiency, HRI becomes activated and phosphorylates

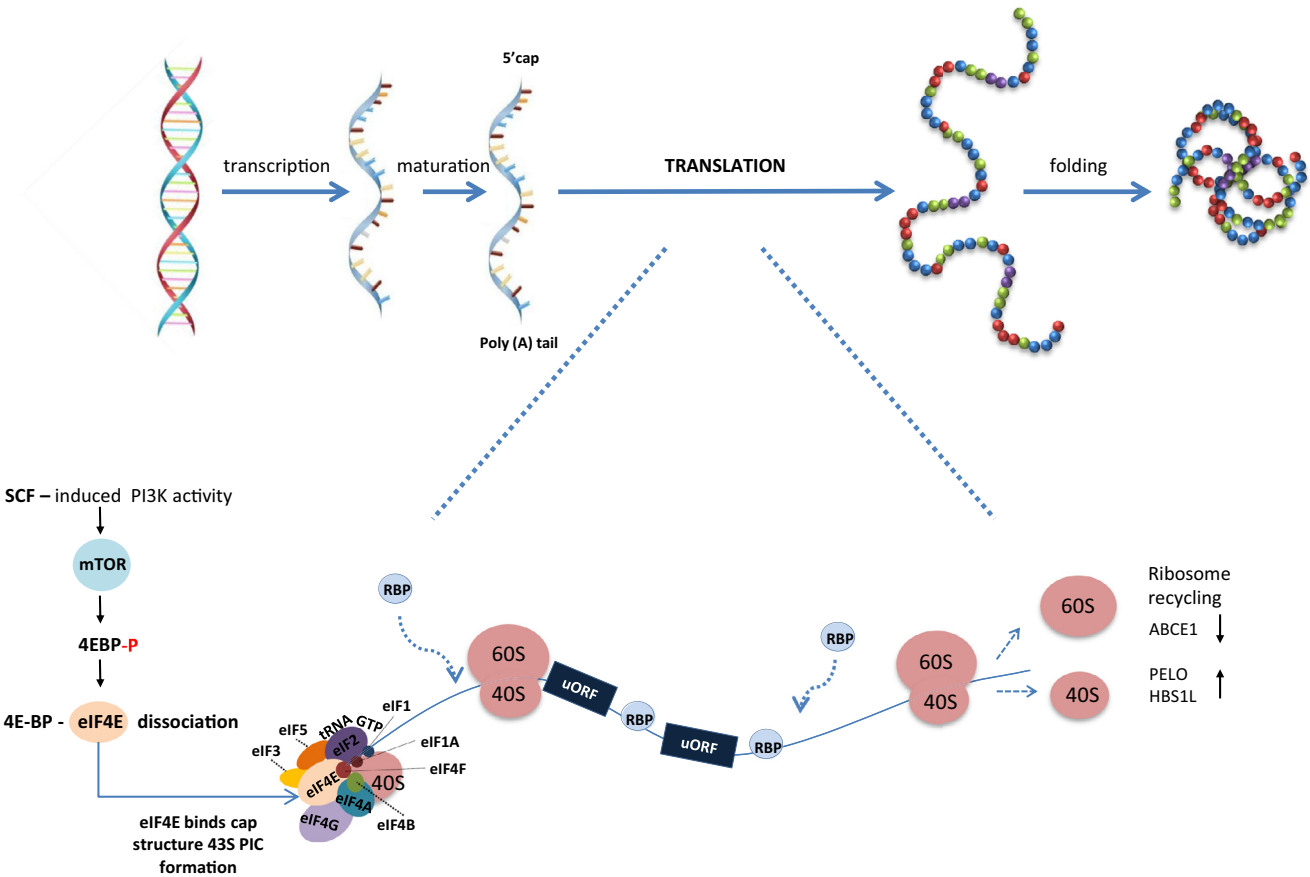

Figure 1. Overview of various levels of translational regulation in erythroid cells, for example, through SCF signaling, uORFs, RBPs, and ribosome recycling (see main text for more details). SCF induces phosphorylation of 4EBP through mTOR signaling, leading to the release of the cap-binding factor eIF4E. After eIF4E binding, eIF4F complex is formed by the recruitment of eIF4A and eIF4G proteins. Then the ternary complex (eIF2-GTP, tRNA) is recruited together with the 40S ribosomal subunit and eIF1, eIF1A, eIF3, and eIF5 factors to form the 43S pre-initiation complex (PIC).

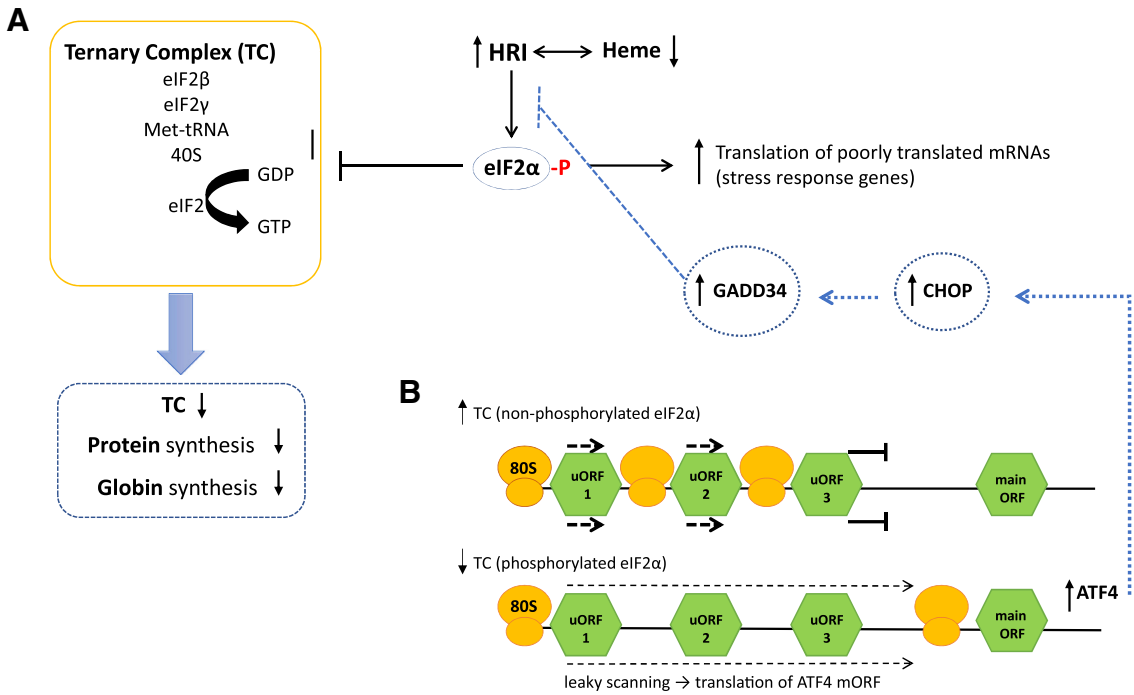

Figure 2. Mechanisms through which heme-regulated inhibitor (HRI) regulates translation in erythroid cells. **(A)** In the absence of heme, HRI phosphorylates eIF2α, resulting in the reduction of globin chain translation. When available, heme binds HRI, blocking its activity and activating the ternary complex and globin chain synthesis. **(B)** Parallel to the downregulation of globin chain translation, eIF2α phosphorylation increases the translation of poorly translated transcripts, such as that for ATF4. ATF4 activates the transcription factor CHOP and expression of GADD34, which promotes protein phosphatase 1 (PP1)-mediated dephosphorylation of eIF2α. This mechanism results in re-initiation of protein synthesis.

eIF2α, thus blocking its productive recycling and reducing the translation of globin chains (Fig. 2A). By contrast, when there is availability, heme binds to HRI and prevents its activity, thus allowing the recycling of eIF2α and the synthesis of globin chains. This translational regulatory mechanism safeguards against an imbalance in the synthesis of hemoglobin components which would otherwise cause proteotoxicity [31,32].

Iron deficiency

Translational regulatory mechanisms involving eIF2α phosphorylation also safeguard against ineffective erythropoiesis caused by iron deficiency (ID). ID results in decreased heme production, HRI activation [32,33], and oxidative stress [31,34]. Phosphorylation of eIF2α by HRI selectively increases the translation of poorly translated mRNAs for adaptation to oxidative stress [31]. One such mRNA codes for activating transcription factor 4 (ATF4), which induces the expression of stress response genes [35]. Translational regulation of ATF4 expression is controlled by the presence of uORFs in the 5′ UTR of its mRNA [36]. Under normal conditions, the presence of uORFs does not allow the translation of the ATF4mORF. However, stress conditions favor leaky scanning and translation of the ATF4mORF, which, in turn, activates the transcription factor C/EBP homologous protein-10 (CHOP) [37] (Fig. 2B). This leads to the expression of GADD34, which promotes dephosphorylation of eIF2α through its interaction with protein phosphatase 1 (PP1) [38], thus reactivating IF2α and reinitiating protein synthesis during the later stages of stress response [39] (Fig. 2B). The importance of this translational regulatory mechanism is highlighted by the fact that exposure of HRI knockout mice to ID results in inhibition of erythroid differentiation at the basophilic erythroblast stage and exacerbated protoporphyria and thalassemia phenotypes [35,40]. In both cases, HRI seems to moderate the severity of the disease by modulating protein expression in erythroid cells. In protoporphyria, the production of PPIX is affected, whereas in β-thalassemia an accumulation of α-globin is observed. Importantly, both HRI copies are necessary to mitigate the severity of the diseases.

Iron deficiency also leads to hypoxia as a result of the decrease in heme. Hypoxic conditions trigger elevated erythropoietin (Epo) expression in the kidney and upregulation of the mammalian target of rapamycin complex 1 (mTORC1) signaling in erythroid cells [41]. mTORC1 is a protein complex that controls protein synthesis by sensing the resources available for protein production [42]. The complex comprises mTOR, mammalian lethal with SEC13 protein 8 (MLST8), PRAS40, and DEPTOR [43]. mTORC1 is

activated when the cells have adequate energy resources, nutrient availability, oxygen abundance, and the appropriate growth factors for mRNA translation to begin [44].

In erythroid cells, the HRI–eIF2α–ATF4 pathway fulfills an additional regulatory role by suppressing mTORC1 signaling, which is increased as a result of elevated Epo during ID in reducing ineffective erythropoiesis. Importantly, $Hri^{-/-}$ and $Atf4^{-/-}$ mice suffer from ineffective erythropoiesis when challenged with ID, presenting with defects in differentiation and elevated mTORC1 signaling in erythroid precursors. HRI therefore plays a key role in erythropoiesis through regulation of the two key pathways of eIF2α and mTORC1 [33]. The HRI–eIF2α–ATF4 pathway is active in erythroid differentiation and is very important for the regulation of protein expression and, hence, for erythroid differentiation [31]. Accordingly, HRI expression increases with erythroid commitment differentiation, with the highest expression levels observed in $CD71^{+}/GPA^{+}$ erythroblasts [45].

The key role that the mTORC1 pathway plays in erythroid cell growth and proliferation has been verified through different studies, including an unbiased analysis of proteomic and transcriptomic changes during human erythropoiesis [46], in in vivo and in vitro experiments with $Foxo3^{-/-}$ mice [47] and a screen for cells with high levels of signaling through mTORC1 [48]. Liu et al. [46] reported that mTORC1 inhibition impaired translation of mitochondria-associated proteins during erythropoiesis, suggesting that mitochondria-associated mRNAs are selectively sensitive to enhanced protein translation in erythroid cells. In another study, the investigation of mechanisms underlying alterations of Foxo3-deficient erythroid cell cycling led to identification of the overactivation of the JAK2/AKT/mTOR signaling pathway, suggesting that FOXO3 and mTOR together may be part of a metabolic network during erythroid cell maturation [47]. A screen for cells with high levels of signaling through mTORC1 resulted in identification of the regulatory role the mTORC1 pathway plays in red cell growth and proliferation and established that perturbations in this pathway result in anemia [48].

RNA-binding proteins in erythroid cells

RNA-binding proteins (RBPs) are an integral part of the translation machinery and are essential for the translation of mRNAs. In general, RBPs work by identifying particular motifs in the 5′ and 3′ UTRs and by interacting with the translation machinery [6]. RBPs may also function in a selective way by regulating the translation of specific mRNAs. As outlined above, the archetypical RBPs are the IRP1 and IRP2 proteins in iron metabolism. In addition to IRPs, which have been extensively reviewed previously, other RBPs also play an important role in the control of translation in erythropoiesis. For example, CPEB4 is an RBP that is strongly induced during terminal erythroid differentiation by the joint action of the GATA1 and TAL-1 erythroid transcription factors [49]. CPEB4 binds to the translation initiation factor eIF3 in erythroid cells, thus repressing the translation of a large subset of mRNAs involved in processes like chromatin structure and cell cycle regulation, which undergo dramatic changes during terminal erythroid differentiation [49]. Importantly, loss-of-function studies, as well as ectopic expression of CPEB4, led to the impairment of erythroid differentiation, highlighting the essential functions it fulfills in erythropoiesis [49]. CPEB4 is subjected to translational regulation itself as it binds its own mRNA to repress its translation as a mechanism to maintain CPEB4 protein at a level that is essential for proper erythroid differentiation [49].

RBM38 is another RBP that is highly induced with terminal erythroid differentiation, and its expression appears to be regulated by GATA1 and TAL-1 [20]. Erythroid functions for RBM38 were previously suggested by the Rbm38 gene knockout in mice, which showed defective erythropoiesis and symptoms of anemia [47]. Importantly, in one study [20], interrogation of the UTR sequences of mRNAs that are dynamically controlled during erythropoiesis identified the RBM38 motif as being the one that is most highly represented. Further analysis revealed that mRNAs strongly bound by RBM38 in mouse erythroleukemia (MEL) cells were enriched for proteins that are highly synthesized in late differentiating erythroid cells and that include proteins involved in cell maintenance and specialized functions, including translation initiation factors. Thus, it appears that RBM38, at least in part, functions to boost translation of select mRNAs with important functions in later stages of erythroid differentiation, when new mRNA synthesis winds down or is altogether absent. The functional significance of these findings is supported by the observations that the RBM38 knockdown in MEL cells severely impairs terminal erythroid differentiation and enucleation [20].

CSDE1 (cold shock domain protein e1) is a widely expressed RBP implicated in the translational control of many mRNAs such as Apaf1and Cdk11B in human cells [50,51]. Interestingly, Csde1 expression increases in erythroblasts compared with other hematopoietic cells, whereas the Csde1 knockdown in a murine erythroid cell model impairs erythroblast proliferation and differentiation [52]. CSDE1-bound transcripts in murine erythroblasts are enriched for functions related to ribogenesis, mRNA translation, and protein stability and to mitochondrial function, whereas deletion of CSDE1's functional domains revealed functions in transcript stability and translation and protein homeostasis [53]. Recent work revealed that the STRAP

protein functionally interacts with CSDE1 [54]. STRAP belongs to the WD40 family of proteins, which generally function as a platform for protein–protein interactions. The STRAP knockdown led to reduced protein translation of many CSDE1-bound transcripts, while enhancing expression of others. Affected transcripts included mRNAs for genes involved in terminal erythroid differentiation, cell cycle regulation, and the hypoxic response, thus providing a potential molecular basis for CSDE1's erythroid-specific functions [54].

Regulation of ribosome levels and recycling in erythropoiesis

In mammals, ribosome biogenesis requires equimolar production of all ribosomal proteins (RPs) and ribosomal RNAs (rRNAs) and coordinated activity of all three RNA polymerases orchestrated by several ribosome assembly factors [55]. Tight regulation of ribosome biogenesis is a key aspect of cell growth control, as ribosome biogenesis consumes a lot of cellular energy and molecular building blocks. Thus, as growth conditions change, cells must rapidly adapt their ribosome production based on the available resources. The regulation of ribosome biogenesis occurs primarily at the transcriptional level and involves all three nuclear RNA polymerases. Pol I transcribes rDNA encoding the 35S rRNA precursor, Pol II transcribes the RP genes, and Pol III produces 5S rRNA (and tRNA) [56]. A central sensor and regulator of cell growth and metabolism in all eukaryotes is the mTORC1 complex (see **Iron Deficiency**).

Mutations in genes that encode either RPs or ribosome biogenesis factors result in ribosomopathies, which have a particular impact on the erythroid lineage giving rise to Diamond–Blackfan anemia (DBA) and $5q^-$ syndrome. Recent work investigating the molecular basis of DBA-associated pathologies led to the discovery that ribosome levels, rather than RP composition, are critical for physiological erythropoiesis [57]. Initially, it was found that the ribosomal protein chaperone TSR2 is essential for terminal erythroid maturation. Because TSR2 plays an important role in ribosome production, but does not itself participate in the mature ribosome, the implication of this observation was that ribosome levels are important in erythropoiesis. Quantitative polysome profiling of differentiating erythroid cells bearing DBA-related RP gene knockdowns indeed indicated that these resulted in reduced ribosome levels, whereas quantitative proteomic analysis of ribosome protein composition revealed that this was largely invariant between control cells and cells bearing different RP knockdowns [57]. It is of interest that individual RP gene knockdowns resulted in a global reduction in the translational efficiency of RP gene transcripts, suggesting that they are translationally coregulated in erythroid cells, presumably to maintain an appropriate RP stoichiometry and ribosome levels in addressing the increased translational needs of erythropoiesis [57].

In the light of the importance of maintaining ribosome levels in erythroid cells, perhaps it is not surprising that ribosome recycling also plays a role in translational regulation in erythropoiesis. In fact, ribosome recycling is a key step in translational regulation as it determines the availability of ribosomes and thus the cell's translational capacity. Briefly, during translation termination, eRF1 recognizes all three stop codons and induces release of the nascent polypeptide, and eRF3 (a GTPase) enhances polypeptide release. Subsequently, the ribosome is split in a process mediated by ABCE1. This binds in the ribosomal space between the two ribosomal subunits, interacting extensively with different sites on the 40S subunit and at a single site (rpL9) on the 60S subunit. Cycles of binding of ATP to the binding sites in ABC proteins (yielding a "closed" state), hydrolysis of ATP, and release of ADP (leading to an "open" state) induce conformational changes in these proteins that drive structural changes in associated domains or macromolecules which finally lead to ribosomal splitting [58]. Overall, recycling enables ribosomes and mRNAs to participate in multiple rounds of translation [59].

Recent work described the dynamic regulation of ribosome recycling in erythroid cells [60]. Specifically, ribosome profiling revealed an unexpectedly high ribosome occupancy of 3′ UTRs in human reticulocytes, almost 30-fold higher compared with that of nucleated peripheral blood mononuclear cells. This appears to be a result of declining levels of the ribosome recycling factor ABCE1 in terminal erythroid differentiation, most likely caused by the programmed elimination of mitochondria and consequent cessation in the biosynthesis of an iron–sulfur cluster that is coordinated by the ABCE1 protein and is essential for its function [61]. To compensate for this, there appears to be a transient increase in the levels of the ribosome rescue factors PELO and HBS1L in earlier stages of erythroid differentiation. PELO and HBS1L function as a release mechanism for stalled or unrecycled ribosomes, thus making them available again for translation [62]. The transient activation of the ribosome rescue pathway appears to be an erythroid-specific compensatory mechanism against the decline in ABCE1 levels, thus safeguarding ribosome levels and translation of critical mRNAs, including those for hemoglobin synthesis, in erythroid terminal differentiation [60].

Ribosomopathies and defects in erythropoiesis

Mutations in RP genes, or any genetic abnormality that impairs biogenesis and function of the ribosome, result in specific clinical phenotypes collectively known as ribosomopathies, which differ significantly in mechanism, clinical presentation, and treatment options [5]. The erythroid lineage is particularly sensitive to ribosomal dysfunction in ribosomopathies, as evidenced by the hematological disorders DBA and $5q^-$ syndrome. DBA was the first

ribosomopathy to be identified and is characterized by anemia, macrocytosis, reticulopenia, and selective reduction or absence of erythroid precursors in an otherwise normocellular bone marrow [63]. More than 200 constitutive heterozygous mutations in 19 RP genes have been recorded to date, accounting for more than 70% of the DBA cases [64]. RPS19 was the first and most frequently mutated RP gene in DBA, accounting for approximately 25% of DBA cases [65]. The $5q^-$ syndrome is a subtype of myelodysplastic syndrome (MDS) in which somatic deletion of one allele of chromosome 5q results in haplo-insufficiency for the RPS14 gene [66]. Although 5q deletions are large, encompassing many gene loci, defective erythropoiesis is known to be a result of the RPS14 haplo-insufficiency [66,67].

Why erythropoiesis is particularly sensitive to deregulation of the universal process of translation is still not fully understood. It has been established that free RPs in the cytosol resulting from an imbalance in RP biosynthesis in DBA lead to p53 activation, cell cycle arrest, and apoptosis of erythroid cells [68]. It has been proposed that the erythroid selectivity in p53 activation in DBA reflects the highly proliferative rates and large protein translation demands of erythroid progenitors, which massively synthesize globin chains and may thus be more susceptible to RP imbalance and p53 activation [69]. However, the extent of p53 activation and the accompanying impact on erythropoiesis appear to differ depending on which RP gene is mutated in DBA [70]. In addition, ribosomal deficiencies may result in defective translation of mRNAs with essential functions in human and murine erythropoiesis such as Bag1, Csde1, and gata1 [52]. Furthermore, Sankaran et al. [71,72] reported that RP haplo-insufficiency in DBA affects translation of the mRNA for the critical erythroid transcription factor GATA1. These observations led to the suggestion of the ribosome concentration hypothesis [60]. This postulates that the sensitivity of the erythroid lineage to translational (ribosomal) imbalance reflects the selective reduction in the translation of specific mRNAs with inherently low translational efficiencies, such as that for GATA1, which may become very poor substrates for translation when ribosome availability becomes limited because of impaired RP gene expression, as opposed to mRNAs with high translation rates such as those for globin chains [60].

The ribosome concentration hypothesis received experimental support from the recent study of [57], which used ribosome profiling and quantitative proteomics to illustrate that ribosome levels, rather than protein composition, are important in physiological erythropoiesis (see **Regulation of Ribosome Levels and Recycling in Erythropoiesis**). The same study identified several transcripts that were translationally downregulated by DBA-associated RP deficiencies, which included transcripts of genes that are essential for growth in hematopoietic cells. DBA-sensitive transcripts shared common structural features in that they were, on average, shorter in length with shorter 5′ UTRs that were predicted to have less complex secondary structures and fewer uAUGs [57]. Such features are generally associated with efficient ribosome initiation and translation and may reflect underlying regulatory mechanisms employed upon commitment to the erythroid lineage to rapidly upregulate proteins that are important for erythroid differentiation and functions.

In seeking to explain the susceptibility of the erythroid lineage to RP deficiencies in DBA, several studies identified the translational deregulation of the erythroid master transcription factor GATA1 as a key contributing factor. Mutations in the X-linked human *GATA1* gene resulting in reduced GATA1 protein levels were identified in DBA patients in the absence of RP gene mutations, suggesting that a reduction in GATA1 protein was sufficient to cause disease [71]. Furthermore, deficiencies in RPs in DBA act upstream of GATA1 in downregulating its protein levels (while mRNA levels remain unaffected), with subsequent defects in erythroid differentiation similar to those observed in DBA [57,72]. The GATA1 mRNA has an unusual 5′ UTR sequence that shares characteristics with the 5′ UTRs of transcripts that are translationally sensitive to RP deficiencies; that is, they are shorter in length and predicted to have less complex secondary structures, facilitating translation initiation [57]. Remarkably, by use of a functional complementation assay, it was found that the very presence of the GATA1 5′ UTR is critical for GATA1 functions in erythroid differentiation [57], providing an explanation for the sensitivity of GATA1 translation to ribosomal levels in erythroid cells. Recent evidence has also implicated the ribonuclease inhibitor 1 (RNH1) protein as providing essential, as yet not fully characterized, erythroid-specific functions by associating with ribosomes and enhancing translation of GATA1 and other important erythroid regulatory factors, such as FOG-1 [73]. Lastly, GATA1 protein levels may also be indirectly affected by ribosomal deficiencies, at least in some cases of DBA. Specifically, it was recently reported that in RPL11- and RPL5-deficient human primary erythroid cells, but not in RPS19-deficient cells, HSP70 protein becomes degraded, resulting in defects in the proliferation and differentiation of erythroid cells [74]. HSP70 is a chaperone that protects GATA1 from degradation by caspase-3 during erythroid differentiation [75]. Hence, increased HSP70 degradation in RPL11- and RPL5-deficient cells results in increased GATA1 protein cleavage, which presumably underlies the observed erythroid defects [74].

Conclusions

It has become clear in recent years that translational regulation is a major feature of erythroid differentiation and a reflection of the peculiar physiology of the red blood cell. Erythroid differentiation is regulated at many levels of the protein translation pathway, from the specific structure of 5′ UTRs of mRNAs coding for erythroid functions to the differential utilization of uORFs, from the regulation of translation initiation complexes to erythroid-specific RBP functions, and from ribosome levels to erythroid-specific ribosome recycling mechanisms (Fig. 1). As such, the erythroid system has served exceptionally well as a model for studying translational regulation in general, for example, in establishing that ribosome levels rather than composition (as in specialized ribosomes) are important in erythropoiesis. The importance of translational regulation in erythropoiesis is highlighted by the erythroid-specific pathologies of ribosomopathies, such as DBA and $5q^-$ syndrome, which have provided strong motivation and many opportunities for uncovering important aspects of translational regulation in erythropoiesis. For example, studies investigating DBA have converged on the importance of the, direct or indirect, translational (de)regulation of the key transcriptional regulator GATA1 as a critical factor in the erythroid-specific pathologies of ribosomopathies and other conditions of ineffective erythropoiesis. Recent work suggested that GATA1 may in fact act upstream in regulating expression of the translational machinery in erythroid cells [76,77], adding an interesting twist that remains to be investigated in detail. It is also of interest that key players of translational regulation in erythroid cells, such as HRI, may also fulfill additional erythroid functions that are not directly related to protein translation [45]. Taken together, it is clear that erythropoiesis will continue to serve as a central model for elucidating protein translation regulation in physiology and deregulation in human disease.

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