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Erythroid Differentiation: A Matter of Proteome Remodeling

By Francesca Vinchi[1,2]

Correspondence: Francesca Vinchi (e-mail: francesca.vinchi@libero.it).

Mature red blood cells (RBCs) transport oxygen throughout the body from pulmonary capillaries to peripheral tissues. The maturation of RBCs from hematopoietic stem cells features a highly specialized and sophisticated process of cellular differentiation, which requires a combination of selective protein synthesis, protein degradation, and organelle extrusion. To make space for the oxygen-carrying protein hemoglobin, erythrocyte precursors shed most organelles, including nucleus, mitochondria, and ribosomes, and intensively remodel the proteome. In fact, during their terminal maturation, erythroblasts produce and accumulate prodigious amounts of hemoglobin, which eventually comprises 98% of the total cellular protein. To eliminate unnecessary proteins, erythrocyte precursors activate a program of degradation of pre-existing cellular proteins. This is accomplished through the function of the ubiquitin-proteasome system (UPS). In most cells, this degradation pathway removes misfolded, aged, and damaged proteins to avoid their accumulation and cell toxicity, whereas in erythroid precursors it mainly acts to specifically remodel the proteome and enrich the cell with hemoglobin. "Trash" proteins are selected for degradation via covalent attachment of ubiquitin by ubiquitin-conjugating enzymes (E1, E2, E3). Ubiquitin tagging allows the proteasome to properly recognize proteins destined for destruction. However, the molecular mechanism that determines which proteins get destroyed and which are spared during differentiation has remained elusive.

Recently, the ubiquitin conjugating enzyme UBE2O has moved into focus, as it is highly expressed at the same time with globins, in terminally differentiating reticulocytes. To gain mechanistic insight into the function of UBE2O during RBC maturation, Nguyen et al[1,2] took advantage of a mouse model of UBE2O deficiency, carrying a nonsense mutation in the gene encoding this protein. As described in their recent Science paper, UBE2O-null mice exhibit microcytic hypochromic anemia, hallmarked by an elevated number of small RBCs with reduced hemoglobin content. Surprisingly, the lack of a single ubiquitinating factor produced a drastic reduction in the overall ubiquitin-conjugated protein species in reticulocytes, suggesting a prominent role for UBE2O in the regulation of protein degradation during RBC maturation. The authors specifically identified ribosomal proteins as the main target of UBE2O, accounting for almost 90% of all the ubiquitinated proteins within reticulocytes. Proteomic approaches revealed that, among several ribosomal and nonribosomal proteins, RPL29 and RPL35 are 2 of the most relevant substrates of UBE2O (Fig. 1).

Erythroid differentiation requires a dynamic balance between active protein synthesis and selective protein degradation. If on one side the synthetic process is mostly centered on hemoglobin production, the degradative one is extended to a vast number of pre-existing relatively stable molecules, which are unneeded to the mature RBC to exert its oxygen-carrying function. In particular, together with stimulated hemoglobin synthesis, the loss of ribosomal materials is a primary hallmark of the maturation process of reticulocytes to erythrocytes. This publication shows that UBE2O plays a central role in this process by selectively ubiquitinating ribosomal proteins and promoting their degradation. The loss of UBE2O in reticulocytes leads to a defective mechanism of protein ubiquitination, causing extensive intracellular accumulation of ribosomal proteins and ribosomes. Conversely, the overexpression of UBE2O in nonerythroid cells significantly reduced the levels of ribosomal proteins and promoted extensive proteome remodeling through an accelerated process of protein degradation. The addition of recombinant active UBE2O to UBE2O-null reticulocyte lysate, but not its catalytically inactive mutant form, successfully reconstitutes ribosomal protein degradation. Interestingly, in nonerythroid cells UBE2O was sufficient to drive ribosome turnover by itself, without the need of other erythroid

Francesca Vinchi
[1]*Molecular Medicine Partnership Unit, University of Heidelberg and European Molecular Biology Laboratory, Heidelberg, Germany*
[2]*Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY.*
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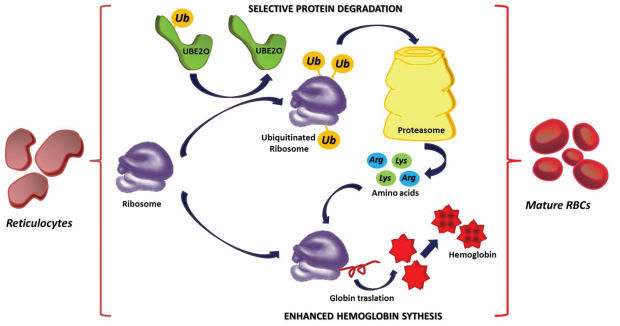

Figure 1. Crucial role of UBE2O in erythroid proteome remodeling during terminal differentiation. The transition from reticulocytes to erythrocytes requires a fine balance between protein degradation and synthesis. UBE2O is an E2-E3 hybrid enzyme that mediates protein ubiquitination, thus dictating selective elimination of proteins by the proteasome. Major substrates of UBE2O are ribosomal proteins, recognized in a free form or assembled ribosomal complex. Protein degradation results in the generation of a flux of free amino acids, which then support the translation of hemoglobin mRNA in the last stage of erthroid differentiation. By promoting extensive proteome remodeling, where hemoglobin is enriched and unwanted proteins eliminated, UBE2O critically contributes to erythroid maturation. Arg = arginine, Lys = lysine, RBC = red blood cells, Ub = ubiquitin.

factors. Indeed, UBE2O emerges as a critical regulator of ribosome breakdown and therefore proteome remodeling. Importantly, the inhibition of the proteasome in wild-type reticulocytes phenocopied the UBE2O-null defect, indicating that UBE2O-stimulated protein degradation requires proteasome activity.

According to Nguyen et al, UBE2O cooperates with few other ligases, to remodel the erythroid proteome and induce RBC terminal differentiation.[1,3] UBE2O mediates a specific, yet broad ubiquitination program, determining the degradation of ribosomes and hundreds of other proteins by the proteasome. In such a way, UBE2O shapes the erythroid proteome, controlling the transition from a complex proteome in the immature reticulocyte to a simple one in the mature hemoglobin-rich erythrocyte (Fig. 1). In vitro experiments with purified proteins demonstrated that UBE2O is an E3 enzyme fused to an E2 ligase, where the E3 domain recognizes the site to be ubiquitinated on the substrate and the E2 region mediates the ubiquitination itself. This hybrid E2-E3 enzyme adds multiple ubiquitin groups to its substrates as mono-ubiquitin modification. Despite an active degradation, during terminal differentiation most ubiquitin factors are transcriptionally down-regulated, suggesting that the UPS is reconfigured during this stage and a small set of specific enzymes is able to entirely support RBC maturation.[1,3] The E2-E3 hybrid nature allows UBE2O to efficiently and autonomously work in the final erythroid differentiation stages, when most E2 enzymes are depleted.

The analysis of UBE2O-deficient reticulocytes also revealed a close connection between the synthetic and degradative processes occurring during erythroid differentiation: protein degradation critically supplies free amino acids needed for globin synthesis. Upon proteasome inhibition or UBE2O loss, free amino acid pools are depleted. The reduced availability of amino acids, such as lysine and arginine, which are abundant in ribosomal proteins, decreases globin production. This explains the hypochromic anemia hallmarked by low hemoglobin levels associated with UBE2O deficiency. Therefore, UBE2O determines the flux of ubiquitinated proteins through the proteasome and, as a consequence, the supply of free amino acids required for mRNA translation (mostly globin mRNA) in the late stage of erythroid differentiation (Fig. 1). It is of note that the loss of UBE2O ameliorates the anemic phenotype of β-thalassemia. The precipitation of excessive α-globin chain caused by the diminished synthesis of β-globin, promotes cell damage and shortens RBC lifespan. The overall reduction of globin synthesis upon UBE2O deficiency limits this process, thus improving RBC numbers and hemoglobin levels. In summary, the work by Nguyen et al[1] proposes UBE2O as a key player of proteome remodeling and RBC maturation. Finally, these observations support the concept of UBE2O targeting for therapeutic application in diseases where metabolic reprogramming and proteome remodeling have a role, from β-thalassemia to different forms of cancer.[4,5]

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