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Mortensen M., Ferguson D.J.P., Simon A.K.

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Mitochondrial clearance by autophagy in developing erythrocytes

Clearly important, but just how much so?

Monika Mortensen,[1] David J.P. Ferguson[2] and Anna Katharina Simon[1,3,*]

[1]Nuffield Department of Medicine; Weatherall Institute of Molecular Medicine; [2]Nuffield Department of Clinical Laboratory Sciences; and [3]National Institute for Health Research Biomedical Research Centre; John Radcliffe Hospital; Oxford, UK

Erythrocytes are anucleated cells devoid of organelles. Expulsion of the nucleus from erythroblasts leads to the formation of reticulocytes, which still contain organelles. The mechanisms responsible for the final removal of organelles from developing erythroid cells are still being elucidated. Mitochondria are the most abundant organelles to be cleared for the completion of erythropoiesis. Macroautophagy, referred to as autophagy, is a regulated catabolic pathway consisting of the engulfment of cytoplasmic cargo by a double membraned-vesicle, the autophagosome, which typically then fuses to lysosomal compartments for the degradation of the sequestered material. Early electron microscopic observations of reticulocytes suggested the autophagic engulfment of mitochondria (mitophagy) as a possible mechanism for mitochondrial clearance in these. Recently, a number of studies have backed this hypothesis with molecular evidence. Indeed, the absence of Nix, which targets mitochondria to autophagosomes, or the deficiency of proteins in the autophagic pathway lead to impaired mitochondrial clearance from developing erythroid cells. Importantly, however, the extent to which the absence of mitophagy affects erythroid development differs depending on the model and gene investigated. This review will therefore focus on comparing the different studies of mitophagy in erythroid development and highlight some of the remaining controversial points.

Key words: autophagy, Nix, Ulk1, Atg7, erythrocyte, mitochondria, anemia, apoptosis, ROS

Abbreviations: BM, bone marrow; MOMP, mitochondrial outer membrane permeabilisation; RBC, red blood cell; ROS, reactive oxygen species; PE, phosphatidylethanolamine; Ptds, phosphatidylserine

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*Correspondence to: Anna Katharina Simon; Email: katja.simon@ndm.ox.ac.uk

Red Blood Cell Maturation Involves the Removal of Mitochondria

Erythrocytes differentiate from hematopoietic stem cells in the bone marrow (BM).[1] Erythroblastic islands, niches for erythroid differentiation, are found in fetal liver, spleen and BM. These islands consist of a central macrophage surrounded by at least one synchronously maturing cohort of erythroid cells.[2] The pronormoblast, earliest recognisable erythroid precursor, divides and gives rise to increasingly smaller normoblasts around the central macrophage. Normoblasts contain progressively less RNA but more hemoglobin and more condensed chromatin. The nucleus is finally extruded from the late-normoblast and displays phosphatidylserine (Ptds) on its surface, which triggers its phagocytosis by the erythroblastic island's macrophage.[3] An anucleated reticulocyte results and exits the BM, circulates in peripheral blood for a couple of days and then matures, mostly in the spleen, into an erythrocyte.[1] Reticulocytes retain mitochondria, endocytic vesicles, ribosomes, golgi cisternae and rough endoplasmic reticulum (ER).[4] These organelles are then lost as reticulocytes mature into erythrocytes.[5] Until recently, the mechanisms by which these organelles are removed remained unclear. An accumulation of autophagosomes was observed in early electron micrographs of human peripheral blood cells,[6] as well as in rat erythroblasts and reticulocytes.[7] This led to the suggestion that autophagy could be one such mechanism, responsible for the removal

of organelles during red blood cell (RBC) development.

Autophagy

Autophagy is a regulated, catabolic pathway responsible for the lysosomal degradation of cytoplasmic constituents, such as long-lived proteins and organelles. It is characterized by the formation of a double-membrane vesicle, the autophagosome, which engulfs the cargo to be degraded and delivers it to lysosomal compartments.[8]

Autophagy is regulated developmentally[9] and by environmental triggers such as nutrient and energy availability, hormones, growth factors,[10] pathogens and immune mediators.[11-13] The signals that regulate autophagy converge on two main signaling nodes: mammalian target of rapamycin (mTOR)-Atg1 and Beclin 1-Vps34. While mTOR plays a role upstream of the Atg1 complex in the negative control of autophagy, the Beclin 1 (yeast Atg6 homologue) complex is directly targeted by signaling pathways.[14] As mTOR is activated by the abundance of nutrients and energy, autophagy is induced upon nutrient starvation.[15] The activity of Beclin 1, on the other hand, is negatively regulated by interaction with the anti-apoptotic molecules Bcl-2 or Bcl-x_L in normal conditions. Dissociation of Bcl-2/Bcl-x_L from Beclin 1 therefore induces autophagy.[16,17] Autophagosome elongation requires two ubiquitin-like conjugation systems, whereby Atg12 is covalently linked to Atg5 and Atg8 is conjugated to phosphatidylethanolamine (PE), known as Atg8 lipidation.[18] Atg7 encodes the E1-like enzyme needed for both of these conjugation systems and is therefore essential for autophagy.[19,20]

Mitochondrial Quality Control by Mitophagy

Mitochondria are essential for life as they carry out the energy-producing oxidative reactions that give rise to most of the ATP required for cellular function.[21] However, mitochondria, also participate in the regulation and amplification of apoptosis. Their involvement in apoptosis is regulated by proteins of the Bcl-2 family.[22] Mitochondria possess two membranes: the outer one, separating them from the cytosol and the inner one, which is the site of oxidative phosphorylation. Between the two mitochondrial membranes, lies the intermembrane space, where proteins such as the soluble and pro-apoptotic protein cytochrome *c*, are stored.[22] The intrinsic pathway of apoptosis is defined by mitochondrial outer membrane permeabilization (MOMP), resulting in the release of cytochrome *c* and other pro-apoptotic co-factors, which can activate the apoptosome and, in turn, effector caspases.[23] In addition, reactive oxygen species (ROS), a by-product of the electron transport chain, can leak out of damaged mitochondria. Mitochondria produce the majority of cellular ROS, with the superoxide radical being the most common. ROS are known to cause mitochondrial DNA mutations, dysfunction and therefore also apoptosis.[24] ROS can also damage nuclear DNA leading to the activation of DNA damage pathways, but unlike nuclear DNA, mitochondrial DNA is not protected by histones and is therefore more susceptible to damage.[25] Mitochondrial quality control mechanisms are therefore indispensable to cell survival and these include the removal of damaged mitochondrial proteins by an intraorganelle proteolytic system, the repair of damaged mitochondria by healthy ones through fission and fusion events and the removal of severely damaged mitochondria by autophagy (mitophagy).[26,27] Of these, mitophagy is the major degradative pathway for mitochondrial turnover.[28]

Long assumed to be random, increasing evidence indicates that mitophagy may be a selective process. The mitochondrial-resident protein Atg32, which can bind Atg11 and Atg8, was recently discovered to be a "tag" for mitophagy in yeast.[29,30] However, no Atg32 homologue has yet been found in metazoans.[31] In mammalian cells, the loss of mitochondrial membrane potential is a common trigger of mitophagy.[32] One particular mitophagy pathway has now been elucidated, which involves the selective recruitment of the ubiquitin ligase Parkin to dysfunctional mitochondria with low membrane potential.[33] The recruitment of Parkin to depolarized mitochondria relies on the function of the PTEN-induced putative kinase 1 (PINK1).[34-36] Parkin then mediates the poly-ubiquitylation of voltage-dependent anion channel 1 (VDAC1), which in turn recruits the LC3-binding protein p62/SQSTM1 and thereby directs the formation of autophagosomes to the damaged mitochondrion.[35,37] Whether these molecules are also important for the developmental removal of mitochondria from erythroid cells has not yet been investigated.

The Role of Mitophagy in Red Blood Cell Maturation

The first molecular evidence confirming mitophagy as a mechanism for the developmental removal of mitochondria from RBCs came from the study of $Nix^{-/-}$ mice. Nix is a BH3-only-like pro-apoptotic member of the Bcl-2 family upregulated during terminal erythroid differentiation[38] and dispensable for autophagosome formation.[39,40] $Nix^{-/-}$ mice are viable but display anemia and reticulocytosis,[38-40] due to the fact that $Nix^{-/-}$ RBCs produce more ROS, undergo apoptosis and have a shortened life span[40] (**Table 1**). A proportion of $Nix^{-/-}$ erythrocytes retain mitochondria despite having cleared the rest of their organelles.[39,40] The remaining mitochondria in $Nix^{-/-}$ RBCs lie in the proximity of autophagosomes,[39,40] whereas wild type RBCs contain mitochondria within autophagosomes, which are eliminated by exocytosis.[39] Importantly, mitochondria within wild type reticulocytes depolarize during their maturation, while those in $Nix^{-/-}$ reticulocytes maintain their membrane potential.[40,41] Mitochondrial clearance is restored in $Nix^{-/-}$ reticulocytes in which the loss of mitochondrial membrane potential is chemically induced.[40] Nix was therefore suggested to be essential for the loss of mitochondrial membrane potential, which would act as a signal to target mitochondria for autophagy.[40,41] In agreement with this, Nix was recently found to bind the mammalian Atg8 homologues MAP1-LC3 and GABARAP and thus recruit forming autophagosomes to depolarized mitochondria[42] (**Fig. 1**); as such Nix may represent the closest mammalian homologue of the yeast Atg32,[37,42] at least in erythroid mitophagy.

Table 1. Summary of the different gene knockout mouse models used to study the involvement of autophagy in mitochondrial clearance from developing red blood cells

Phenotype				Gene targeting		
Model	**Lethal**	**Severity of anemia**	**Presence of RBCs retaining mitochondria in total blood (max %)**	**Essential autophagy gene targeted**	**Restriction of gene deletion**	**Stress during erythropoiesis due to type of mouse model**
Vav-Atg7$^{-/-}$[47]	Yes	Severe	Yes (up to 40%)	Yes	Hematopoietic system	None
ErGFP-Atg7$^{-/-}$	No	Mild	Not determined (See Table 2)	Yes	Erythroid cells	None
Atg5$^{-/-}$ fetal liver chimeras	No*	Not reported	No	Yes	Hematopoeitic system	Lethal irradiation of recipients & preparation of donor fetal liver cells
Atg7$^{-/-}$ fetal liver chimeras	No**	Moderate	No	Yes	Hematopoeitic system	Lethal irradiation of recipients & preparation of donor fetal liver cells
Ulk1$^{-/-}$	No	Mild	Yes (up to 35%)	No	None***	None
Nix$^{-/-}$	No	Moderate	Yes (up to 33%)	No	None***	None

*No lethality among recipients of Atg5$^{-/-}$ fetal liver was reported.[53] **Half of the recipients of Atg7$^{-/-}$ fetal liver died, but no death was reported among successfully transplanted Atg7$^{-/-}$ fetal liver recipients.[46] Ulk1$^{-/-}$,[43] and Nix$^{-/-}$,[39,40] were both viable. ***Although the gene deletion is not tissue specific, the expression of these genes was reported to be upregulated during erythroid development.[38-40,43]

The studies of Nix$^{-/-}$ mice also showed that autophagy is induced during terminal erythroid maturation[39] and that chemical inhibition of autophagy, or Atg7 knockdown in reticulocytes selectively suppresses the removal of mitochondria.[34,40]

The autophagy-specific gene, Ulk1, was then also shown to be important in mitochondrial and ribosomal removal from developing RBCs[43] (**Table 1**). Ulk1 is a ubiquitously expressed mammalian homologue of the yeast Atg1-related serine-threonine kinase that is not required for Atg8 lipidation or for typical macroautophagy in response to nutrient starvation.[43] Its expression in reticulocyte cultures is similar to that of Nix and correlates with the loss of mitochondrial proteins. Ulk1$^{-/-}$ mice are viable but have a very mild decrease in RBC counts and reticulocytosis.[43] A proportion of Ulk1$^{-/-}$ erythrocytes retain polarized mitochondria and ribosomes. Ulk1$^{-/-}$ reticulocytes in culture have delayed mitochondrial clearance but treatments to induce the loss of mitochondrial membrane potential enable cells to overcome this defect.[43] Loss of RNA is also impaired in Ulk1$^{-/-}$ mice.[43] Notably, the mitochondrial removal defect in Ulk1$^{-/-}$ mice is not restricted to RBCs, as murine embryonic fibroblasts isolated from these mice have a higher mitochondrial mass than normal.[43]

Atg7 in Mitochondrial Clearance from Developing Erythrocytes: Different Models, Different Phenotypes

Although the studies, described so far, provided compelling evidence for the importance of mitophagy in erythroid development, as neither Nix nor Ulk1 are essential for starvation-induced autophagy, the role of the classical macroautophagic machinery in erythroid mitophagy remained unknown. The fact that circulating erythrocytes from Atg5$^{-/-}$ neonates appear normal[44] would indeed rather suggest that the two ubiquitin-like conjugation systems are dispensable for programmed mitophagy in RBCs. Recently, the role of the essential autophagy gene, Atg7, was studied in RBC development using murine models engineered to bypass the neonatal lethality resulting from constitutive Atg7 deficiency.[45] Although both studies find a mitochondrial removal defect in erythroid cells in the absence of Atg7,[46,47] the severity of the defect differs considerably between models.

Firstly, Zhang et al. (2009) transplanted Atg7$^{-/-}$ fetal liver into lethally irradiated hosts.[46] Interestingly, half of the Atg7$^{-/-}$ fetal liver recipients die; those successfully engrafted survive but develop anemia, reticulocytosis and lymphopenia. Secondly, in our own study, the role of Atg7 in erythroid development was studied using Vav-Atg7$^{-/-}$ mice,[47] which were obtained by crossing Atg7$^{Flox/Flox}$ [45] to Vav-iCre[48] mice expressing the Cre recombinase under the control of the pan-hematopoietic, Vav gene promoter.[47] Vav-Atg7$^{-/-}$ mice are moderately anemic early in life and go on to develop severe anemia until their death at a median age of 12 weeks.

In Atg7$^{-/-}$ fetal liver transplanted mice, mitochondria are cleared at a reduced rate from Atg7$^{-/-}$ reticulocytes,[46] yet Atg7$^{-/-}$ fetal liver chimeras have no circulating abnormal RBCs retaining mitochondria.[46] Their mitochondrial removal defect was milder than in Nix$^{-/-}$ cells. Moreover, reticulocytes from Atg7$^{-/-}$ fetal liver transplanted mice display mitochondria within degradative vacuoles, resembling autophagosomes, and indistinguishable in number and appearance from those seen in wild type reticulocytes.[46] In sharp contrast, mitochondria accumulate in both nucleated and anucleated erythroid cells from Vav-Atg7$^{-/-}$ mice, and a significant proportion of their circulating RBCs retain mitochondria, which can be seen in the proximity of structures resembling pre-autophagosomes.[47] This suggests that unlike in Nix$^{-/-}$ cells, where the targeting of mitochondria to autophagosomes is

Table 2. Percent mitochondria-retaining reticulocytes and erythrocytes in the peripheral blood of Vav-Atg7$^{-/-}$,[47] and ErGFP-Atg7$^{-/-}$ mice (unpublished data)

Model	Cell population	Percent cells containing mitochondria	
		Conditional Atg7 knockout mice	Littermate controls
Vav-Atg7$^{-/-}$ (Atg7 deletion restricted to the hematopoietic system)	TO^{+} Reticulocytes	77%	15%
	TO^{-} mature red blood cells	15%	4%
ErGFP-Atg7$^{-/-}$ (Atg7 deletion restricted to the erythroid lineage)	TO^{+} Reticulocytes	>50%	10–15%
	TO^{-} mature red blood cells	1%	<1%

Thiazole orange (TO)-positive (reticulocytes) and TO-negative cells (erythrocytes) were flow-sorted and analysed by electron microscopy and the percentage of cells retaining mitochondria was determined.

defective, mitochondria within RBCs from Vav-Atg7$^{-/-}$ mice are targeted to forming autophagosomes, yet as autophagosome elongation is impaired, mitochondrial engulfment cannot take place. ER and RNA, on the other hand, are efficiently cleared from Vav-Atg7$^{-/-}$ RBCs, suggesting that mitochondrial clearance is selectively affected by the absence of Atg7.[47]

Interestingly, the mitochondria within reticulocytes from Atg7$^{-/-}$ fetal liver transplants remained polarized, leading the authors to suggest that mitochondrial depolarization is a consequence, rather than a cause, of autophagosome recruitment and that Nix may not promote mitochondrial depolarization.[46] Overall, the authors conclude that, although Atg7-dependent autophagy is involved in mitochondrial clearance from reticulocytes, other Atg7-independent degradative pathways must be in place.[46] Again, in contrast with the findings by Zhang et al.[46] loss of mitochondrial membrane potential occurs in BM erythroid cells from Vav-Atg7$^{-/-}$ mice.[47] The results obtained with Vav-Atg7$^{-/-}$ mice suggest that Atg7-dependent autophagy is at least as important as Nix for the programmed mitochondrial clearance from erythroid cells.[47]

Furthermore, we reproduced the anemia of Vav-Atg7$^{-/-}$ in another in vivo model lacking Atg7 only in the erythroid compartment (ErGFP-Atg7$^{-/-}$ mice). The phenotype of ErGFP-Atg7$^{-/-}$ mice, however, was milder than that of Vav-Atg7$^{-/-}$.[47] The less efficient excision of Atg7^{Flox} in ErGFP-Atg7$^{-/-}$ potentially leading to some Atg7$^{+/+}$ erythroid cells giving rise to healthy, mature RBCs can explain the less severe phenotype.[47] But, despite the leakiness of this conditional knockout model, ErGFP-Atg7$^{-/-}$ mice also have abnormal circulating reticulocytes and erythrocytes retaining mitochondria (unpublished data, **Table 2**).

The difference in phenotype between Atg7$^{-/-}$ fetal liver chimeras and Vav-Atg7$^{-/-}$ mice is surprising and makes the role of Atg7, in the programmed removal of mitochondria from RBCs, still uncertain. Although, the two models target the same gene and the gene deletion in both is restricted to the hematopoietic system, one is lethal and the other is not (**Table 1**). In addition, Atg7$^{-/-}$ fetal liver transplanted mice do not have a population of abnormal circulating erythrocytes retaining their mitochondria[46] compared to up to 40% of total circulating RBCs containing mitochondria in Vav-Atg7$^{-/-}$ mice.[47] A few reasons for this can be envisaged. Firstly, it is possible that in Atg7$^{-/-}$ fetal liver chimeras, a few normal recipient erythroid precursors may have survived and given rise to healthy, mitochondria-free RBCs. Indeed, any healthy erythroid precursors would be under strong selective pressure considering the significant survival defect that we found in Atg7$^{-/-}$ RBCs from Vav-Atg7$^{-/-}$.[47] However, as the authors claim to have confirmed the donor origin of erythrocytes in their Atg7$^{-/-}$ fetal liver chimeras at ten weeks post-transplantation, time at which all analyses were undertaken,[46] this is unlikely to explain the phenotypic differences between models. Secondly, the conditions in which these two models were obtained differ considerably (**Table 1**). The hematopoietic system of Atg7$^{-/-}$ fetal liver transplanted mice is reconstituted from fetal liver hematopoietic precursors under stress hematopoiesis triggered by the lethal irradiation of the hosts. The hematopoietic system of Vav-Atg7$^{-/-}$ mice, on the other hand, is Atg7-deficient throughout their development making these mice a more physiological model. In fact, some of the differences between the two models could be attributed to the origin of the hematopoietic system of Atg7$^{-/-}$ fetal liver chimeras being fetal. There are indeed hints from studying lymphoid progenitors, that fetal stem cells may be intrinsically different from adult stem cells and that these may not acquire adult characteristics when exposed to adult environmental cues.[49] In addition, the stress factors released in the lethally irradiated recipients of the Atg7$^{-/-}$ fetal livers could affect erythropoiesis; under such stressful conditions, other survival pathways may be stimulated overriding a requirement for autophagy.

Alternative Pathways for the Degradation of Mitochondria and Other Organelles from Developing Erythrocytes

Other organelle degradation mechanisms, such as cytosolic degradation systems are known to participate in organelle removal from reticulocytes. Indeed, the 15-lipooxygenase (15-LOX) enzyme, highly upregulated in reticulocytes, functions in cytosolic degradation by integrating specifically into organelle membranes and permeabilising them, thereby allowing access to the cytosolic proteolytic system, the ubiquitin/proteasome.[50] It was also shown that inhibition of 15-LOX in cultured reticulocytes results in delayed organelle clearance.[51] This degradation pathway, as well as participating in the clearance of mitochondria, may partly explain the efficient removal of other organelles from Atg7$^{-/-}$ RBCs in Vav-Atg7$^{-/-}$ mice[47] (**Fig. 1**).

Blocking of 15-LOX, however, caused only a modest mitochondrial clearance defect compared to Nix deficiency,[39] suggesting that the Nix-dependent pathway for mitochondrial clearance is more important than the 15-LOX degradation of mitochondria in developing erythroid cells. One can speculate, mitochondria being the last thing to be removed from reticulocytes,[47] that the 15-LOX system may only degrade organelles up to a certain stage, after which autophagy may be the main pathway responsible for removal of leftover mitochondria. However, as a significant proportion of RBCs achieved mitochondrial clearance in Nix$^{-/-}$,[40] Ulk1$^{-/-}$[43] and Atg7$^{-/-}$,[47] RBCs and as the 15-LOX pathway only accounts for a relatively small amount of mitochondrial degradation, there must be yet more mechanisms participating in this developmentally regulated organelle removal. Such an alternative pathway was recently discovered to be the Atg5/Atg7-independent autophagic degradation.[52] This suggested alternative macroautophagy pathway is induced by cell stress other than starvation, is regulated by Ulk1 and Beclin 1, takes place in the absence of LC3 lipidation and is responsible for the near complete clearance of mitochondria from Atg5$^{-/-}$ fetal liver erythroid cells[52] (**Fig. 1**). Whether mitochondrial engulfment by this pathway is also dependent on autophagosome recruitment to depolarized mitochondria by Nix, remains to be elucidated. The facts that Atg5$^{-/-}$ fetal liver chimeras do not develop anemia[53] and Atg5$^{-/-}$ reticulocytes clear their mitochondria normally[44] support the existence and importance of this pathway. However, the anemia and strong mitochondrial removal defect seen in Vav-Atg7$^{-/-}$ mice highlight the weight of classical Atg7-dependent autophagy in erythroid mitophagy.[47] Our findings with Vav-Atg7$^{-/-}$ would indeed suggest that Atg7 is more important than Atg5 and that Atg7-dependent macroautophagy is essential for mitophagy in erythroid cells. Important differences between Atg5 and Atg7 have also been previously noted in the mitochondrial degradation from lymphocytes.[53,54] Nevertheless, both Atg5 and Atg7 are essential for neonatal survival, which suggests that in other tissues, perhaps only in non-hematopoietic tissues, both genes are required.[45,55]

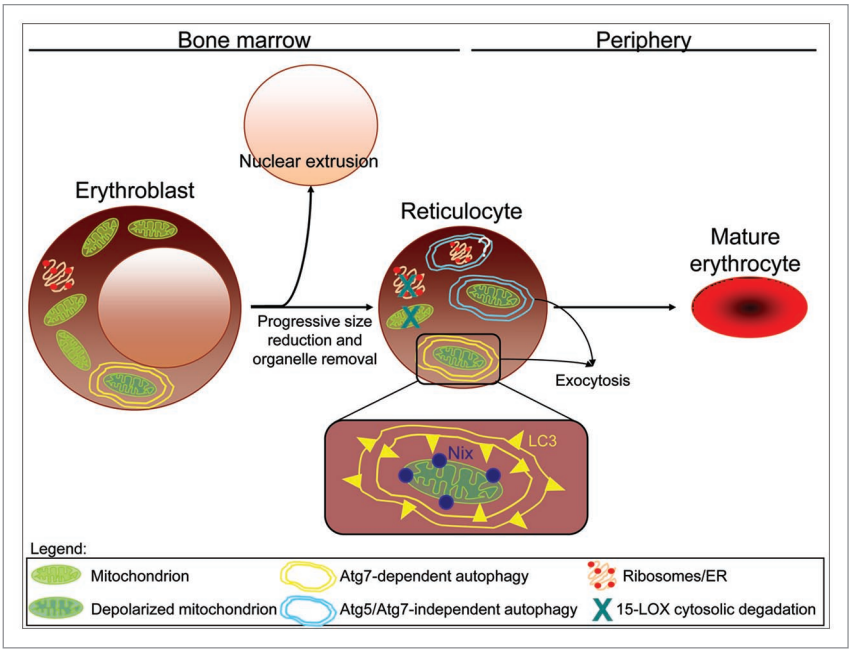

Figure 1. Pathways involved in mitochondrial and organelle clearance from maturing erythrocytes. Mitochondria are progressively lost throughout erythroid maturation, from the erythroblast until the mature erythrocyte stage. Mitochondria lose their membrane potential (shown as depolarized mitochondria) which targets them to autophagosomal vesicles. Loss of mitochondrial membrane potential can be seen as early as in bone marrow erythroblasts. Nix localizes to mitochondria, is responsible for their developmentally-regulated loss of membrane potential and recruits autophagosomes by binding to Atg8/LC3 on autophagosomal membranes. While autophagic engulfment of mitochondria in erythroblasts can still lead to their degradation in lysosomes, mitochondria within autophagosomes can be exocytosed from reticulocytes. The 15-LOX cytosolic degradation pathway degrades mitochondria and other organelles in reticulocytes. The Ulk1-dependent, Atg5/Atg7-independent alternative autophagy pathway may also be important for the removal of ribosomes from reticulocytes.

On the other hand, the ribosome removal defect of Ulk1$^{-/-}$ reticulocytes,[43] suggests that the Atg5/Atg7-independent, Ulk1-dependent alternative macroautophagy[52] may be important in the developmentally regulated removal of other organelles from RBCs. Supporting this is the observation that Atg7$^{-/-}$ erythroid cells from Vav-Atg7$^{-/-}$ mice cleared their RNA and ER normally[47] (**Fig. 1**).

Conclusions

To recapitulate, this review covers the recent molecular evidence for mitochondrial autophagy as one of the mechanisms used by developing erythroid cells to clear their mitochondria and highlighted some contradicting data, particularly regarding the role of the essential autophagy gene Atg7 in this process. The mitochondrial molecule, Nix, is responsible for recruiting autophagosomes to depolarized mitochondria in developing erythroid cells. Then, Atg7-dependent classical autophagy is necessary, to a yet unclear extent, for the engulfment of mitochondria in erythroid cells. Another pathway for autophagosome formation, Ulk1-dependent but Atg5/Atg7-independent also participates in the removal of mitochondria from developing RBCs. The relative contributions of the Atg7-dependent and Atg7-independent pathways to mitochondrial autophagy, in erythroid cells, remain uncertain. Ultimately, investigating erythroid development in models deficient for genes essential to both the classical and alternative autophagy pathways, such as Beclin 1, will reveal the role of mitophagy in RBC development. Overall, the studies of mitophagy in erythroid cells highlight, first of all, the importance of deleting more than one Atg gene to determine the role of autophagy

in any particular setting. Secondly, the differences observed between the fetal liver chimera and Vav-Cre models for hematopoietic-specific gene ablation have important implications for the interpretation of future studies using fetal liver or BM chimeras. Finally, it is envisageable that more cell- and/or organelle-specific pathways for autophagosomal degradation may be discovered. Autophagy being implicated in a vast number of diseases, such as neurodegenerative disorders, infections, cancer and autoimmunity, it is essential to understand which autophagy pathway is predominant in each tissue and under which conditions it is induced. This will help to achieve a targeted modulation of autophagy.

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