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Autophagy in mammalian development and differentiation

Noboru Mizushima and
Department of Physiology and Cell Biology, Tokyo Medical and Dental University, Tokyo 113-8519, Japan.

Beth Levine
Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390-9113, USA, the Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390-9113, USA and the Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9113, USA.

Noboru Mizushima: nmizu.phy2@tmd.ac.jp

Abstract

It has been known for many decades that autophagy, a conserved lysosomal degradation pathway, is highly active during differentiation and development. However, until the discovery of the autophagy-related (*ATG*) genes in the 1990s, the functional significance of this activity was unknown. Initially, genetic knockout studies of *ATG* genes in lower eukaryotes revealed an essential role for the autophagy pathway in differentiation and development. In recent years, the analyses of systemic and tissue-specific knockout models of *ATG* genes in mice has led to an explosion of knowledge about the functions of autophagy in mammalian development and differentiation. Here we review the main advances in our understanding of these functions.

Autophagy is a process by which cytoplasmic components including macromolecules (for example proteins, glycogens, lipids and nucleotides) and organelles (for example mitochondria, peroxisomes and endoplasmic reticulum) are degraded by the lysosome (Fig. 1a)[1,2]. There are at least three different types of autophagy; macroautophagy (delivery of cytosolic contents to the lysosome by autophagosomes), microautophagy (inward invagination of the lysosomal membrane) and chaperone-mediated autophagy (direct translocation across the lysosomal membrane). Among these, macroautophagy (hereafter referred to as autophagy) has been the most extensively studied. Yeast genetic studies identified a set of autophagy-related (*ATG*) genes that are required for macroautophagy and its related processes. These genes are highly conserved among eukaryotes, allowing analyses of the roles of autophagy using reverse genetic techniques (Tables 1–3). Such analyses have revealed various physiological roles and pathological effects of autophagy, including adaptive responses to starvation, quality control of intracellular proteins and organelles, anti-aging, suppression of tumour formation, elimination of intracellular microbes, and antigen presentation[2–9].

Beyond these links to physiology, there is also increasing evidence that autophagy has conserved roles in differentiation and development. As a dynamic and highly inducible catabolic process that responds to environmental and hormonal cues, the autophagy pathway can drive the rapid cellular changes necessary for proper differentiation and/or development.

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Indeed, autophagy-defective organisms, including fungi, protozoa, worms and insects, show various abnormalities in differentiation and development (Table 1). These abnormalities may result from a deficiency in general autophagy, but they may also result from a failure to degrade specific components through selective autophagy, as shown for the degradation of germline P granules in somatic cells during *Caenorhabditis elegans* embryogenesis[10].

In mammals, autophagy is important for preimplantation development, survival during neonatal starvation, and cell differentiation during erythropoiesis, lymphopoeisis and adipogenesis (Fig. 2 and Tables 2 and 3). In addition to this 'remodelling' function, autophagy is also crucial for intracellular 'refreshment', and this homeostatic role is particularly important for the health of terminally differentiated cells such as neurons. In this review we discuss current knowledge about the role of autophagy in development and differentiation in mammals.

Autophagy in mammalian embryonic development

Oocyte-to-embryo transition

The earliest autophagic event in mammalian development is observed in fertilized oocytes[11] (Figs 1b and 2) and the *ATG* gene, *Atg5*, is essential during the early phase of pre-implantation development (Table 3)[11]. The oocyte, one of the most highly differentiated cells, suddenly changes to a highly undifferentiated state after fertilization. This 'reprogramming' occurs in both the nucleus and the cytoplasm. Maternal mRNA and proteins are rapidly degraded after the two-cell stage in the embryos, and new mRNA and proteins encoded by the zygotic genome are synthesized[12,13], leading to marked changes in the protein species synthesized after the four-cell to eight-cell stages[14]. Moreover, the degradation of maternal proteins and RNAs may be necessary for the activation of the zygotic genome[15]. Autophagy occurs at only low levels in unfertilized oocytes but is massively induced within 4 h after fertilization (Figs 1b and 2)[11]. This induction of autophagy is completely dependent on fertilization and is not due to starvation after ovulation, because autophagy is not induced in the ovulated oocyte unless it has been fertilized. This type of autophagy may be triggered by calcium oscillation, because parthenogenetic activation also induces autophagy in oocytes[11]. Interestingly, autophagy is transiently suppressed from the late one-cell to middle two-cell stages, and then reactivated. As suppression of autophagy in mitotic phase is also observed in cultured mammalian cells[16], perhaps there is a common mechanism to avoid the degradation of important nuclear factors during cell division.

Conventional *Atg5*$^{-/-}$ mice survive early embryogenesis (see below), but this is due to the presence of maternally inherited Atg5 protein in *Atg5*$^{-/-}$oocytes. Elimination of the maternal Atg5 protein with the use of oocyte-specific *Atg5*-knockout mice results in embryonic lethality at the four-cell to eight-cell stages[11] (Table 3). The precise role of autophagy during this process is not fully understood. Because the rate of protein synthesis is reduced in autophagy-defective embryos, normal levels of autophagy may be necessary for the production of sufficient amino acids for protein synthesis[11]. However, autophagy may also be required for the active elimination of unnecessary proteins and organelles that accumulate within oocytes or to facilitate 'remodelling' by degrading maternal suppressors of the zygotic gene program. Given that autophagy is an intracellular recycling system, these different possibilities are not mutually exclusive.

Embryo-to-neonate transition

The next wave of massive autophagy in mice is observed during the early neonatal period[17] (Fig. 2). Autophagy is actively induced in all neonatal tissues except the brain until one or two days after birth[17]. Throughout mammalian embryogenesis, necessary nutrients are

supplied through the placenta. At birth, this supply is terminated and neonates inevitably face severe starvation. Accordingly, conventional knockout of *Atg3* (ref. 18), *Atg5* (ref. 17), *Atg7* (ref. 19), *Atg9* (ref. 20) and *Atg16L1* (ref. 21) causes neonatal lethality (within one day) despite almost normal appearance at birth (Table 2). Amino-acid levels are decreased in the plasma and tissues of these *Atg*-knockout neonates[17–19], suggesting that autophagy is necessary to maintain the amino-acid pool during the early neonatal period. It remains undetermined precisely how the resultant amino acids are used in neonates. They may be used for energy production in a cell-autonomous manner to meet high energy demands in certain neonatal tissues; in support of this possibility, autophagy is highly activated in the heart and diaphragm after birth, and Atg5-deficient hearts show activation of the low-energy-sensing kinase AMPK (AMP-activated protein kinase)[17].

However, it is unknown whether the decrease in amino-acid levels is the sole cause of premature death in these *Atg*-knockout neonates, because the mice show additional abnormalities. First, although autophagic activity is not enhanced in neurons by starvation during the neonatal period, the absence of basal autophagy in neurons may contribute to a suckling defect in *Atg*-knockout neonatal mice, which may exacerbate their malnutritional state[17,19]. However, the suckling failure alone cannot explain the early death because *Atg5*$^{-/-}$ and *Atg7*$^{-/-}$ neonates die earlier than wild-type neonates even under non-suckling conditions. Second, autophagy may also be important for the degradation of macromolecules other than proteins. For example, glycogen delivery to the lysosome results in the production of glucose and energy[22,23] in newborn liver and muscle. Third, *Atg5*$^{-/-}$neonates have less adipose tissue mass as a result of defects in adipogenesis (see below)[24], which might prevent sufficient energy production. Fourth, the clearance of apoptotic corpses is defective in *Atg5*$^{-/-}$late embryos, which might also contribute to developmental abnormalities[25]. Finally, as environmental conditions change markedly at birth, autophagy-mediated degradation may also contribute to cellular remodelling by generating a new set of proteins and organelles (somewhat akin to the process of metamorphosis in insects).

Other processes during embryogenesis

Although complete autophagy-defective embryos derived from oocyte-specific Atg5-deficient mice die before implantation[11], conventional *Atg3*$^{-/-}$, *Atg5*$^{-/-}$, *Atg7*$^{-/-}$, *Atg9*$^{-/-}$ and *Atg16L1*$^{-/-}$ embryos survive the entire embryonic period and are born at Mendelian frequency[17–21]. These data suggest that Atg3, Atg5, Atg7, Atg9 and Atg16L1 are not essential for embryogenesis (except during the early stages, when maternally inherited proteins allow survival in conventional knockout mice). Nonetheless, a more subtle role for these *ATG* genes in embryonic development cannot be ruled out. Although high levels of autophagy have not been reported during later stages of embryogenesis in mice expressing the autophagy reporter green fluorescent protein (GFP)–LC3, except in certain tissues such as the developing thymus[26], certain phenotypic abnormalities of *Atg5*$^{-/-}$ mice (such as ubiquitin inclusions in embryonic neurons or decreased adipose tissue mass in neonates) indicate that wild-type levels of autophagy may be important for completely normal embryogenesis. Further studies are therefore required for the careful dissection of the role of *ATG* gene deficiency in more subtle aspects of embryonic development.

In contrast with the embryonic survival observed in conventional *Atg3*$^{-/-}$, *Atg5*$^{-/-}$, *Atg7*$^{-/-}$, *Atg9*$^{-/-}$, and *Atg16L1*$^{-/-}$ mice, the conventional knockout of several other *ATG* genes, including *beclin 1*, *Ambra1* and *FIP200*, produces somewhat different phenotypes (Table 2). Beclin 1, a component of class III phosphatidylinositol-3-OH kinase (PI(3)K) complexes[27], is the mammalian homologue of yeast Atg6/Vps30. *Beclin 1*$^{-/-}$ mice show early embryonic lethality[28,29], and abnormally small embryos are detected at embryonic day 7.5 (E7.5)[29]; these embryos show massive cell death and failure to close the proamniotic canal. *Beclin 1*$^{-/-}$ embryonic stem cells are viable, although *beclin 1*$^{-/-}$ mice are early embryonic lethal,

suggesting that Beclin 1 is dispensable *in vitro* but critically important for development *in vivo*. Ambra1 is a Beclin 1-interacting protein that positively regulates autophagy and is strongly expressed in developing neural tissues. Ambra1-deficient mice produced by gene-trap mutagenesis (*Ambra1*$^{gt/gt}$) are embryonic lethal at days E10–E14 and show defective neural tube development and hyperproliferation of neural tissues[30]. FIP200 (focal adhesion kinase (FAK) family interacting protein of M_r 200K, also known as RB1CC1) is a ULK1 (an Atg1 homologue)-interacting protein and has a molecular function similar to that of yeast Atg17, although it shows no homology with any yeast Atg proteins[31]. *FIP200*$^{-/-}$ mice are also embryonic lethal between E13.5 and E16.5 as a result of defective heart and liver development[32].

It is not completely understood why the phenotypes of different *ATG*-gene knockout mouse models vary. Because Beclin 1-including PI(3) K complexes have several distinct functions, and FIP200 has multiple interacting partners such as FAK, Pyk2, TSC1 (tuberous sclerosis complex 1) and p53, some of the abnormalities found in *beclin 1*$^{-/-}$, *Ambra1*$^{gt/gt}$and *FIP200*$^{-/-}$mice may be related to other factors besides autophagy deficiency. Alternatively, the disparate phenotypes in different *ATG*-gene knockout mouse models may depend on the step in autophagy at which each factor functions. The Beclin 1-containing PI(3)K and the ULK1–FIP200 complexes function earlier in autophagy at the autophagosome nucleation step[2,33], while Atg3, Atg5, Atg7 and Atg16L function later in autophagy in autophagosome elongation. Thus, upstream factors may show more severe phenotypes, or alternatively, as recently reported, downstream factors may be dispensable for a particular type of macro autophagy[34]. However, one exception to this general pattern is Atg9, which acts early (according to hierarchical analyses in yeast) but leads to a less severe phenotype when deficient[35]. There could also be different degrees of functional redundancy between different Atg proteins or different levels of compensatory mechanisms for the knockout of different Atg proteins. Further studies are needed to determine whether the autophagy pathway itself, or merely certain components of this pathway with autophagy-independent functions, are essential for distinct stages of embryonic development after the transition from oocyte to embryo.

Autophagy in cell differentiation

Erythrocyte differentiation and mitochondrial clearance

A long-standing question in developmental biology has been how the erythroblast loses its organelles, and to what extent the autophagy pathway functions in this process. Erythroblasts possess nuclei and intracellular organelles, which are lacking in mature erythrocytes and are replaced by haemoglobin molecules (Fig. 2). During erythroid differentiation, the nuclei are released from the cell, but the mechanism(s) by which other organelles are eliminated are poorly understood. The 15-lipoxygenase enzyme, which is highly expressed in reticulocytes, has been suggested to participate in this degradation[36–38]. Electron microscopy studies have also indicated a potential role for autophagy in the degradation of erythroid organelles[39–43]. However, organelle elimination in erythroid cells of *Atg5*$^{-/-}$ neonates seems normal[44]; thus, the role of autophagy in this process has been uncertain.

Several recent studies suggest that mitochondrial clearance in reticulocytes is partly dependent on autophagy. This concept first emerged from studies with cells and mice deficient in Nix (also known as BNIP3L), a Bcl-2 homology 3 (BH3)-only protein that is present on the mitochondrial outer membrane. Nix is required for the selective removal of mitochondria but not that of ribosomes or nuclei; in *Nix*$^{-/-}$ reticulocytes, autophagosomes are generated but there is a deficiency in the loss of the mitochondrial membrane potential and consequent mitochondrial engulfment by autophagosomes[45–47]. *Nix*$^{-/-}$ mice have lower

numbers of mature erythrocytes (that is, anaemia) and a compensatory expansion of erythrocyte precursors (that is, reticulocytosis)[46,48].

A direct requirement for *ATG* genes in mitochondrial elimination during erythroid cell maturation has also been demonstrated. There are five Atg1-related proteins in mammals, including ULK1, ULK2, ULK3, ULK4 and STK36. Because ULK3, ULK4 and STK36 lack the carboxy-terminal domain that is required for binding to Atg13 and FIP200, ULK1 and ULK2 are the primary candidate mammalian Atg1 orthologues[49,50]. During erythroid differentiation, the expression of ULK1, but not that of ULK2, is induced, suggesting that ULK1 may have a major role in this process[51]. Indeed, *ULK1*$^{-/-}$ mice show an increase in reticulocyte number. Interestingly, in addition to impaired clearance of mitochondria, the erythroid cells in *ULK1*$^{-/-}$ mice also have impaired clearance of RNA-bound ribosomes. Similarly, lethally irradiated wild-type recipients of *Atg7*$^{-/-}$ fetal liver transplants have decreased red blood cell counts and delayed mitochondrial clearance in erythroid cells[52]. Furthermore, haematopoietic cell-specific *Atg7*-knockout mice (*Atg7*$^{flox/flox}$;Vav-*Cre* mice) are severely anaemic and die at 8–14 weeks of age (Table 3)[53]. The *Atg7*$^{-/-}$erythrocytes accumulate selectively damaged mitochondria; this is associated with premature cell death. In contrast with *ULK1*$^{-/-}$mice[51] (but similarly to *Nix*$^{-/-}$ mice[46]), erythroid cells from *Atg7*$^{-/-}$mice do not seem to accumulate any organelles besides mitochondria[53].

Thus, the autophagy pathway seems to function selectively in mitochondrial clearance during erythroid differentiation[46,51–53]. One proposed mechanism for the selective degradation of mitochondria (called mitophagy) is through Nix-mediated mitochondrial recognition. Nix has a typical LC3-interacting WXXL-like motif, WVEL, at the amino terminus, through which Nix interacts with LC3 family proteins such as γ-aminobutyric acid receptor-associated protein (GABARAP) and LC3A on autophagosomal membranes[47,54]. Thus, Nix could serve as a cargo receptor for mitophagy. However, Nix may have additional autophagy-independent roles in mitochondrial clearance. The treatment of *Nix*$^{-/-}$ reticulocytes with an uncoupling reagent restores mitochondrial engulfment by autophagosomes[46], indicating that effects on mitochondrial membrane potential, rather than a specific function as a cargo receptor for mitophagy, may, at least in part, underlie Nix-mediated effects in erythroid maturation.

Future studies are required to delineate more precisely the role of mitophagy and its molecular mechanisms in erythroid differentiation. How other organelles, such as ribosomes and ER, are eliminated during erythroid differentiation is also an interesting question. The normal clearance of ribosomes in *Nix*$^{-/-}$ cells[46] and of ribosomes and ER in *Atg7*$^{-/-}$cells[53] suggest the presence of unknown degradation system(s), one of which may be the recently reported Atg5/Atg7-independent macroautophagy[34]. Mitophagy, and/or alternative forms of macroautophagy, may also have a more general role in the differentiation of other cell lineages (see below).

Lymphocyte differentiation

Haematopoietic-specific, B-lymphocyte-specific and T-lymphocyte-specific deletion of *ATG* genes has revealed specific roles of autophagy in lymphocyte differentiation. In addition to severe anaemia, the haematopoietic cell-specific *Atg7*-knockout mice (*Atg7*$^{flox/flox}$;Vav-*Cre* mice) also have a significant decrease in T and B lymphocyte counts (with no alterations in numbers of cells of the myeloid lineage)[53]. Both CD4 and CD8 T cells from these mice have a greater number of mitochondria, which translates into increased mitochondrial mass, higher levels of superoxide, and increased susceptibility to apoptosis after *in vitro* culture. The *Atg7*$^{-/-}$ fetal liver cell transplant recipient mice discussed above are also lymphopenic[52]. Furthermore, T-cell-specific *Atg5*-knockout and *Atg7*-knockout mice (*Atg5*$^{flox/flox}$;Lck-*Cre* and *Atg7*$^{flox/flox}$;Lck-*Cre*) have decreased numbers of peripheral T

cells, increased T-cell accumulation of mitochondria, and enhanced apoptosis in mature T cells (Table 3)[55,56]. Atg5$^{-/-}$ chimaeric mice also show a similar decrease in T and B lymphocyte numbers and defects in stimulation-induced proliferation[57]. During the normal development of thymocytes to circulating mature T cells, mitochondrial content is decreased, but this developmental change is suppressed in autophagy-defective T cells[55]. Thus, the clearance of mitochondria may represent a developmental process that contributes to the survival of mature T cells. It is not yet fully understood why erythroid and lymphocyte, but not myeloid, survival depends partly on mitophagy.

Another recently identified role of autophagy in T cells is the elimination of autoreactive T cells in the thymus[58]. High levels of autophagy in thymic epithelial cells[26], the only non-haematopoietic cell type that constitutively expresses major histocompatibility complex class II (MHC II) molecules, were initially described in GFP–LC3 reporter mice. Subsequently, autophagy was shown to be essential for the delivery of certain endogenously synthesized antigens to MHC II loading compartments[7,8]. Genetic disruption of *Atg5* in thymic epithelial cells results in altered selection of certain MHC-II-restricted T-cell specificities and in autoimmunity[58]. Thus, this autophagy-dependent antigen presentation pathway participates in self-tolerance.

As noted above, *ATG* gene deletion in mice also decreases B-cell counts. Like *Atg7*$^{flox/flox}$;Vav-*Cre* mice[53] and *Atg5*$^{-/-}$ chimaeric mice[57], B cell-specific *Atg5*-knockout mice (*Atg5*$^{flox/flox}$;CD19-*Cre*) have decreased numbers of B cells[59]. In addition to its role in B-cell development in the bone marrow[59], Atg5 is required for the survival of certain types of B cell in the periphery. The mechanism underlying the B-cell defects is unknown; unlike in T cells, mitochondrial content is not developmentally regulated in B cells[55].

Adipocyte differentiation and energy metabolism

In a similar manner to erythrocyte maturation, adipogenesis also involves marked intracellular remodelling. Adipocytes are differentiated from preadipocytes, which emerge from multipotent mesenchymal precursors. During adipogenesis, the fibroblast-like cells differentiate into round cells containing single large lipid droplets (Fig. 2). Autophagy is actively induced during this differentiation process *in vitro*[24], and both *in vitro* and *in vivo* studies have confirmed that autophagy contributes to cellular remodelling during adipogenesis.

Primary mouse embryonic fibroblasts (MEFs) can be differentiated into adipocytes in the presence of adipogenic factors, but this process is significantly delayed or suppressed in primary *Atg5*$^{-/-}$ MEFs[24]. The knockdown of Atg7 or Atg5 in 3T3-L1 preadipocytes also suppresses triglyceride accumulation and decreases protein levels of mediators and markers of adipocyte differentation[60]. Moreover, treatment with short interfering RNA against LC3 impairs lipid droplet formation in various mammalian cell lines such as HeLa, PC12 and HepG2 cells[61].

Newborn *Atg5*$^{-/-}$ mice possess fewer subcutaneous adipocytes than wild-type neonates[24], and the importance of autophagy in adipogenesis *in vivo* has been further confirmed in adipocyte-specific *Atg7*-knockout mice (*Atg7*$^{flox/flox}$;Ap2-*Cre* mouse)[60,62]. These mutant mice have decreased white adipose tissue mass and have smaller adipocytes containing multi-locular lipid droplets, increased numbers of mitochondria and increased cytoplasmic volume. These adipocyte features are characteristic of brown adipose tissue, and several brown fat-associated enzymes such as UCP-1 and PGC-1α are expressed in the white adipose tissue of these mutant mice[60] (the increase in UCP-1 was not observed in ref. 62).

Interestingly, the adipocyte-specific *Atg7*-knockout mice are leaner than wild-type mice, even though there is no difference in their food intake. Furthermore, they are resistant to obesity induced by a high-fat diet and show higher insulin sensitivity[60,62]. This may be due in part to increased β-oxidation of fatty acids and lower free fatty acid levels in their plasma. Basal physical activity is also higher in the adipocyte-specific *Atg7*-knockout mice[62]. Thus, autophagy is important not only for adipocyte differentiation but also for local and whole-body lipid metabolism.

An important question is whether the anti-obesity and insulin sensitization effects of Atg7 disruption are primarily the result of alterations in the ratios of brown adipose-like tissue versus white adipose tissue. Although the amount of brown adipose tissue declines after birth, recent evidence indicates that significant amounts are found in adult humans[63–65]. There is a growing consensus that the ratio of white to brown adipose tissue may influence the development of obesity, which is characterized by an increased mass of white adipose tissue[66]. Given the defect in adipocyte differentiation in adipocyte-specific *Atg7*-knockout animals, it is difficult to predict how the disruption of autophagy would affect body weight and insulin sensitivity in the fully developed animal. Studies of inducible adipocyte-specific *ATG*-gene knockout mice will be required to address this question.

The involvement of autophagy in hepatocyte lipid droplet accumulation seems more complex than its role in adipocyte lipid metabolism. During starvation, free fatty acids are remobilized from adipose tissue to the liver and accumulate in lipid droplets. One paper reports that there is less formation of lipid droplets in hepatocyte-specific *Atg7*-knockout mice (*Atg7*$^{flox/flox}$;Alb-*Cre* mice)[67]. However, it is also known that suppression of autophagy in hepatocytes leads to an increase in hepatic triglyceride storage in lipid droplets in *Atg7*$^{flox/flox}$;Mx1-*Cre* and *Atg7*$^{flox/flox}$;Alb-*Cre* mouse lines[19,68]. This may be due to impaired autophagic degradation of lipid droplets, because small lipid droplets can be selectively engulfed by autophagosomes in cultured hepatocytes[68]. It is not clear why different phenotypes are observed in different investigations of liver-specific *Atg7*-knockout mice. Perhaps Atg7 is required for the rapid formation of lipid droplets that occurs in the liver during the acute response to starvation, whereas Atg7 may inhibit the chronic accumulation of lipid droplets during normal feeding states. Furthermore, the mechanistic basis for differential effects of autophagy on lipid metabolism in the liver versus the adipocyte remains to be determined.

Homeostatic role in terminally differentiated cells

Autophagy may be dispensable for many types of rapidly proliferating cells, but its housekeeping role may become essential in differentiated or senescent cells (Fig. 2)[69]. Tissue-specific knockout of *ATG* genes causes the accumulation of ubiquitin-positive inclusion bodies/aggregates in neurons (Fig. 1c)[70–72], hepatocytes[19], cardiac muscle[73], skeletal muscle[74,75], pancreatic β-cells[76,77]and podocytes (Table 3)[78]. These inclusions are also positive for the autophagy substrate p62 (ref. 79). These aggregates are observed as early as E15.5 in neurons[70]. In addition to these inclusion bodies, soluble and cytosolic ubiquitylated proteins and p62 accumulate in these cells. The observations that ablation of autophagy leads to the degeneration/dysfunction of each tissue (Table 3) indicate that autophagy has a critical homeostatic role in post-mitotic differentiated cells. The accumulation of p62 may be the primary cause of the cellular toxicity, at least in the liver (but not in the brain), because hepatomegaly and hepatic dysfunction in the liver-specific *Atg7*-knockout mice are restored by simultaneous knockout of p62 (ref. 80). The effect of p62 overexpression is, at least in part, a result of aberrant hyperactivation of the transcription factor Nrf2, which occurs because p62 binds to and inhibits Keap1, an E3-ligase for Nrf2 (refs 81–84).

In non-dividing cells, quality control, not only of proteins but also of organelles, is important. As discussed above, mitochondrial quantity and quality can be controlled by mitophagy. Indeed, the accumulation of mitochondria, most of which are deformed or dysfunctional, is observed in autophagy-defective cells in most tissue-specific *ATG*-gene knockout models (Table 3)[19,53,55,60,62,72,73,75–78]. Recent studies have considered the mechanisms of autophagy-mediated quality control of mitochondria in the context of Parkinson's disease. Parkin is a ubiquitin ligase whose mutation causes familial Parkinson's disease[85]. On mitochondrial damage and depolarization, this cytosolic soluble protein translocates to mitochondria and subsequently induces mitophagy[86]. The recruitment of Parkin to mitochondria requires PINK1, another protein kinase associated with Parkinson's disease[87–92]. However, it is not exactly clear how the PINK1–Parkin pathway promotes mitophagy. Because the E3-ligase activity of Parkin is a prerequisite for mitophagy, the ubiquitylation of some mitochondrial proteins is likely to be essential. So far, VDAC1 (voltage-dependent anion channel 1)[88] and mitofusin (a mitochondrial pro-fusion factor)[91] have been proposed as Parkin substrates. Ubiquitylation of these proteins could recruit the autophagy adaptor p62 (ref. 88), and/or affect mitochondrial fission or fusion, to mediate mitophagy[91]. The failure of any step in this pathway would lead to the accumulation of dysfunctional mitochondria and the excessive production of reactive oxygen species (ROS), resulting in the injury of ROS-sensitive cells such as the dopaminergic neurons.

Conclusion

Through the analysis of systemic and tissue-specific *ATG*-gene knockout mice, great advances have been made in our understanding of the role(s) of autophagy in mammalian development and differentiation. The autophagy pathway seems essential for two critical stages of early development: the pre-implantation period after oocyte fertilization, and the early postnatal period after disruption of the placental food supply. Autophagy could also have a role in other stages of embryogenesis, because the deletion of some *ATG* genes leads to lethality during mid-embryonic development, and the *ATG*-gene knockout mice that survive the postnatal period display some developmental abnormalities. The autophagy pathway is also involved in the differentiation of two cell types that require marked architectural remodelling — erythroid cells, presumably through mitochondrial clearance, and adipocytes — probably through a more complex mechanism. The autophagy pathway is also involved in the differentiation of lymphocytes, although this is not a cell type that undergoes as marked a remodelling. Moreover, autophagy's homeostatic role in protein and organelle quality control may prevent the degeneration of post-mitotic cells throughout embryonic development and postnatal life. The failure of autophagy to perform this specific function in adulthood may underlie the pathogenesis of certain neurodegenerative diseases, such as familial Parkinson's disease. Future studies are likely to reveal a more universal role for autophagy in numerous other developmental and differentiation events that require cellular self-digestion, cellular housekeeping and/or cellular recycling to meet high nutritional and energy demands.

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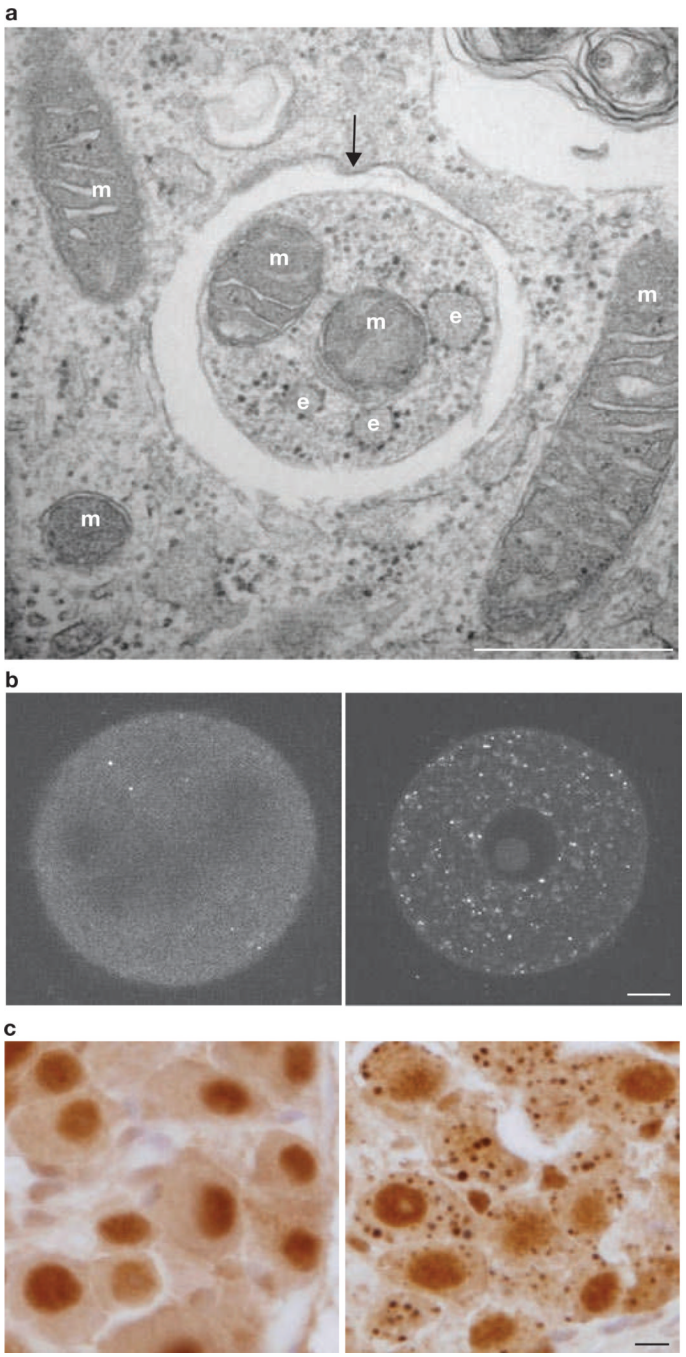

Figure 1.

Representative images of the induction of autophagy and the outcome of its suppression. (**a**) Electron microscopic analysis of mouse embryonic fibroblasts during nutrient starvation. Mitochondria (m) and fragments of endoplasmic reticulum (e) are observed inside an autophagosome (arrow). Mitochondria may be randomly sequestered under conditions of starvation, but can be selectively degraded during erythroid differentiation and Parkin-mediated mitophagy. Scale bar, 500 nm. (**b**) Induction of autophagy after fertilization. Unfertilized (left) and fertilized (right) oocytes from female mice expressing GFP–LC3 to monitor autophagosome formation were observed by confocal microscopy. Small dots indicate GFP–LC3-positive autophagosomes. Scale bar, 10 μm. (**c**) Accumulation of

ubiquitin-positive inclusion bodies in neurons of Atg5-deficient mice. Dorsal root ganglion neurons from wild-type (left) and systemic Atg5-deficient (right) neonates were stained with a monoclonal anti-ubiquitin antibody (1B3, purchased from MBL). Scale bar, 10 μm.

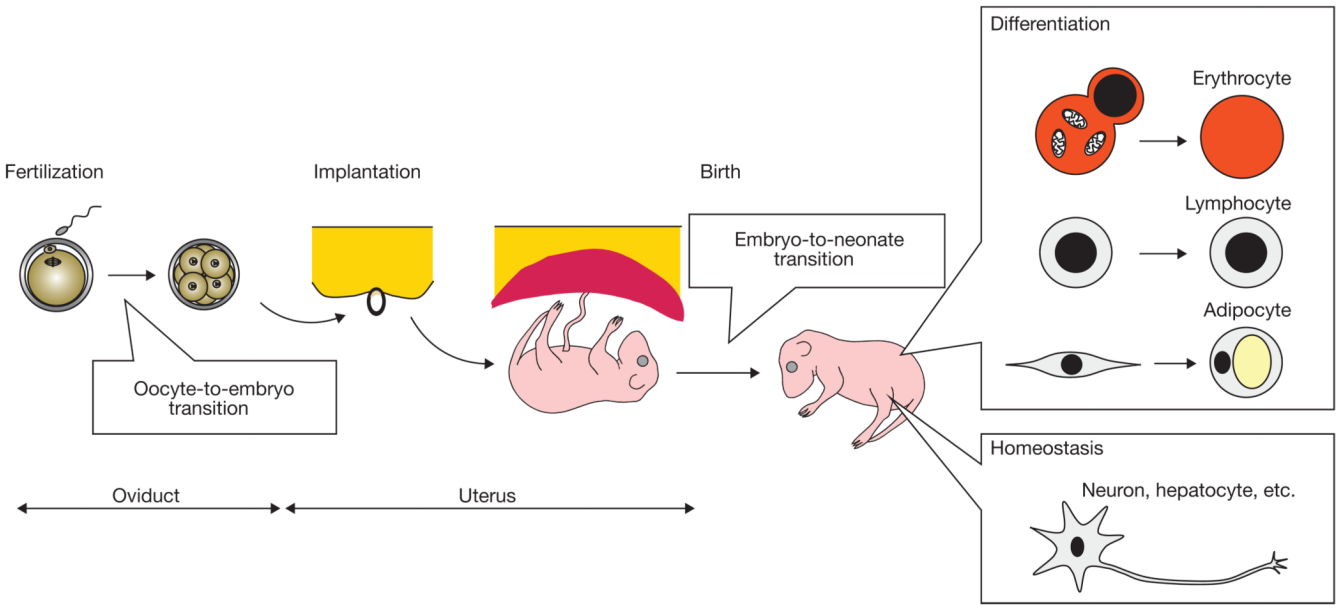

Figure 2.

The role of autophagy in development and differentiation in mammals. Autophagy has critical roles in fertilized oocytes and neonates through protein catabolism and the resulting production of the necessary amino acids. Autophagy is also important in cell remodelling (for example in mitochondrial elimination) during the differentiation of erythrocytes, lymphocytes and adipocytes. Finally, the role of autophagy in housekeeping is important, particularly in terminally differentiated cells such as neurons and hepatocytes, in which continuous renewal of cytoplasmic contents is essential.

Table 1

The roles of autophagy in development and differentiation of model organisms

Organism	Role	Reference
Saccharomyces cerevisiae	Spore formation	93
Schizosaccharomyces pombe	Spore formation	94, 95
Dictyostelium discoideum	Fruiting body formation	96, 97
Sordaria macrospora	Essential for growth; fruiting body formation	98
Podospora anserina	Development of aerial hyphae; differentiation of female reproductive organs	99
Leishmania major	Differentiation into metacyclic promastigote	100
Trypanosoma cruzi	Differentiation into metacyclic trypomastigotes	101
Caenorhabditis elegans	Larval development; dauer formation; degradation of germline P granules in somatic cells	10, 102, 103
Drosophila melanogaster	Larval development; degradation of larval tissues (for example midgut and salivary gland)*; synaptic development	104–107
Arabidopsis thaliana	Dispensable	108–110
Mus musculus	See Tables 2 and 3	

The development-related roles of autophagy are listed. Autophagy may have other roles in each organism. For example, autophagy is important for starvation adaptation in all of these organisms.

* Metamorphosis seems almost normal in the *Drosophila atg7* mutant, although the pupal period is prolonged111

Table 2

Phenotypes of systemic knockout mice of *ATG*-related genes

Genes	Phenotype	References
Atg3$^{-/-}$, *Atg5*$^{-/-}$, *Atg7*$^{-/-}$, *Atg9*$^{-/-}$, *Atg16L1*$^{-/-}$	Neonatal lethal with reduced amino acid levels, suckling defect (Atg9 has an additional role in innate immune responses induced by double-stranded DNA)	17–21
beclin 1$^{-/-}$	Early embryonic lethal (E7.5 or earlier) with defects in proamniotic canal closure (heterozygous mice show increased susceptibility to spontaneous tumours)	28, 29
FIP200$^{-/-}$	Embryonic lethal (E13.5–E16.5) due to defective heart and liver development	32
Ambra1$^{gt/gt}$	Embryonic lethal (~E14) with defects in neural tube development, and hyperproliferation of neural tissues	30
ULK1$^{-/-}$	Increased reticulocyte number with delayed mitochondrial clearance	51
Atg4C$^{-/-}$	Viable, fertile, increased susceptibility to carcinogen-induced fibrosarcoma	112
LC3B$^{-/-}$	Normal phenotype	113
GABARAP$^{-/-}$	Normal phenotype	114

The phenotypes of conventional systemic knockout mice of *ATG*-related genes are listed. *gt*, gene-trapped allele.

Table 3

Phenotypes of tissue-specific knockout mice of *ATG*-related genes

Mouse model	Target tissue/cell	Major phenotype	References
Atg5 F/F;; Nestin-*Cre*, *Atg7 F/F;* Nestin-*Cre*	Neural cell	Neurodegeneration; accumulation of ubiquitin and p62	70, 71, 80
FIP200 F/F; Nestin-*Cre*	Neural cell	Neurodegeneration; accumulation of ubiquitin, p62 and mitochondria (more severe than *Atg5/7 F/F;* Nestin-*Cre*)	72
Atg5 F/F; Pcp2-*Cre*, *Atg7 F/F;*; Pcp2-*Cre*	Purkinje cell	Axonal degeneration; accumulation of p62	115, 116
Atg5 F/F; Mx1-*Cre*, *Atg7 F/F;*; Mx1-*Cre*, *Atg7 F/F;*; Alb-*Cre*	Hepatocyte	Hepatic failure, hepatomegaly; accumulation of ubiquitin, p62 and mitochondria	19, 67, 70, 80
Atg5 F/F; MLC2v-*Cre*	Cardiomyocyte	Minimal abnormal phenotype (sensitive to pressure overload-cardiac failure)	73
Atg5 F/F; Mer*Cre*Mer	Cardiomyocyte	Cardiac hypertrophy and dysfunction; accumulation of ubiquitin, p62 and mitochondria	73
Atg5 F/F; HSA-*Cre*	Skeletal muscle	Atrophy of fast muscle fibres; accumulation of ubiquitin and p62	74
Atg5 F/F; Mlc1-*Cre*	Skeletal muscle	Muscle atrophy and weakness; accumulation of ubiquitin, p62 and mitochondria	75
Atg7 F/F; Ap2-*Cre*	Adipocyte	Decreased white adipose tissue mass, resistant to obesity; accumulation of p62 and mitochondria	60, 62
Atg5 F/F; EL-*Cre*	Pancreatic acinar cell	No abnormal phenotype (resistant to acute pancreatitis)	117
Atg7 F/F; Rip-*Cre*	Pancreatic β-cell	Impaired β-cell function, reduced β-cell mass; accumulation of ubiquitin, p62 and mitochondria	76, 77
Atg5 F/F; Zp3-*Cre*	Oocyte	Embryonic lethal at 4–8-cell stage (if fertilized with *Atg5*$^{-}$sperm)	11
Atg5 F/F; Lck-*Cre*, *Atg7 F/F;* Lck-*Cre*	T cell	Decreased T-cell numbers; accumulation of mitochondria	55, 56
Atg5 F/F; CD19-*Cre*	B cell	Reduced B-1a B-cell numbers	59
Atg7 F/F; Vav-*Cre*	Haematopoietic cell	Severe anaemia, lymphopenia (T and B cells); accumulation of mitochondria	53
Atg5 F/F; CD11c-*Cre*	Dendritic cell	(Succumbed to viral infection due to defects in antigen presentation)	118
Atg5 F/F; Podocin-*Cre*	Podocyte	Late-onset glomerulosclerosis; accumulation of ubiquitin, p62 and mitochondria	78

Phenotypes of tissue-specific knockout mice of *ATG*-related genes are listed. *F*, floxed allele; MLC2v, myosin light chain 2v; Mlc1, myosin light chain 1; HAS, human skeletal actin; EL, elastase I; Rip, rat insulin promoter. ‘Accumulation of ubiquitin’ refers to the accumulation of either ubiquitylated proteins or ubiquitin aggregates. ‘Accumulation of p62’ refers to the accumulation of either p62 protein or p62-positive aggregates.