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Mitochondrial homeostasis in adipose tissue remodeling

Svetlana Altshuler-Keylin[1,2,3] and **Shingo Kajimura**[1,2,3,*]

[1]UCSF Diabetes Center, University of California, San Francisco, San Francisco, CA 94143–0669, USA

[2]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California, San Francisco, San Francisco, CA 94143–0669, USA

[3]Department of Cell and Tissue Biology, University of California, San Francisco, San Francisco, CA 94143–0669, USA

Abstract

Mitochondrial homeostasis is regulated by a balance between mitochondrial biogenesis and degradation. Emerging evidence suggests that mitophagy, a selective form of autophagy that degrades mitochondria, plays a key role in the physiology and pathophysiology of mitochondria-enriched cells, such as brown and beige adipocytes. This review discusses findings regarding the roles of autophagy and mitophagy in cellular development, maintenance, and functions of metabolic organs, including adipose tissue, liver, and pancreas. A better understanding of the molecular links between mitophagy and energy metabolism will help to identify promising targets for the treatment of obesity and obesity-associated disorders.

Introduction: Autophagy and Mitophagy

Mitochondria are double-membrane organelles that serve as the central source of ATP (adenosine 5′-triphosphate) with which cells carry out various functions. The cell must undergo both mitochondrial biogenesis and mitochondrial degradation to maintain “healthy” mitochondria in response to varying energetic demands of the cell (Fig. 1). On one end, mitochondrial biogenesis is tightly regulated by various transcriptional regulators encoded by nuclear genes, including peroxisome proliferator–activated receptor γ (PPARγ) coactivator 1α (PGC-1α), nuclear respiratory factor 1 (Nrf1) and Nrf2, and mitochondrial transcription factor A (Tfam). Transcriptional control of mitochondrial biogenesis has been previously discussed in detail (1).

On the other end, mitochondrial degradation is carried out through autophagy, a process of intracellular degradation to break down unwanted or damaged cellular components. The main hallmark of autophagy that distinguishes it from other degradation processes is the formation of a double-membrane vesicle, the autophagosome, to deliver large cytoplasmic components to the lysosome for degradation. The detailed processes of autophagosome formation are described elsewhere (2, 3). In short, the molecular signal from the mechanistic

*Corresponding author. skajimura@diabetes.ucsf.edu.

target of rapamycin complex 1 (mTORC1) triggers the activation of unc-51–like autophagy activating kinase 1 (ULK1) complex, consisting of ULK1, autophagy-related protein 13 (ATG13), and focal adhesion kinase family interacting protein of 200 kDa (FIP200), to initiate the formation of the isolation membrane from existing membrane sources such as the endoplasmic reticulum (ER) or Golgi (Fig. 2A). The membrane further expands to produce a completely enclosed, double-membraned vesicle known as the autophagosome. Autophagosome formation is orchestrated by a number of core autophagy-related proteins. A key step for autophagosome formation is the conjugation of phosphatidylethanolamine (PE) to microtubule-associated protein 1 light chain 3 (LC3), an ATG8 homolog, to generate a lipidated form of LC3, LC3-PE. This conjugation is mediated in part by ATG7 and the ATG5-ATG12-ATG16L1 complex (3). LC3 is retained inside the autophagosome and, when expressed as a green fluorescent protein (GFP) fusion protein, serves as a common marker of autophagy (4). Once the autophagosome is developed, it fuses with the lysosome, forming an autolysosome, a single-membraned acidic vesicle where lysosomal hydrolytic enzymes, such as cathepsins, degrade the enclosed contents. Lysosome biogenesis is an important component of autophagy machinery and regulated by the microphthalmia/transcription factor E (MiT/TFE) family of transcription factors, which includes microphthalmia-associated transcription factor (MITF), transcription factor EB (TFEB), and transcription factor binding to IGHM enhancer 3 (TFE3) (5–8). Although the later elements of the autophagy machinery are pivotal in the regulation of degradation, the initial selective degradation of cytoplasmic components through autophagy is worth exploring in depth.

A particularly interesting example is mitophagy, the selective clearance of mitochondria through autophagy. Selectivity is driven by specific proteins that physically connect the intended target (such as mitochondria) with the autophagosomal protein LC3. These receptors interact with the autophagosome through the LC3-interacting region (LIR) (Fig. 2B). Mitochondrial damage is a major physiological trigger for selective mitochondrial clearance. Damage-induced mitophagy can occur through two different mechanisms: (i) adapter-mediated, ubiquitin-dependent mitophagy and (ii) direct, ubiquitin-independent mitophagy (Fig. 2B). Adapter-mediated mitophagy, which is mediated by phosphatase and tensin homolog (PTEN)–induced putative kinase 1 (PINK1) and the E3 ubiquitin ligase Parkin, requires the ubiquitination of the target. Damage to the mitochondria leads to reduced mitochondrial membrane potential, stabilization of PINK1 on the outer mitochondrial membrane, and subsequent recruitment of Parkin, which ubiquitinates outer mitochondrial proteins (9). The ubiquitinated substrates are then recognized by autophagy adapter proteins including p62, optineurin, NDP52 (nuclear dot protein 52 kDa), and NBR1 (neighbor of Brca1 gene 1), which link the ubiquitinated targets to LC3 (10–13). Adapter proteins contain two defining domains: a ubiquitin-binding domain for cargo recognition and an LIR domain that interacts with LC3 to promote encapsulation by the autophagosome. Whether adapter proteins have tissue- or cell type–specific functions have yet to be revealed. Damage-induced mitophagy can also occur through the direct interaction of mitochondria-localized proteins with LC3 independent of ubiquitination. For example, BCL2/E1B 19 kDa–interacting protein 3 (BNIP3) and FUN14 domain-containing protein 1 (FUNDC1) directly interact with LC3 to promote mitophagy in response to hypoxia-triggered mitochondrial damage (14, 15). The mitophagy receptor BCL2-like 13 (BCL2L13), which is

a mammalian homolog of Atg32, directly interacts with LC3 through the LIR domain, but the mechanism that activates BCL2L13 remains to be determined (16).

Mitophagy can take place independently of mitochondrial damage during developmental processes, although fewer models of this process have been established. For instance, the BNIP3 homology NIP3-like protein X (NIX; also known as BNIP3L) is required for mitochondrial clearance during erythrocyte maturation (17, 18). NIX mediates mitophagy in a ubiquitin-independent manner, and blocking the direct interaction between NIX and LC3 leads to accumulation of mitochondria in maturing erythrocytes (19). In addition, mitochondrial degradation occurs when sperm mitochondria are removed during fertilization. The mechanism of paternal mitochondrial degradation is not conserved between species. In *Caenorhabditis elegans*, this process requires autophagosome formation and is independent of ubiquitination (20, 21). In *Drosophila*, paternal mitophagy also requires autophagosome formation that is ubiquitin- and p62-dependent but does not require Parkin (22). Examples in *C. elegans* and *Drosophila* suggest that there are uncharacterized proteins that target mitochondria for degradation. In mammals, including the mouse, pig, and rhesus monkey, mitochondrial degradation appears to occur through the ubiquitin-proteosome system independently of LC3-mediated autophagy (23–25). In a slightly different vein, we have identified a developmentally important process of mitochondrial clearance: selective mitochondrial degradation during beige adipocyte conversion to white adipocytes after the withdrawal of cold exposure or β_3-adrenergic receptor (β3-AR) stimulation. The mechanism underlying the recognition of mitochondria for selective degradation awaits future investigation.

The Role of Autophagy in Adipose Biology

Mammals have two functionally distinct types of adipocytes: white adipocytes, which store excess energy as triglycerides, and brown adipocytes, which dissipate energy in the form of heat and thus can counteract obesity and obesity-associated diseases such as type 2 diabetes (26). Adult humans and rodents have a “recruitable” form of brown adipocytes, termed “beige adipocytes,” the development of which can be induced by certain environmental stimuli such as chronic cold exposure (27–29). Although brown and beige adipocytes have similar biochemical and morphological characteristics, including the brown and beige fat-specific protein uncoupling protein 1 (UCP1), high mitochondrial content, and multilocular lipid droplets, their developmental origins are distinct (29–34). Brown adipocytes arise early during development from a subset of dermomyotome precursors, and their development is stalled during postnatal stages. On the other hand, beige adipocytes arise postnatally in part through the action of the transcription factor progesterone receptor (PR) (PRD1-BF1-RIZ1 homologous) domain–containing 16 (PRDM16) in response to environmental cues from precursors that are positive for early B cell factor 2 (Ebf2), platelet-derived growth factor receptor α (Pdgfrα), and stem cell antigen–1 (Sca1) (Fig. 3) (35–37). Cold exposure, which stimulates the β3-AR signaling pathway, is a dominant activator of brown and beige adipocyte development. Notably, beige adipocytes lose their morphological and thermogenic characteristics and acquire “white-like” characteristics shortly after the requisite stimuli (cold exposure or β3-AR stimulation) are removed (38, 39). We have reported that the conversion from beige adipocyte to white adipocyte is direct, circumventing an intermediate

precursor state, and involves active mitochondrial clearance (Fig. 3) (39). Given the crucial role of the mitochondria in the thermogenic function of beige and brown adipocytes, it is important to understand the mechanisms underlying the regulation of mitochondrial homeostasis in brown and beige adipocytes.

Autophagy has been implicated in remodeling mitochondrial contents and thus regulating adipocyte differentiation as well as the maintenance of differentiated adipocytes. Accordingly, it is important to use an appropriate Cre mouse line that can target specific adipocytes at different differentiation stages to dissect the roles of autophagy in defined cell types (for example, preadipocytes or differentiated adipocytes). Several genetic autophagy-deficient animal models have been used to study the function of autophagy in adipose tissues but exhibit inconsistent phenotypes (Table 1). For instance, a total knockout of *Atg5* results in a differentiation defect of white adipose tissue (WAT), whereas deletion of *Atg7* through a muscle- and brown adipocyte–specific Cre, *Myf5*-Cre, promotes beige adipocyte development and impairs brown adipocyte differentiation (40, 41). These models assess the role of autophagy during adipogenesis and preclude insight into autophagy function in terminally differentiated adipocytes. Similarly, *aP2*-Cre–mediated deletion of *Atg7* or *p62* affects not only mature brown and white adipocytes but also some nonadipose tissues (42–44). Nonspecific expression of *aP2*-Cre in skeletal muscle, liver, brain, and macrophages can cause indirect effects on adipocyte differentiation and/or function (45, 46). In addition, inhibiting autophagy in proopiomelanocortin (POMC) neurons and skeletal muscle causes browning of WAT (41, 47). We have used *Ucp1*-Cre to selectively target autophagy in differentiated brown and beige adipocytes to show that autophagy is required specifically for beige-to-white adipocyte conversion after cold or β3-AR withdrawal (39).

Another potential cause for various phenotypes observed in autophagy-deficient models may reside in the promiscuity of autophagy machinery components, many of which have broad cellular functions beyond autophagy (48). Deletion of either *Atg5* or *Atg12* using *Ucp1*-Cre results in a consistent phenotype: retention of mitochondrial content and high UCP1 abundance in inguinal WAT containing beige adipocytes even after withdrawal of β3-AR stimulation (39). In contrast, deletion of *p62*, a cargo receptor that mediates selective autophagy, results in impaired mitochondrial function including reduced UCP1 abundance in both brown adipose tissue (BAT) and inguinal WAT (44). These apparently conflicting phenotypes can be explained by the role that p62 plays in various signaling pathways, including those of nuclear factor κB (NF-κB), extra-cellular signal–regulated kinase 1 (ERK1), and nuclear factor erythroid 2–related factor 2 (Nrf2) (49, 50). Assessing multiple components of the autophagy process using the mature adipocyte-specific Cre lines, such as *Adiponectin*- or *Ucp1*-Cre, would clarify the specific biological processes that are specifically regulated by each autophagy regulator.

Physiological Regulation of Autophagy in Adipose Tissues

Autophagy is initiated in concordance with responses to nutrient availability and is thus tightly regulated by the mTORC1 complex of the mTOR signaling pathway, which acts as a nutrient sensor to coordinate cellular responses (Fig. 4). High nutrient abundance leads to the activation of mTORC1 and its downstream targets, including ribosomal S6 kinase 1

(S6K1) to promote protein synthesis for anabolic functions. In the absence of nutrients, mTORC1 is inhibited, leading to autophagy activation through the regulation of the ULK1, FIP200, and ATG13 complex (Fig. 4A) (51).

Another form of physiological regulation of autophagy is β3-AR signaling, which triggers protein kinase A (PKA) signaling. PKA signaling functions to inhibit autophagy in yeast and mammals (52), and feedback loops between autophagy and PKA have been uncovered (53, 54). Notably, the β3-AR signaling pathway is a key mediator of beige adipocyte biogenesis in the face of cold exposure. Beige adipogenesis is promoted when environmental cues, such as cold exposure, trigger the release of norepinephrine from the sympathetic nervous system, which, in turn, acts on the β3-AR, increases intracellular cyclic adenosine 3′,5′-monophosphate (cAMP) concentrations, and triggers the PKA signaling pathway, leading to transcriptional activation of the thermogenic program in brown and beige fat (Fig. 4B) (55). In response to cold or β3-AR stimulation, PKA directly phosphorylates mTOR and its binding partner, regulatory-associated protein of mTOR (RAPTOR), to stimulate the activity of mTORC1, a major regulator of autophagy (Fig. 4C) (56). Our group has further shown that PKA, in turn, represses autophagy in beige adipocytes (Fig. 4D), which is partially mediated through repression of *Mitf*, a member of the MiT/TFE family of transcription factors that regulates lysosome biogenesis. Pharmacological inhibition of PKA relieves repression of *Mitf* and genes encoding autophagy and lysosome components, confirming that PKA is responsible for inhibiting autophagy in beige adipocytes. In addition, regulation of *Mitf* and autophagy-related genes by PKA occurs even under starvation conditions, suggesting that PKA represses autophagy independently of nutritional signals (39). Notably, mTORC1 inhibits transcription of autophagy and lysosome target genes by phosphorylating TFEB, another member of the MiT/TFE family, which blocks its translocation to the nucleus (Fig. 4E) (57–59). It is conceivable that the PKA-mTORC1 signaling axis simultaneously regulates beige adipocyte development and autophagy through MiT/TFE transcription factors.

How is mitochondrial clearance by mitophagy initiated during the beige-to-white adipocyte conversion? One possibility is that the mitophagy activation may be mediated through the PINK1-Parkin pathway. To support the notion, PKA inhibits PINK1-Parkin–mediated mitophagy through phosphorylation of a mitochondrial membrane protein, MIC60 (also known as mitofilin) (60). Another compatible possibility is that the mitochondrial degradation is regulated by the BAT-specific mitochondrial protein UCP1, which reduces mitochondrial membrane potential through proton “leak.” During the beige-to-white adipocyte conversion, the loss of UCP1 protein correlates with the loss of mitochondrial respiratory chain complexes. Inhibition of autophagy by genetic ablation of *Atg5* or *Atg12* maintains expression of mitochondrial DNA transcripts and abundance of mitochondrial proteins, including UCP1 (39). Hence, it is possible that mitochondrial removal through autophagy during the beige-to-white adipocyte conversion requires the transient induction of UCP1 protein, which acts as an upstream regulator of mitophagy by uncoupling the proton gradient in the mitochondria. To support the hypothesis, UCP1 abundance is tightly associated with mitochondrial content and cristae density in adipose tissues (61, 62).

In addition, the cAMP-PKA signaling pathway has been implicated in regulating adipocyte lipophagy. Lipophagy, which is reviewed by Evans *et al.* (63) in the same issue, is a selective autophagic degradation of lipids. Although it was first demonstrated in hepatocytes, where pharmacological and genetic inhibition of autophagy in cultured hepatocytes lead to increased triglyceride accumulation (64), lipophagy has also been detected in hepatic stellate cells, neurons, and brown adipocytes (65–69). Lipophagy in brown adipocytes is regulated by cold exposure, although the effect appears to be dependent on the length of exposure. Whereas chronic cold exposure inhibits lipophagy and associated autophagy components through the cAMP-PKA signaling pathway (70), acute cold exposure activates autophagy-mediated lipid degradation (71). An aspect of concern is the shortage of tools to monitor lipophagy, making it difficult to assess selective lipophagy. The biological importance of lipophagy over lipolysis for lipid breakdown awaits future studies.

Pathological Regulation of Autophagy in Metabolic Disease

Autophagy plays a central role in the function and maintenance of metabolic tissues such as liver, pancreas, and adipose tissues. Emerging evidence suggests that dysregulation of autophagy contributes to the initiation or progression of metabolic disorders in the following organs.

Adipose tissue

Obesity (increased adiposity or body mass index) is inversely correlated with the thermogenic activity of BAT in response to cold exposure (72). Beige adipocyte biogenesis is impaired in obese mice partly because of the activation of negative regulators such as the transforming growth factor–β (TGF-β) and Notch signaling pathways (26). In turn, blockade of the TGF-β or Notch signaling pathways by genetic or pharmacological approaches promotes beige adipocyte biogenesis and protects mice from diet-induced obesity (73–75). Furthermore, autophagy blocks beige adipocyte development. Inhibiting autophagy through deletion of *Atg7* in adipocytes leads to increased beige adipocyte differentiation, resistance to diet-induced obesity, and improved insulin sensitivity (42, 43).

Dysregulation of beige adipocyte maintenance is likely to contribute to the development of obesity. We have found that diet-induced obesity accelerates the conversion of beige adipocytes to white adipocytes after β3-AR agonist withdrawal, which correlates with increased autophagy in subcutaneous WAT. Genetic deletion of *Atg12* or *Atg5* specifically in UCP1-positive adipocytes substantially prolongs the retention of beige adipocytes in vivo. The maintained beige fat is thermogenically active and suppresses diet-induced obesity and obesity-induced insulin resistance (39). Notably, increased adipose tissue autophagy has been observed in human obesity and type 2 diabetes (76–79). In addition, activation of autophagy in human subjects with type 2 diabetes and obesity is partly attributed to repression of mTORC1 activity (76). These studies suggest that dysregulation of autophagy in the adipose tissues of obese subjects may contribute to the accelerated beige-to-white adipocyte conversion.

Pancreas

Defects in pancreatic islet β cell function are the fundamental cause for type 1 and type 2 diabetes. Pancreatic β cells rely heavily on mitochondria and the ER to maintain glucose-stimulated insulin production and secretion, and autophagy maintains β cell homeostasis by removing damaged mitochondria and/or ER. Mice with a β cell–specific deletion of *Atg7* accumulate defective mitochondria and distended ER in their β cells, leading to impaired glucose tolerance and reduced insulin secretion (80, 81). Autophagy-deficient β cells fail to proliferate as an adaptation to increased insulin demand in obesity (81, 82). The mechanism by which autophagy controls β cell proliferation remains unclear.

A limited number of studies have investigated the metabolic consequences of dysfunctional mitophagy in β cells. Total Parkin-null mice display reduced insulin secretion after glucose challenge under pathological stress conditions such as streptozotocin exposure, whereas Parkin overexpression in pancreatic β cell lines maintains insulin secretion under diabetic conditions (83). In addition, C-type lectin domain family 16 member A (Clec16a) has been implicated in β cell mitophagy through a Parkin-dependent mechanism. Pancreas-specific deletion of *Clec16a* using *Pdx1*-Cre leads to accumulation of abnormal mitochondria, impaired glucose tolerance, and insulin secretion in response to glucose challenge (84). Surprisingly, *Clec16a*-deficient pancreas have more Parkin, which raises questions about the role of Parkin in regulating mitophagy in β cells. Although the role of Parkin in β cell mitophagy remains unclear, these results emphasize that dysfunction in selective mitophagy alone could lead to β cell failure and diabetes.

Liver

Obesity is closely associated with hepatic steatosis. Mice with diet-induced obesity or a genetically induced form of obesity (*ob/ob* mice) display reduced hepatic autophagy, as assessed by decreased numbers of GFP-LC3 puncta, reduced LC3-II abundance, and accumulation of p62 (85). Liver-specific deletion of *Atg7*, *Vps34* (vacuolar protein sorting 34), or *Tfeb* results in lipid accumulation and enlarged livers (64, 86, 87). Conversely, overexpression of *Atg7* or *Tfeb* by adenovirus reverses hepatic lipid accumulation, reduces liver size, prevents body weight gain in response to genetic or high-fat diet–induced obesity, and improves glucose tolerance and insulin sensitivity (85, 87). However, contrary to these studies, liver-specific deletion of *Atg7* leads to reduced hepatic lipid accumulation (47, 88). Because the diverging phenotypes come from the same genetic model, the discrepancies are presumably due to differences in experimental conditions or analyses.

Starvation is a powerful stimulus that induces autophagy in the liver to control hepatic gluconeogenesis. During the early neonatal period, when the placental nutrient supply is cut off at birth, autophagy-deficient ($Atg5^{-/-}$) pups die soon after birth from severe hypoglycemia and hypolipidemia (89). Dysregulation of autophagy in a liver-specific fashion in adults leads to a defect in intracellular lipid degradation, enlarged liver, and increased hepatic lipid content under starvation conditions (64, 87). In addition, liver-specific deletion of *Atg7* using inducible *Mx1*-Cre results in hypoglycemia due to reduced gluconeogenesis (90).

Positive regulators of the starvation-induced autophagy include TFEB and PPARα; for instance, liver-specific deletion of *Tfeb* prevents transcription of various autophagy-related genes and leads to increased hepatic lipid content, increased circulating free fatty acids (FFAs), and impaired FFA oxidation in vitro (87). Similarly, PPARα activates transcription of autophagy components. Pharmacological activation of PPARα induces autophagy in nutrient-replete cells, which mimics a starvation response, whereas the liver of fasted *Pparα*$^{-/-}$mice cannot induce autophagy and exhibits increased lipid content (91). On the other hand, farnesoid X receptor (FXR) functions to repress autophagy in the fed state. Pharmacological activation of FXR blunts starvation-induced hepatic autophagy, whereas *Fxr*$^{-/-}$mice maintain high amounts of autophagy in the liver even in the fed state (65, 91). It would be important to determine whether the same regulation applies to non–starvation-induced autophagy in other metabolic organs.

Methodologies for Detecting Mitophagy in Adipocytes

Mitophagy structures were initially identified by electron microscopy. Studies from the 1950s described double-membrane vesicles, later termed autophagic vacuoles, which contained recognizable mitochondrial cristae (92). Identification and quantification of mitophagy have been challenging due largely to the substantial overlap with autophagy machinery and an absence of a universally defined marker for mitophagy per se. Despite the current limitations, there are several tools available for monitoring mitophagy as described below.

Colocalization of mitochondria-localized proteins with GFP-LC3

Mitophagy can be assessed on the basis of colocalization of the autophagosome with mitochondria. To this end, GFP-LC3 transgenic mice have been successfully used to visualize autophagosomes in vivo and cultured cells (93). Vital dyes, such as MitoTracker Red are reliable options for labeling mitochondria in vitro. However, these dyes label less than 50% of the existing mitochondria and lose signal after fixation, limitations that preclude their use in vivo (94). An alternative approach to vital dyes is to label mitochondria with mitochondria-localized proteins and assess their sequestration into the autophagosome using GFP-LC3. During the beige-to-white adipocyte conversion, we have observed the colocalization of the mitochondria protein translocase of outer mitochondrial membrane 20 (TOM20) with GFP-LC3; analyzed in conjunction with mitochondria-autophagosome structures observed by electron microscopy, as well as with changes in mitochondrial content, these data suggest that selective mitophagy takes place during the beige-to-white adipocyte conversion (39).

Monitoring mitochondrial turnover: Indirect measurement of mitophagy

Mitophagy is a transient event that cannot be fully examined through snapshots of the mitochondria using mitochondria-localized proteins. MitoTimer is a fluorescent reporter that can measure the kinetics of mitochondrial biogenesis and degradation. A mutant of the red fluorescent protein is attached to the mitochondrial localization sequence of cytochrome c oxidase subunit VIII (COX VIII), which fluoresces to green when the protein is newly synthesized and then gradually transitions to red as the protein matures (95, 96). This system

has been used to assess mitochondrial turnover in skeletal muscle and heart (97, 98). Quantifying the loss of red fluorescence determines the kinetics of mitophagy process. This method is an indirect assessment of mitophagy, and as such, a major concern would be that protein degradation is also measured as well as mitophagy flux.

Monitoring delivery of mitochondria to lysosomes

A more direct way of measuring mitophagy is to assess the delivery of mitochondria to lysosomes. A tandem mCherry-GFP fusion protein is attached to the mitochondrial localization sequence of the mitochondrial fission 1 protein (FIS1). Different chemical properties of mCherry and GFP allow the identification of mitochondria that are undergoing lysosomal degradation; GFP is immediately degraded in the acidic lysosomal environment, whereas mCherry persists (99). A transgenic mouse model called *mito*-QC (quality control), which uses this system, has been generated (100).

A similar strategy for assessing mitophagy directly has been developed using the mt-Keima transgenic mouse system. A tandem repeat of COX VIII tagged with the fluorescent protein Keima is targeted to the mitochondria. Keima fluorescence is pH-dependent; it emits at different wavelengths at neutral or acidic pH, making it possible to determine whether mitochondria are in the cytosol (neutral pH) or lysosomes (acidic pH). In addition, Keima fluorescence is resistant to lysosomal degradation because it is derived from corals (101, 102). The dual fluorescence of this reporter allows direct and quantitative assessment of mitophagic flux using fluorescence-activated cell sorting (FACS), which often is a more sensitive and quantitative tool than imaging analysis (103). At least two potential shortcomings of using mt-Keima system exist: (i) unfixed tissues are required for visualizing changes in Keima fluorescence, and (ii) some spectral overlap of the red and green fluorescence exists (101, 102). The *mito*-QC system mentioned above could be a better system for imaging analysis because it overcomes these limitations, but it has not yet been optimized for FACS analysis (100). These direct tools of assessing mitophagy could be applied to metabolic organs including adipose tissue.

Future Directions

An intriguing observation from our study is that autophagy-mediated degradation of mitochondria in beige adipocytes maintains the fate of these cells. Whereas the role of the nucleus in regulating mitochondrial mass and function has been examined extensively in the past, the above result indicates, in turn, that mitochondria immensely influence the cellular maintenance of beige adipocytes. What is the molecular signaling that mediates the mitochondria-to-nucleus communication? Mitochondrial clearance could regulate cellular maintenance and function by altering nuclear gene expression by limiting metabolite availability. For instance, several mitochondrial metabolites (such as α-ketoglutarate, succinate, and fumarate) regulate chromatin-modifying enzymes, such as Jumonji demethylases, which control the adipocyte differentiation program (104). In addition, mitochondrial stress in *C. elegans* activates another type of mitochondrial quality control, UPR^{mt} (unfolded mitochondrial protein response), which alters the chromatin structure, causing persistent changes in gene expression (105). Further studies are needed to uncover

the molecular mechanisms and biological roles of the mitochondria-nuclear communication in adipocytes.

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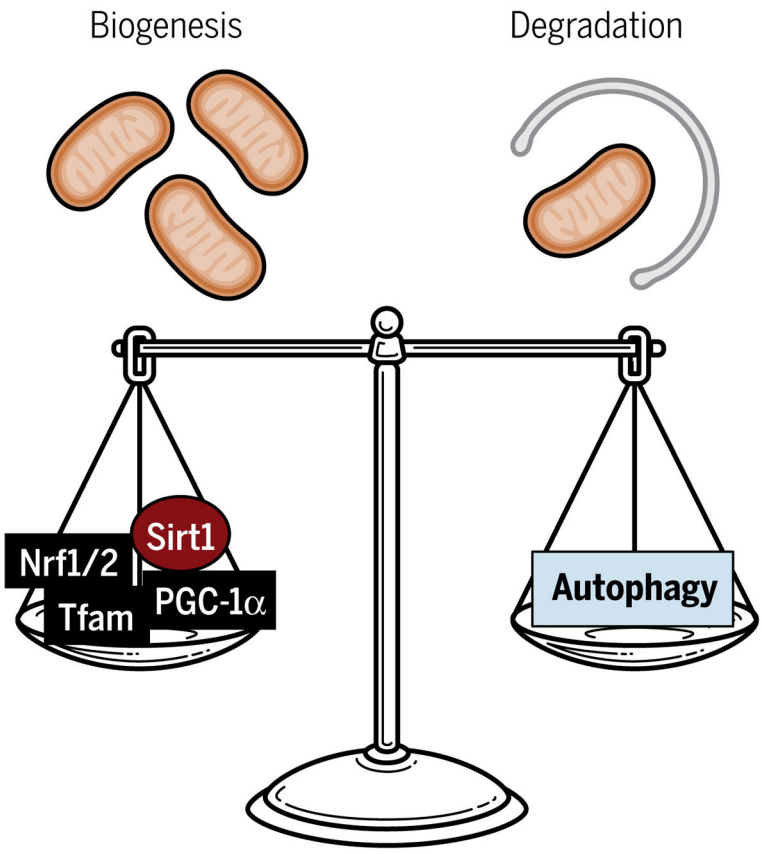

Fig. 1. Regulation of mitochondrial dynamics

Mitochondrial content is regulated by abalancebetweenmitochondrialbiogenesisanddegradation. Nuclear-codedtranscriptional regulators, such as PGC-1α, Nrf1 and Nrf2 (Nrf1/2), and Tfam, control mitochondrial biogenesis, whereas autophagy removes damaged or unwanted mitochondria. Sirt1, sirtuin 1.

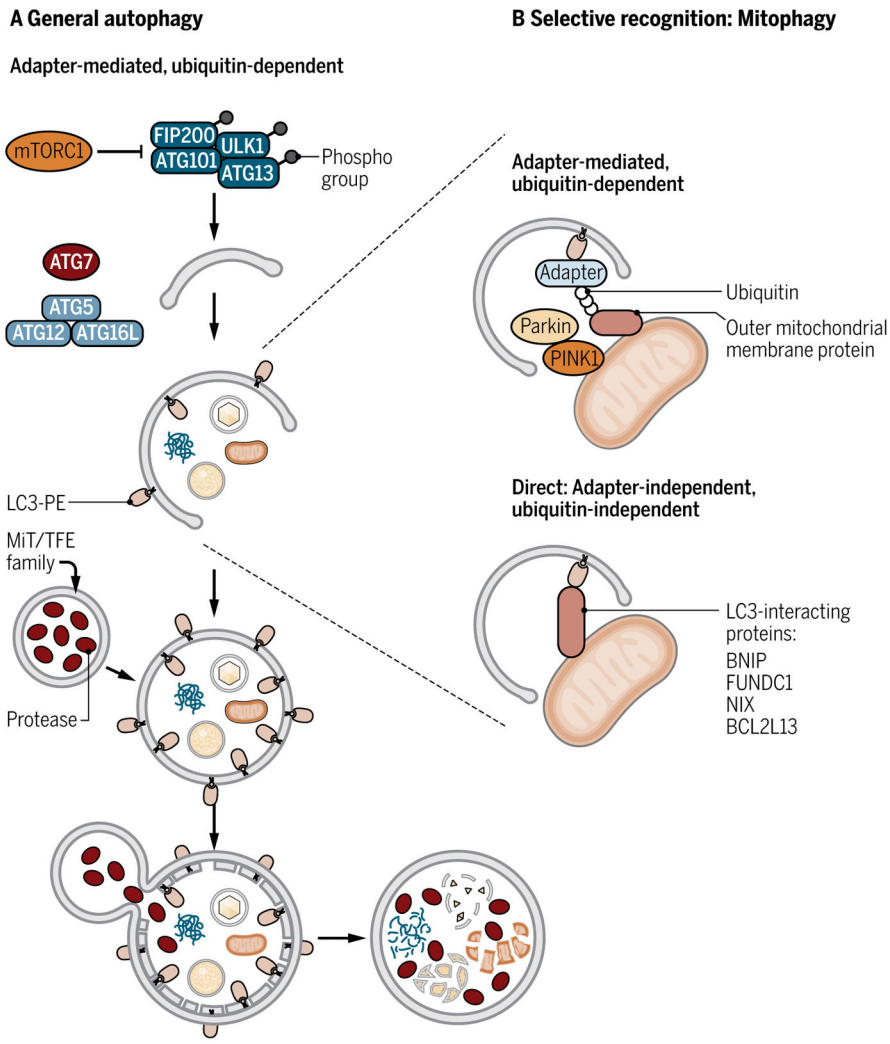

Fig. 2. Overview of the autophagy and mitophagy pathways

(**A**) Autophagy begins with the formation of the isolation membrane. Initiation of the isolation membrane requires the ULK1 complex, which is regulated by mTORC1. The isolation membrane then encloses cytosolic components and elongates to completely enclose and form the autophagosome. The elongation and closure of the autophagosome involve two ubiquitin-like conjugation systems: One forms the ATG5-ATG12-ATG16L complex, and the other one forms the PE-conjugated LC3 (LC3-PE). LC3-PE is required for autophagosome formation and serves as a marker of autophagy. Subsequently, the autophagosome fuses with the lysosome, and the enclosed components are degraded by the lysosomal enzymes. The MiT/TFE family of transcription factors regulates transcription of lysosomal autophagy genes. (**B**) Selective mitochondrial degradation, or mitophagy, relies on autophagy receptors that can interact with LC3-PE proteins (green). In adapter-mediated, ubiquitin-dependent mitophagy (top), PINK1 stabilization recruits Parkin and promotes ubiquitination of proteins in the outer mitochondrial membrane. Ubiquitin chains are recognized by adapter proteins that also contain the LIR and promote encapsulation of the mitochondria by the

autophagosome. In adapter-independent, ubiquitin-independent mitophagy, specific mitochondrial proteins, several of which have been identified, directly interact with LC3.

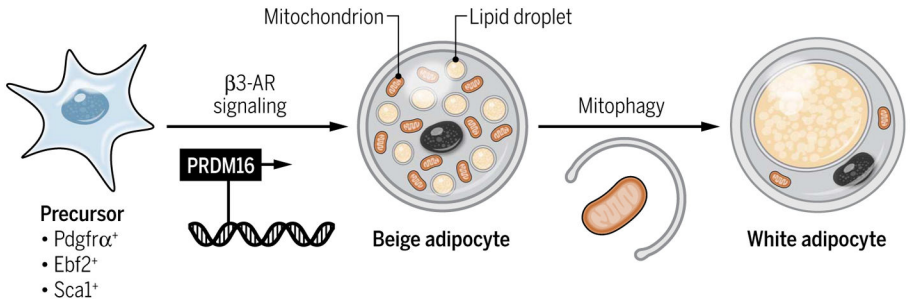

Fig. 3. Beige adipocyte development

Activation of the β3-AR signaling by cold exposure or agonists induces differentiation of precursors into UCP1-positive beige adipocytes. Activation of autophagy after the withdrawal of the stimulus triggers loss of mitochondrial content and conversion from beige to white adipocytes.

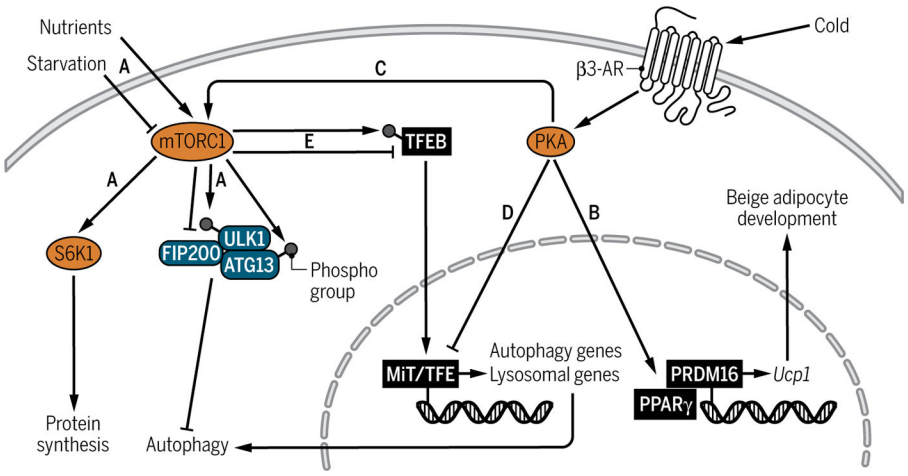

Fig. 4. Cross-talk between the mTOR and β3-AR signaling pathways in beige adipocytes

(**A**) Under nutrient-rich conditions, mTORC1 is activated and phosphorylates ULK1 and ATG13 to repress the ULK1 complex and block autophagy. In response to starvation, mTORC1 is inhibited, inducing autophagy. (**B** and **C**) Activation of PKA in response to β3-AR stimulation induces transcription of brown/beige adipocyte program and promotes mTORC1 activity to inhibit autophagy partly through regulation of MiT/TFE family of transcription factors. (**D**) PKA activation suppresses the expression of genes encoding MiT/TFE transcription factors, and its lysosomal and autophagy targets. (**E**) mTORC1 alters lysosomal and autophagy gene expression through regulating the nuclear-cytoplasmic shuttling of TFEB (an MiT/TFE family member). Active mTORC1 phosphorylates TFEB and blocks its translocation to the nucleus, preventing transcription of lysosomal and autophagy targets.

Table 1

Overview of adipose tissue phenotypes in animals that lack autophagy-related genes.

Molecule	System	Adipocyte phenotype	References
Atg5	Total knockout	WAT development is impaired.	(40)
Atg5	MEFs in vitro	MEFs from total knockout mice have defects in white adipocyte differentiation in culture.	(40)
Atg5	*Ucp1*-Cre	Beige adipocytes are maintained in the absence of browning stimuli and retain mitochondria and UCP1.	(39)
Atg7	*aP2*-Cre	WAT is reduced and adipocytes have increased mitochondrial content with small lipid droplets. WAT in knockout mice contains increased numbers of beige adipocytes. Mice are protected from diet-induced obesity and insulin resistance.	(42, 43)
Atg7	*Myf5*-Cre	Deletion in $Myf5^+$ progenitors impairs BAT development and promotes beige fat development.	(41)
Atg7	*Mlclf*-Cre	Deletion in skeletal muscle induces browning of WAT.	(47)
Atg7	*Pomc*-Cre	Deletion in POMC neurons induces browning of WAT.	(71)
Atg12	*Ucp1*-Cre	Beige adipocytes are maintained in the absence of browning stimuli and retain mitochondria and UCP1. Mice are protected from diet-induced obesity and insulin resistance.	(39)
p62	Total knockout	Enlarged adipocytes due to lipid accumulation. Mice are obese, glucose intolerant, and have decreased insulin sensitivity.	(106)
p62	*aP2*-Cre	Brown adipocytes accumulate defective mitochondria and have more lipid droplets. Mice are obese, glucose intolerant, and have decreased insulin sensitivity.	(44)
Parkin	Total knockout	Mice are resistant to high-fat diet–induced obesity. EpiWAT and BAT of these mice accumulate less lipid in response to a high-fat diet.	(107)
Raptor	*Adiponectin-Cre*	Browning of WAT is disrupted in response to chronic cold exposure.	(56)