

Mini Review

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Crosstalk between Autophagy and Obesity: Potential Use of Avian Model

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ABSTRACT

Autophagy, a self-eating mechanism for recycling cellular constituents, is essential for maintaining cellular homeostasis and its malfunction is associated with diverse diseases including neurodegeneration, cancer, immunity and metabolic syndrome. Human Obesity is a devastating multifactorial disease with continuous increasing prevalence and, thus, there is a need for more extensive research using several experimental models to understand its underlying molecular mechanisms. Emerging evidence indicates a key role of autophagy in the development of obesity and has been a focus of research interest in recent years. This review will briefly describe the autophagy processes and provide insight into metabolic characteristics of avian species that make birds a model of choice for investigation of autophagy particularly with respect to obesity.

KEYWORDS: Autophagy; Obesity; Avian species.

ABBREVIATIONS: AMPK: AMP-activated protein kinase; ApoB: Apolipoprotein B; Atg: Autophagy related gene; db/db: leptin deficient mouse; ER: Endoplasmic Reticulum; GLUT: Glucose transporter; HFD: High Fat Diet; LC3: microtubule-associated protein light chain 3; mTOR: mechanistic Target of Rapamycin; MVB: Multivesicular bodies; ob/ob: obese mouse; PI3P: Phosphatidylinositol 3-phosphate; PI: Phosphatidylinositol; Ulk: Unc-51-Like Kinase; UVRAG: UV radiation resistance associated gene; Vps: Vacuolar protein-sorting.

INTRODUCTION

Autophagy is a highly conserved cellular mechanism that is responsible for the degradation and recycling of damaged organelles. In recent years though, autophagy has appeared to play critical roles in several cellular functions and physiological processes. Although originally described in 1960 by Christian de Duve, the founding father of autophagy,¹ first autophagy-related proteins were only recently identified (in 2008) which makes autophagy a relatively new and thriving field of study. Autophagy was used to distinguish the 'eating' (phagy) of part of the cell's self (auto) from the breakdown of extracellular material (heterophagy).² The name was coined from the observation of electron microscopy studies that showed novel single or double-membrane vesicles containing organelles in various stages of degradation^{1,3} and, therefore, distinguishes it from the ubiquitine (Ub)-proteasome pathway that is specific for the degradation of short-lived proteins.

There are three major types of autophagy; micro-, macro-autophagy, and chaperone-mediated autophagy.^{4,5} Micro- and macro-autophagy can selectively engulf large structures such as mitochondria and endoplasmic reticulum (referred to as mitophagy or reticulophagy, respectively^{6,7}) or by non-selective mechanisms (e.g. bulk cytoplasm), whereas chaperone-mediated autophagy degrades only soluble proteins.⁸ Micro-autophagy refers to the sequestration of cytosolic components directly by lysosomes through invaginations in their limiting membrane. However, macro-autophagy that we will address in the present review refers to

the sequestration of material within an autophagosome, a unique double membrane cytosolic vesicle. Autophagosomes fuse with late endosomes and lysosomes, promoting the delivery of organelles, aggregated proteins and cytoplasm to the luminal acidic degradative milieu that enables their breakdown into constituent molecular building blocks that can be recycled by the cell.⁹

Although autophagy was first observed in mammalian cells, the molecular mechanisms were delineated in yeast. A number of protein complexes and signaling pathways that regulate autophagy have been identified in yeast and many of these have mammalian orthologs. A breakthrough for studying the molecular basis of this pathway was through identifying the Atg (Autophagy-related) genes.¹⁰ There are currently more than 30 Atg genes that have been identified in yeast as well as functionally characterized orthologs of the Atg gene products in higher eukaryotes including: mammals, insects, worms, and plants.^{11,12}

Knowledge gained over the past decade about the mechanisms that underpin autophagy has provided a universal framework for studies of diverse (patho)-physiological processes. Of particular interest is the emerging role of autophagy in the

regulation of energy homeostasis.¹³ Dysregulation of autophagy might contribute to the development of metabolic disorders such as obesity and insulin resistance. Using different experimental animal models may shed light on these underlying molecular mechanisms and may help to develop new therapeutic strategies. In the following sections we will briefly describe the autophagosome formation from initiation to maturation, their interaction with nutrition in the development of metabolic syndrome and unique metabolic characteristics of avian species that argue for birds becoming a model of choice to study the molecular mechanisms involved in obesity.

Autophagosome Process

The autophagy process contains genes that function in key stages of the pathway: initiation (or induction), elongation, maturation, and fusion with the lysosomes are shown in figure 1. First, Atgs are concentrated on single lipid bilayer membranes called phagophores that bud from pre-existing mitochondria or ER and modulate membrane elongation to form cup-shaped structures that engulf cytoplasmic components to generate spherical autophagosomes.¹⁴

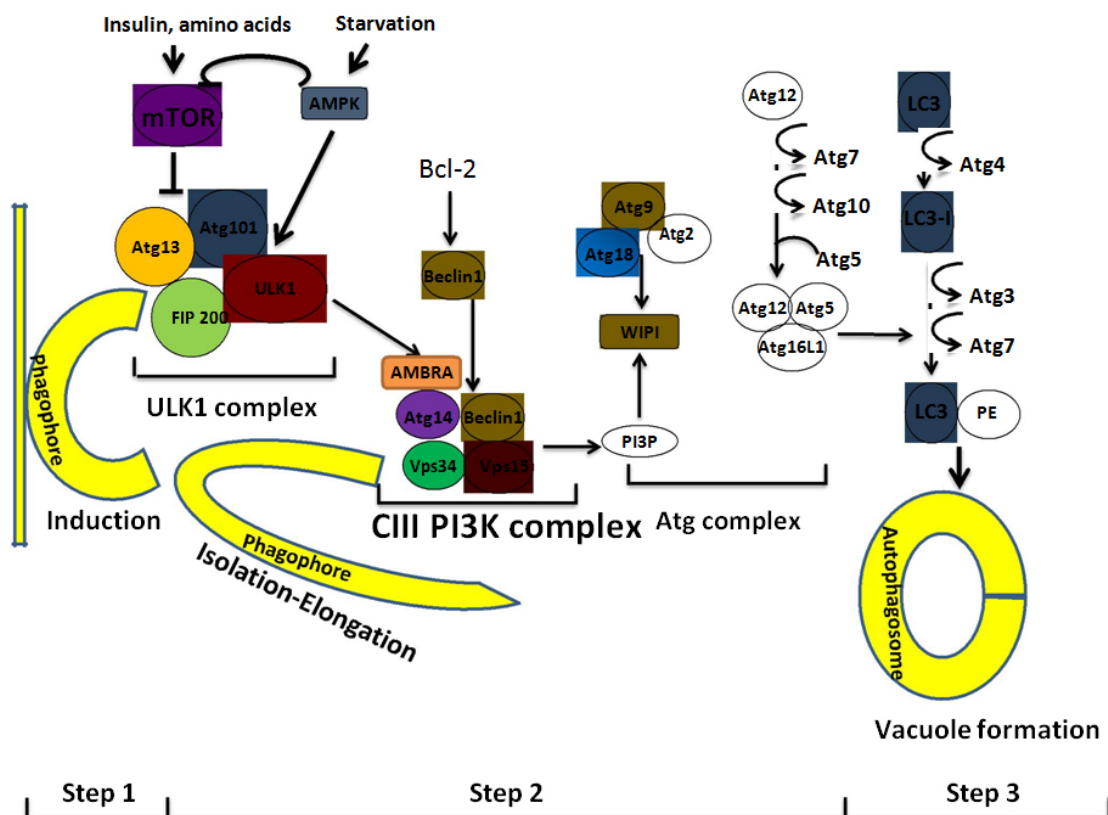


Figure 1: Steps of autophagosome formation: Autophagosome formation can be initiated via mTOR inhibition or AMPK activation during starvation or nutrient limitation. This results in the activation of ULK1 which in turn phosphorylates Atg13, Atg101 and FIP200. When autophagy is activated, Beclin 1 is liberated from Bcl-2 and is associated with Vps34, Vps15 and Atg14. ULK1 phosphorylates also AMBRA, a component of the PI3K CIII complex enabling it to relocate from the cytoskeleton to the isolation membrane. The activation of Vps34 generates PI3P which catalyzes the first of two types of ubiquitination-like reactions that regulates membrane elongation. Firstly, Atg5 and Atg12 are conjugated to each other in the presence of Atg7 and Atg10. Attachment of the Atg5-Atg12-Atg16L1 complex on the isolation membrane induces the second complex to covalently conjugate PE to LC3 which facilitates in turn the closure of the isolation membrane. The complex Atg9-Atg2-atg18 cycles between endosomes, the Golgi and the phagophore possibly carrying lipid components for membrane expansion. LC3-II is formed by LC3 conjugation to its lipid target PE and Atg4 removes LC3-II from the outer surface of newly formed autophagosome, and LC3 on the inner surface is degraded when the autophagosome fuses with lysosomes. AMBRA, autophagy/beclin-1 regulator 1; Atg, autophagy-related genes; LC3, microtubule-associated protein light chain; PE, phosphatidylethanolamine; PI3K, phosphatidylinositol 3 kinase; PIP3, phosphatidylinositol 3-phosphate; ULK1, UNC51-like kinase 1.

The autophagosome-lysosome fusion releases the autophagosome content into the lysosome lumen for degradation. Under fed (normal nutrient-energy adequate) state, the nutrient sensor mechanistic target of rapamycin (mTOR) is activated and in turn phosphorylates ULK1 and thereby sequestering the ULK1-Atg13-FIP200 complex in an inactive state at the mTOR complex.¹⁵ In contrast when nutrients are limited (e.g. during stress or starvation), the energy sensor AMPK is activated. AMPK activation inhibits mTOR activity leading to a reduced ULK1 phosphorylation and consequently releases the ULK1-Atg13-FIP200 complex from mTOR to the site of autophagosome formation and induction of autophagy. In the second step of autophagy, Beclin1 forms a lipid kinase complex with vacuolar sorting proteins Vps15, Vps34 and Atg14 that phosphorylates phosphatidylinositol (PI) to form inositol-3-phosphate (PI3P) and is essential for induction of autophagy.¹⁴ Accumulation of PI3P in specific sub-domains of the ER increases membrane curvature at the site of autophagosome formation. The elongation step involves two ubiquitin-like reactions of the pre-autophagosomal structures. First, the ubiquitin-like protein Atg12 is conjugated to Atg5 by the action of Atg7 and Atg10 after which Atg16 multimerizes to form the Atg12-Atg5-Atg16 complex. Next, Atg4 cleaves soluble microtubule-associated protein light chain 3-I (LC3-I) to form the membrane-bound LC3-II. Both of these two ubiquitin-like systems are required for elongation and closure of the phagophore. During maturation and fusion, autophagosomes will first fuse with endosomes then with lysosomes. Any mutation or loss of proteins important for formation of multivesicular bodies (MVBs) can lead to inhibition of maturation of autophagosomes.^{16,17} Some genes involved in this step include UVRAG, a Beclin 1 interacting protein that recruits the fusion machinery on the autophagosomes. Another Beclin 1 interacting protein, Rubicon, also functions in the maturation of autophagosomes where it is thought to be a part of a distinct Beclin 1 complex containing Vps34, Vps15, and UVRAG that suppresses autophagosome maturation.^{18,19} Working together, these steps complete the formation of the autolysosome and its lysis, that releases proteins and amino acids that can be used as an energy source during times of low energy availability or increased energy demand (stress) for the organism (Figure 1).

Autophagy and Development of Obesity

Obesity is a devastating multifactorial disease that continues to rise and has become an epidemic in the world with rates in the U.S. being among the highest.²⁰ Fat accumulation and fat cell hypertrophy in human are strongly associated with autophagy, and autophagy elevation is particularly prominent in the omental fat of individuals who developed obesity-related insulin resistance.²¹ Using the chicken and egg analogy, it is not known which came first; obesity or adipose autophagy.

Current evidence implicates autophagy in the regulation of lipid stores within the two main organs involved in maintaining lipid homeostasis, namely the liver and adipose tissue.²²

In the hepatocytes, cytoplasmic lipid droplets were found to be subject to breakdown by autophagy, a process referred to as lipophagy. Pharmacological or genetic inhibition of Atg5 increased triglyceride levels and decreased β -oxidation in a rat hepatocyte cell line.²³ Moreover, hepatic lipid droplets were found to be co-localized with LC3 under basal and autophagy-induced conditions. Specific inhibition of hepatic Atg7 caused an accumulation of lipid within the mouse liver²³ indicating that autophagy regulate hepatic lipid turnover.

In adipocytes, however, autophagy appears to have an opposite effect on lipid stores. Indeed, autophagy is required for adipocyte differentiation and lipid droplet accumulation.^{24,25} Atg7 adipocyte-specific knockout reduced white adipose tissue and increased brown adipose tissue along with increased rate of β -oxidation. Recent studies showed also that autophagy regulates lipid metabolism through lipoprotein assembly and apoB degradation.^{26,27} ApoB was found to be co-localized with autophagosomes in docosahexaenoic acid-treated McA cells, and knockout of Atg7 gene increased apoB recovery.²⁸

It is well known that a high fat diet and increased obesity induces insulin resistance in liver, adipose tissue and muscle resulting in hyperglycemia.²⁹ It has been shown that an excess in nutrient supply down-regulated autophagy *via* insulin signaling.³⁰ Recent studies found that hepatic autophagy is defective and its up-regulation improves insulin sensitivity in common rodent obese models (ob/ob, db/db and HFD).^{31,32,33} Furthermore, insulin inhibited autophagy *via* mTOR activation and mTOR inhibition by rapamycin lead to a hyperinsulinemic and hyperglycaemic states in rat skeletal muscle.^{34,35} These results indicate that insulin and autophagy might participate in a feedback mechanism of reciprocal regulation. Thus, autophagy may regulate insulin sensitivity in a tissue specific manner. For instance, HFD decreased and increased LC3-II levels in mouse liver and adipose tissue, respectively.²¹

Autophagy and Avian Species: A Model of Choice

As the incidence of obesity or metabolic syndrome continues to rise, there is a clear demand to identify new and efficient therapeutic strategies. Therefore, insights into the molecular mechanisms of this devastating disease using different experimental models are of uppermost interest. Rodents are very useful models for the study of obesity, but it could be suggested that another equally good model for this study would be chickens (*Gallus gallus*).

Whereas lipogenesis occurs in both adipose tissue and liver in rodents,^{36,37,38} chickens are similar to humans in that lipogenesis occurs exclusively in the liver and is exported *via* the circulatory system to adipose tissue.³⁹ In addition, chickens are characteristically hyperglycemic compared with mammals, with their blood glucose levels averaging three times that found in humans (300 vs. 100 mg/dl).⁴⁰ Genetic selection for production ef-

iciency (rapid growth rate and feed efficiency) necessitates feed restriction in commercial meat-type chicken (broiler) breeders that are hyperphagic, heavy, and prone to obesity. Broilers voraciously consume approximately 4.1 kg of feed to achieve a 40-fold increase in body weight after hatch that is concomitant with tremendous increase in muscle development as well as abdominal fat during a period of 42 days.^{41,42} Both meat and egg producing chickens (broilers and layers) are insulin resistant^{43,44} and lack the glucose transporter protein GLUT4.⁴⁵ They require insulin doses greater than four times that required in mammals to achieve hypoglycaemia.⁴⁶

Obesity is often considered to be a result of either excessive food intake or insufficient energy expenditure. Brown Adipose Tissue (BAT), a specialized fat that dissipates energy to produce heat, plays an important role in the regulation of energy balance. Interestingly, chickens do not have functional BAT. Thus, these unique metabolic characteristics make chickens an interesting model for understanding the role of autophagy in obesity development.

Recently, we characterized several genes involved in autophagosome initiation, elongation and maturation in chickens (*Gallus gallus*) and Japanese quail (*Coturnix coturnix japonica*).⁴⁷ These genes are ubiquitously expressed and showed high similarity with their mammalian orthologs indicating that autophagy is a conserved mechanism in different species. Interestingly, we found that the expression of autophagy-related genes is tissue- and gender-dependent.⁴⁷ For instance, it was found that hypothalamic Beclin1, UVRAC, Atg9a, Atg13, Atg4b, Atg7 and Atg5 mRNA levels were significantly higher in female compared to male chickens suggesting that hypothalamic autophagy might be involved in the regulation of feed intake. Recently, Kaushik and colleagues showed that autophagy regulates lipid metabolism within hypothalamic neurons which in turn modulate neuropeptide levels that control feed intake and energy homeostasis.⁴⁸ Furthermore, using two experimental male quail lines divergently selected over 40 generations for low (resistant line) or high (sensitive line) stress response⁴⁹ it was found that the expression of several autophagy-related genes are higher in several tissues including adipose tissue in the resistant compared to the sensitive line.⁴⁷ Since autophagy has been shown to play a key role in stress response and fat metabolism and since the regulation of energy homeostasis and the stress response are coupled physiological processes, the differential expression of autophagy-related genes between the two quail lines indicated that these lines would be a very useful model to study the interaction between stress response, autophagy and fat metabolism.

In conclusion, studies using avian models may provide critical information on the role of autophagy in lipogenesis and lipid metabolism because the liver is the primary site of de novo fatty acid synthesis in chickens and may help for targeting new therapeutic strategies to treat obesity and its related diseases.

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