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Feedback on fat: p62-mTORC1-autophagy connections

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Abstract

Metabolic homeostasis requires integration of multiple signals and cellular activities. Without this integration, conditions of obesity and diabetes often develop. Recent in vivo studies explore the molecular basis for metabolic homestasis, showing that p62 links autophagy and mTORC1 activation to regulate adipogenesis and energy control.

To confer specificity and plasticity to signal transduction cascades, adapter proteins act as hubs or nodes, organizing and connecting myriad cellular processes. One example of such a signal-organizing hub is the protein known as p62, or sequestosome 1. p62 was initially identified by its ability to interact with the atypical PKCs (aPKCs), but was later found to bind a relatively long list of critical signaling intermediates (Figure 1A)(Moscat and Diaz-Meco, 2009). For example, it associates with TRAF6, regulating NF-κB signaling during osteoclastogenesis and bone homeostasis, and it interacts with caspase-8 via its UBA domain (Moscat and Diaz-Meco, 2009). This interaction efficiently activates the extrinsic apoptotic pathway, which is required during TRAIL-induced cell death. With its UBA domain, p62 also binds polyubiquitinated proteins destined for degradation through autophagy (Moscat and Diaz-Meco, 2009). For this activity, p62 must recruit LC3, a critical component of the autophagic machinery (Moscat and Diaz-Meco, 2009). By associating with a wide variety of proteins, p62 fulfills two distinct biochemical roles: it is a signaling organizer that regulates essential cellular functions and it is also involved in the cellular quality-control mechanisms underlying the disposal of misfolded proteins (Moscat and Diaz-Meco, 2009).

p62 and the control of metabolic homeostasis and inflammation

As a signaling hub, p62 coordinates the processes required for metabolic homeostasis. It does this, in part, through its connections with autophagy. Importantly, p62 not only binds proteins destined for disposal by autophagy, it also gets constitutively degraded by autophagy. This has important functional repercussions in vivo. For example, genetic inhibition of autophagy in the liver leads to poorly characterized hepatotoxicity and p62 accumulation. This phenotype is rescued by the genetic inactivation of p62 (Moscat and Diaz-Meco, 2009). Moreover, chronic increases in p62 levels cause liver cell damage, which, overtime, leads to hepatocarcinogenesis (Inami et al., 2011; Takamura et al., 2011). In this context, p62 promotes tumorigenesis by activating two ROS scavenger systems, NRF2 and NF-κB, which reduce oxidation-induced tumor cell death and promote cancer cell

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survival and proliferation (Moscat and Diaz-Meco, 2009). These results illustrate that context-specific overproduction of p62 has important functional repercussions in vivo, but what is the physiological role of p62? Recent analyses of p62-deficient mice provide insight into this key question. Interestingly, the loss of p62 at an organismal level resulted in mature-onset insulin resistance and obesity (Rodriguez et al., 2006). These unexpected findings suggest a role for p62 in the control of metabolic homeostasis. Consistent with this, p62-deficient mice exhibit reduced energy expenditure and thermogenesis, along with decreased levels of transcripts involved in these processes (Rodriguez et al., 2006). In addition, young mice that lack p62 function exhibit increased levels of the adipogenic master regulatory gene, PPARγ, in white adipose tissue long before obesity or increased adiposity are apparent (Rodriguez et al., 2006). These results indicate that the loss of p62 recapitulates all the characteristics of metabolic syndrome, including glucose intolerance, insulin resistance, and systemic and adipose tissue-specific inflammation.

Interestingly, p62 deficiency leads to obesity independently of its interaction with atypical protein kinase C proteins (aPKCs) (Lee et al., 2010). For example, PKCζ null mice do not exhibit obesity, although they are more prone to insulin resistance and glucose intolerance. These symptoms stem from increased production of inflammatory cytokines not by immune cells, but by the mutant adipocytes (Lee et al., 2010). These observations indicate that the p62-PKCζ cassette controls two key aspects of physiology, which directly impinge on metabolic syndrome. p62 normally represses obesity and enhances energy expenditure, whereas PKCζ represses the pro-inflammatory actions of obesity. Consistent with this notion, PKCζ/IL-6 double knockout mice exhibit normal glucose tolerance and insulin responses, along with reduced hepatosteatosis, even when fed a high-fat diet (Lee et al., 2010). Consequently, both signaling proteins may play positive roles in preventing metabolic syndrome and type 2 diabetes.

Much evidence suggests that p62 represses adiposity in an ERK1-dependent cell-autonomous manner (Rodriguez et al., 2006). Embryo fibroblasts from p62-deficient mice and 3T3-L1 cells lacking p62 display high levels of activated ERK concomitant with enhanced adipogenesis (Rodriguez et al., 2006). p62 interacts preferentially with ERK1 over ERK2, and the reduction of ERK1, but not of ERK2, completely reversed adipogenesis in cultured cells. Importantly, this phenomenon is recapitulated in vivo with p62/ERK1 double knockout mice displaying normal adipogenesis and adiposity, and no obesity, hepatosteatosis, or insulin resistance (Moscat and Diaz-Meco, 2009).

Altogether, these data suggest that p62's associations with ERK1 and PKCζ differentially regulate metabolic homeostasis. The p62-ERK1 pair regulates mature-onset obesity and type 2 diabetes whereas the p62-PKCζ pair regulates obesity-induced inflammation and type 2 diabetes. According to this model, decreased p62 expression emerges as a risk factor for obesity and type 2 diabetes. As obesity and glucose intolerance are often associated with aging, it is tempting to speculate that reduced levels of p62 might constitute a risk factor for the alterations in metabolic homeostasis that go hand-in-hand with the ageing process.

Compartmentalization of mTORC1 and autophagy

Recent data demonstrate that p62 impinges on another critical regulator of metabolic homeostasis: the primary nutrient-sensing complex, mTORC1. The core components of mTORC1 include the mTOR kinase, Raptor, and mLST8/GβL (Guertin and Sabatini, 2007). Recent results have begun to shed light on the upstream mechanisms that connect mTORC1 to nutrient availability and couple its response to the lysosomal compartment (Sancak et al., 2010). Hterodimers of GTP-bound RagA/B bindraptor and direct mTORC1 to the lysosomal surface where it can interact with its activator, Rheb (Sancak et al., 2010). An unbiased

proteomic analysis identifed raptor as a p62-interacting protein (Duran et al., 2011). The p62-raptor interaction explains why cells require p62 to activate mTORC1 in response to cell stimulation by amino acids (Duran et al., 2011). Without p62 function, autophagy is upregulated in mammalian cells and in *C. elegans*, similar to the upregulation of autophagy that accompanies decreased mTORC1 activity (Duran et al., 2011). This unexpected finding implies that p62, which is degraded by autophagy, also regulates autophagy, creating a feed-forward loop by which p62 activation of mTORC1 results in higher p62 levels. These increased levels of p62 thereby promote even more mTORC1 activity (Figure 1B). The physiological significance of this loop is not completely clear, but it suggests that when amino acids levels are low, mTORC1 activity is reduced and autophagy is upregulated. Moreover, it suggests that the p62-mTORC1-autophagy feed-forward loop negatively regulates mTORC1 activation during chronic nutrient deprivation. A potential role for this negative regulation could be to ensure the irreversibility of cell death upon nutrient starvation. Thus, we envision a model in which a prolonged lack of nutrients produces chronic activation of autophagy and long-term reduction of p62. This permanent reduction in p62 would make it impossible for the cell to reactivate the mTORC1 pathway if nutrients become available again (Figure 1B). According to this mechanism, cells that reach a critical level of nutrient stress-induced damage would not recover.

It is likely that p62 carries out additional roles that enablemTORC1's response to amino acid flux related to the intracellular localization of mTORC1 and p62. Recent data support an mTORC1-mediated link between autophagy and inflammatory cytokine synthesis during oncogene-induced senescence (Narita et al., 2011). While autophagy and protein synthesis may seem to oppose each other, in this context they may work in concert, with autophagy generating the amino acid building blocks needed for mTORC1-mediated cytokine synthesis. Importantly, ULK1, the kinase that activates autophagy, is most likely localized in a different compartment of the cell from mTORC1 and p62 in senescent cells. During senescence, p62 may interact with mTORC1, which is localized on the Golgi, away from the autophagosomes. This localization would ensure mTORC1 activation and function, and, at the same time, protect its partner, p62, from degradation by autophagy.

The p62-mTORC1 connection is also implicated in lysosome biogenesis following the termination of autophagy. In this scenario, mTORC1 is reactivated during prolonged starvation by the products generated by the autolysosomes (Yu et al., 2010). This second phase of mTORC1 activation requires p62 and the regeneration of functional lysosomes. In keeping with this notion, recent reports demonstrate the critical role of the transcription factor TFEB in connecting autophagy and lysosomal biogenesis (Settembre et al., 2011). TFEB drives the expression of autophagy and lysosomal gene products as well as that of p62 (Settembre et al., 2011). Although p62 is a substrate of autophagy it is also a critical component of the cellular remodeling that occurs post-autophagy. Testing these hypotheses will yield a better understanding of p62's role as a critical regulator of cellular homeostasis and provide new insight into how p62 modulates autophagy and mTORC1 activation in response to nutrient starvation and refeeding in various physiological contexts.

Autophagy, p62, mTORC1, and metabolic control

The evidence that p62 acts as a cellular metabolic switch in autophagy is also important on the organismal level. For instance, p62, mTORC1, and autophagy play important roles in adipogenesis. When the autophagy regulator, ATG7, is specifically inactivated in adipocytes of mice, obesity and adiposity decrease while glucose tolerance and insulin sensitivity increase (Zhang et al., 2009). Considering that inhibition of autophagy provokes the accumulation of p62 (Zhang et al., 2009), and that the overexpression of p62 inhibits adipogenesis (Rodriguez et al., 2006), we hypothesize that simultaneous inactivation of p62

and Atg7 would restore normal adipogenesis. In this regard, it should be emphasized that the loss of p62 not only increases adipogenesis but also results in decreased energy expenditure and the downregulation of genes involved in energy utilization (Rodriguez et al., 2006). In contrast, the loss of Atg7 in adipose tissue results in increased energy expenditure as mesaured by increased β-oxidation rates. Moreover, Atg7−/− white adipose tissue acquired some characteristics of brown adipocytes, including increased mitochondrial content and multilocular lipid droplets, key characteristics of brown adipocytes (Zhang et al., 2009). Conversely, p62−/− brown adipose tissue looks more like white adipose tissue with a clear reduction in UCP1 mRNA levels (Rodriguez et al., 2006). The studies described above suggest that autophagy regulates systemic metabolic homeostasis and cell-autonomous adipogenesis through p62 inhibition. We suggest that the simple regulation of autophagy, p62 levels, or both in adipose tissue is sufficient to influence whole-body metabolic homeostasis. However, recent data also implicate the brain is this process. Genetic ablation of Atg7 in agouti-related peptide (AgRP) neurons resulted in decreased body weight and fat mass, likely due to reduced food intake (Kaushik et al., 2011). The mechanisms underlying this phenomenon seem to be complex, and might involve the generation of circulating free fatty acids during starvation that are taken up by orexigenic hypothalamic AgRP neurons that synthesize triglycerides and activate autophagy, which in turn upregulates AgRP levels and triggers the appropriate homeostatic response (Kaushik et al., 2011). It is unclear how autophagy in these neurons regulates the generation of AgRP, but p62 does not seem to be involved in this process because systemic loss of p62 in mice does not affect food intake (Rodriguez et al., 2006). Therefore, although autophagy may be involved in the control of obesity in more than one organ or tissue, the role of p62 seems to be restricted to the adipose compartment.

All these recent data support a model where autophagy and p62 play opposing roles in adipogenesis and obesity with adipocytes functioning as key mediators of obesity-associated inflammation and whole-body metabolic homeostasis. Connecting the mTORC1 pathway with p62 and autophagy uncovers additional complexity. In this regard, it is surprising that inhibition of mTORC1 in adipose tissue, by genetic inactivation of raptor, leads to a lean phenotype with resistance to high-fat-diet-induced obesity (Polak et al., 2008). Although these results agree with previous data demonstrating that loss of the mTORC1 target, S6K1, results in an obesity-resistant phenotype (Um et al., 2004), they are at odds with mTORC1 negatively regulating autophagy and promoting adipogenesis and adiposity in vivo (Zhang et al., 2009). These effects of the mTORC1 activity seem to be tissue specific, since the selective inactivation of raptor in muscle results in decreased oxidative capacity and reduced mitochondrial gene expression (Bentzinger et al., 2008) while raptor deletion in adipocytes promotes increased energy expenditure (Polak et al., 2008). This is consistent with experiments in cultured cells showing that mTOR controls mitochondrial function in muscle cells by regulating the activity of PGC1α via a raptor-mTOR-YY1 complex (Cunningham et al., 2007). Although the actions of mTORC1 in energy homeostasis are complex, these results suggest that adipose tissue, but not muscle, is central to the regulation of metabolism and obesity. Moreover, inactivation of mTORC1 in fat recapitulates the lean phenotype of total S6K1-deficient mice, but inactivation of mTORC1 in muscle does not produce lean mice (Polak et al., 2008). Taken together, all these findings point to mTORC1 as a positive modulator of adiposity and lower energy expenditure, and are in agreement with the fact that obesity, both genetic and dietary, promotes mTORC1 activity (Um et al., 2004).

One mechanism whereby mTORC1 might activate adipogenesis is by regulating the expression of the key adipogenic transcriptional regulator SREBP-1. One function of SREBP-1 is to promote the synthesis of PPARγ activators. Interestingly, the selective inactivation of raptor in liver correlates with resistance to diet-induced obesity (Sengupta et al., 2010), which would imply that permanent activation of the mTORC1 pathway should

result in hepatosteatosis as a consequence of hyper-production of SREBP-1 Although it is clear that mTORC1 activates the SREBP pathways through a novel mechanism involving Lipin1 (Peterson et al., 2011), the constitutive activation of mTORC1 in liver results in protection from age- and diet-induced hepatosteatosis, most likely due to the defective synthesis of SREBP1c and lipogenesis (Yecies et al., 2011). These surprising results illustrate the complexity of metabolic homeostasis at an organismal level and its regulation by specific signaling cascades. A confounding factor in many analyses of the mTORC1 pathway is its crosstalk with another mTOR-associated pathway, the mTORC2-Akt signaling cascade. For example, the permanent activation of mTORC1 in the liver leads to a complete shutdown of the Akt pathway, which is necessary for inactivation of the SREBP1c repressor, INSIG2 (Yecies et al., 2011). This could explain why, under certain circumstances, inactivation of the mTORC1 pathway gives rise to the same metabolic phenotype as its constitutive activation. These and other questions are important to consider when analyzing the whole-body phenotypes of mice with ablation of negative regulators of the mTORC1 pathway.

Concluding remarks and speculations

When we survey the literature, we are left with contradictory conclusions about the relationship between autophagy, p62, and mTORC1 function. The most recent data upends the prevailing view of mTORC1, suggesting that instead of mTORC1 acting as an upstream negative regulator of autophagy, autophagy acts as an upstream positive regulator of mTORC1. Some examples of this include lysosomal biogenesis and oncogene-induced inflammation associated with senescence. This apparent paradox may be explained by the distinct compartmentalization of ULK1 and autophagy, and p62 and mTORC1 inside the cell (Figure 1C). Careful biochemical purification of different p62 complexes and determination of their subcellular localization will provide clarification of this model.

Another inconsistency is that while mTORC1 appears to negatively regulate autophagy, both mTORC1 and autophagy positively regulate lipogenesis and adiposity. A potential explanation for this paradox is that a high-calorie diet induces mTORC1, causing increased adiposity and lower energy expenditure, while also prevents autophagy in an mTORC1-dependent manner. This type of regulation could provide a negative feedback mechanism that would result in increased p62 levels. High levels of p62 would inevitably lead to restriction of adipogenesis and increased energy expenditure (Figure 1D). In this way, metabolic homeostasis is maintained with adipocytes and the whole organism keeping fat content and insulin responses in a relatively normal physiological range, despite having to cope with excessive food intake or age-induced deregulated metabolic homeostasis. Future studies using mice with double deficiencies in autophagy and p62 should be helpful in rigorously testing this hypothesis.

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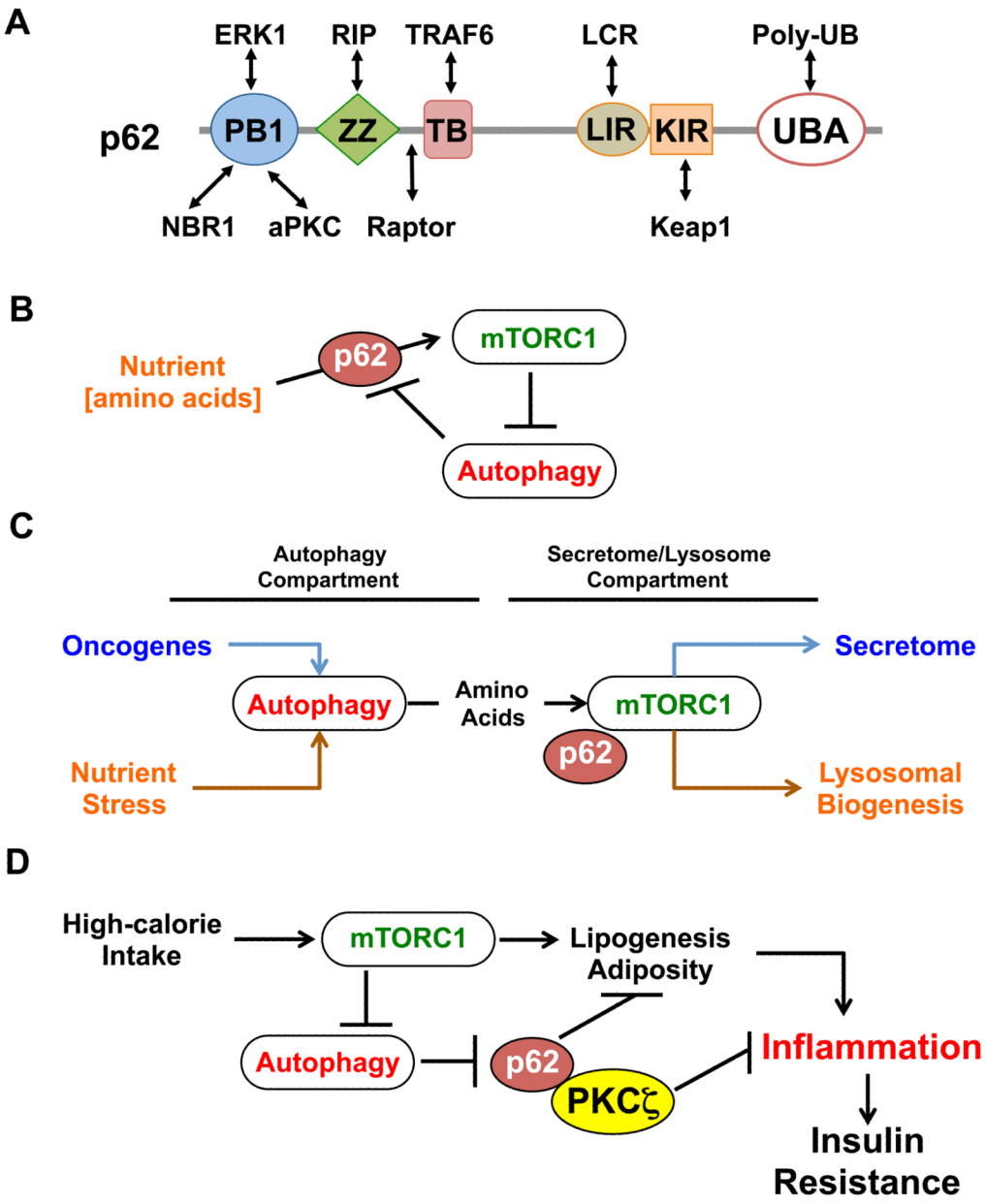

Figure 1. Role of p62 in autophagy and mTORC1 regulation

(A) Domain organization and interacting partners of p62. PB1, Phox/Bem domain 1, interacts with ERK1 to control adipogenesis and, with aPKCs, to control NF-κB; the interaction with NBR1 is also through the PB1 but its role needs to be clarified. ZZ, atypical zinc-finger, governs the interaction with RIP and might be relevant for TNFα-activated NF-κB. TB, TRAF6-binding, accounts for p62's role in IL-1, NGF and RANK towards NF-κB. LIR, LC3-interacting region, locates p62 in the autophagosomes; KIR, Keap-interacting region, serves to regulate NRF2 activation; UBA, ubiquitin-associated, mediates the interaction with poly-ubiquitinated proteins, including caspase-8, and modulates TRAF6 interaction and activity.

(B) p62 senses nutrient signals and activates mTORC1, inhibiting autophagy and creating a loop that results in enhanced p62 levels.

(C) During senescence and lysosomal biogenesis, p62 and mTORC1 are most likely separated from the autophagosome, which generates amino acids that can, in turn, regulate mTORC1 activation.
(D) High-calorie diets promote lipogenesis and adiposity through mTORC1 activation, which is antagonized by p62. p62, itself, is modulated by autophagy and that likely controls the anti-inflammatory actions of PKCζ.