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The lysosome: A crucial hub for AMPK and mTORC1 signalling

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Abstract

Much attention has recently been focussed on the lysosome as a signalling hub. Following the initial discovery that localisation of the nutrient sensitive kinase, mTORC1, to the lysosome was essential for mTORC1 activation, the field has rapidly expanded to reveal the role of the lysosome as a platform permitting the coordination of several homeostatic signalling pathways. Much is now understood about how the lysosome contributes to amino acid sensing by mTORC1, the involvement of the energy sensing kinase, AMPK, at the lysosome and how both AMPK and mTORC1 feedback to lysosomal biogenesis and regeneration following autophagy. This review will cover the classical role of the lysosome in autophagy, the dynamic signalling interactions which take place on the lysosomal surface and the multiple levels of cross-talk which exist between lysosomes, AMPK and mTORC1.

Keywords: mTORC1, AMPK, lysosome, autophagy, signalling

Abbreviations

AMPK - AMP-activated protein kinase; ATG – autophagy related; BORC - BLOC-one-related complex; DEPTOR - DEP Domain Containing MTOR-Interacting Protein; ERK - extracellular signal–regulated kinase; 4E-BP1 - eukaryotic translation initiation factor 4E-binding protein 1; FLCN – folliculin; FNIP – folliculin interacting protein; GAP – GTPase activating protein; GATOR - GTPase-activating protein activity toward Rags; GDI – guanine nucleotide dissociation inhibitor; GEF – guanine nucleotide exchange factor; LAMP - Lysosomal-associated membrane protein; LAMTOR - late endosomal/lysosomal adaptor, MAPK and MTOR activator; LC3 - Microtubule-associated protein 1A/1B-light chain 3; MITF - microphthalmia-associated transcription factor; mLST8 - mammalian lethal with SEC13 protein 8; mTORC1 – mechanistic (mammalian) target of rapamycin complex 1; PAT – proton-assisted amino acid transporter; PI3K - phosphoinositide 3-kinase; PRAS40 - proline-rich Akt substrate of 40 kDa; raptor - regulatory-associated protein of mTOR; Rheb – Ras homolog enriched in brain; rictor – rapamycin insensitive companion of mTOR; rpS6 – ribosomal protein S6; SESN – sestrin; SNAP – synaptosomal-associated protein; SNARE - Soluble N-ethylmaleimide-sensitive factor activating protein receptor; STX – syntaxin; TFEB – transcription factor EB; TSC – tuberous sclerosis complex; ULK1 – Unc51 like kinase; v-ATPase – vacuolar H⁺-ATPase; UVRAG - UV irradiation resistance-associated gene; VAMP - Vesicle associated membrane protein

1. What is the lysosome?

The lysosome was first identified from tissue fractionation studies which aimed to analyse the intracellular distribution of rat liver enzymes [1]. Following the realisation that five acid hydrolase enzymes all were present in the same membrane-limited structure, it was proposed that these organelles were involved in intracellular digestion and hence termed lysosomes (Greek for 'digestive body') [2].

Lysosomes are classically associated with the process of autophagy. Macroautophagy (hereafter referred to as autophagy) is a highly conserved process used to clear long-lived proteins and organelles from the cell, in which the lysosome forms the ultimate 'acceptor' of the material to be degraded (reviewed in [3]). Cargo can also be delivered to lysosomes from the endocytic pathway and phagocytosis. Upon reaching the lysosome, the acid hydrolases it contains function to degrade the cargo. This releases breakdown products which can be recycled into new biomolecules. The lysosome itself must also be reformed, through the maturation of endolysosomes or reformation/budding from autolysosomes [4,5].

Lysosome biosynthesis requires the coordinated transcription of many genes encoding lysosomal proteins. These genes have a common palindromic motif, called the Coordinated Lysosomal Expression and Regulation (CLEAR) element, which is a target of transcription factor EB (TFEB) [6]. More than 100 proteins are contained in the lysosome membrane, including the vacuolar H+-ATPase (v-ATPase). This is a multimeric protein pump which is required to pump H+ ions and thus provide the acidic environment required by lysosomal hydrolases (reviewed in [7]).

It is becoming clear that the lysosome does not solely function as a recycling centre. There is now evidence that lysosomes can undergo secretion, which appears to be important for plasma membrane repair and defence from parasites (reviewed in [8]). The lysosome is also gaining prominence as a nutrient signalling hub, with the v-ATPase playing an important role in sensing amino acid availability. This review will focus on the expanded cellular role of the lysosome, describing both its well-known role in autophagy as well as new research linking it to nutrient signalling more broadly (Figure 1).

2. The role of the lysosome in autophagy

The autophagic process begins with the formation of an isolation membrane (phagophore). This membrane expands through the coordinated action of autophagy related (ATG) proteins to form an autophagosome and engulf the cytoplasmic cargo requiring degradation (reviewed in [9]).

The Unc51 like kinase, ULK1 (known in yeast as Atg1), forms part of a complex which is key to early autophagy signalling (recently reviewed in [10]). The ULK1 complex becomes active under starved conditions and coordinates with parallel signals from the Beclin1 complex to initiate autophagosome formation. Following the generation of phosphatidylinositol-3-phosphate (PtdIns(3)P) via ULK1 and Beclin1 signalling, further ATG proteins are recruited to the nascent autophagosome. Microtubule-associated protein 1A/1B-light chain 3 (LC3), a member of the ATG8 family of ubiquitin-like proteins, becomes lipidated, allowing LC3 to associate with the autophagosomal membrane and drive autophagosomal maturation [11]. The expanded membrane loses association with ATG proteins prior to closure around its cargo, but retains LC3 on the inner surface (reviewed in [12]).

Once sequestered, the cargo within the autophagosome is degraded through autophagosome-lysosome fusion. The exact mechanism which ensures timely fusion is not fully elucidated, but it was hypothesised that soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein receptor

(SNARE) proteins were likely to be involved due to their known role in vesicle fusion [13]. Two studies then showed that the SNARE protein, syntaxin 17 (STX17), was important for autophagosome-lysosome fusion [14,15]. The proposed mechanism is that STX17 becomes tethered onto the outer surface of an autophagosome, where it recruits synaptosomal-associated protein 29 (SNAP29) [15]. ATG14 associates with STX17 and SNAP29 to stabilise this complex on autophagosomes, priming it for interaction with vesicle associated membrane protein (VAMP) 8 [16]. VAMP8 is localised on endosomes and lysosomes, thus the formation of a STX17-SNAP29-VAMP8 complex allows autophagosome-lysosome fusion [15]. Furthermore, lysosomal-associated membrane protein (LAMP) 2, a commonly used marker of lysosomes, has recently been shown to be essential for STX17 expression on autophagosomes and the absence of LAMP2 also alters the recruitment of accessory proteins required for tethering and fusion [17].

Interestingly, autophagosome-lysosome fusion has been revealed as important for activating lysosomal function, as chemical inhibitors of the fusion process lead to a reduction in cathepsin B activity (as a read-out of lysosomal function) under starved conditions [18]. Additionally, cathepsin B activity fails to increase under starvation in cells with constitutively active mammalian target of rapamycin complex 1 (mTORC1) signalling [18]. mTORC1 signalling (covered in detail in Section 3 below) drives anabolic activities within the cell, and so functions in contrast to catabolic processes such as autophagy. There are several layers of cross-talk between these pathways in which the lysosome plays a role, which will be covered in Section 4.

3.1 mTORC1 regulates protein homeostasis and cell growth

The mammalian target of rapamycin complex 1 (mTORC1) plays a fundamental role in the integration of metabolic, energy, hormonal and nutritional signals to promote biosynthetic pathways and suppress the catabolic process of autophagy. mTORC1 is a major regulator of protein homeostasis and thus cell growth. It exerts its effects by phosphorylating and controlling the activity of key ribosomal proteins such as ribosomal protein S6 (rpS6) and inhibiting the translation repressor, eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1). In addition, mTORC1 controls lipogenesis, glycolysis and mitochondrial biogenesis [19]. mTORC1 is a multi-protein complex consisting of mTOR protein kinase, regulatory-associated protein of mTOR (Raptor) (a scaffold protein involved in substrate recruitment), mammalian lethal with SEC13 protein 8 (mLST8)/GβL (which stabilises mTOR-Raptor interaction) [20] and the negative regulators proline-rich Akt substrate of 40 kDa (PRAS40) [21] and DEP Domain Containing MTOR-Interacting Protein (DEPTOR) [22]. The core kinase protein mTOR (as well as mLST8/GβL and DEPTOR) also exists along with rapamycin insensitive companion of mTOR (Rictor) and Sin1 as part of a second complex called mTORC2 [23]. The function and regulation of this complex is less well understood but has been demonstrated to influence the actin cytoskeleton. mTORC2's activity is under the influence of growth factors and it can phosphorylate Akt [23].

The signals controlling mTORC1 originate from various sources in the cell including the plasma membrane (growth factors, hormones and cytokines via receptor tyrosine kinases and G-protein coupled receptors), cytoplasm (energy status via AMP-activated protein kinase (AMPK)), mitochondria (glutaminolysis, reactive oxygen species and energy status) and the lysosome (following degradation and liberation of nutrients). Well described phosphorylation signalling cascades, including phosphoinositide 3-kinase (PI3K)/Akt and Ras/extracellular signal–regulated kinase (ERK) can transduce these signals to mTORC1. In addition and downstream of these events, it

is becoming increasingly clear that mTORC1 is further controlled by the differential localisation of a range of proteins and organelles, including itself, in response to mitogenic signals. Indeed the cell biology of mTORC1 signalling is expanding rapidly and at the very centre of this is the lysosome.

3.2 mTORC1 at the lysosome

The central role of the lysosome in regulating mTORC1 activity emerged from the identification that mTORC1's localisation to Rab7-positive vesicles is required for its activation [24]. Due to the lack of highly specific markers, the distinction between, and the relative importance of, the late endosome versus the lysosome for mTORC1 activity is currently not clear. However, as the literature has primarily focussed on the lysosome as the site of mTORC1 activation, for simplicity we will refer to the site of mTORC1 activation as the lysosome for the remainder of the review. mTORC1 localisation to lysosomes is dependent on a group of small GTPases called Rag GTPases and a pentameric complex called Ragulator (made up of late endosomal/lysosomal adaptor, MAPK and MTOR activator (LAMTOR) 1-5) [24–26]. Mammalian Rag GTPases are atypical members of the Ras superfamily of small GTPases and function via cycling between GTP and GDP states to control their function. They are larger in size (30-50kDa vs 20-25kDa) and lack post-translational prenylation modifications required for membrane localisation that are characteristic of typical small GTPases. Instead, they are anchored to the lysosomal membrane via the resident Ragulator complex that further acts as a guanine nucleotide exchange factor (GEF) for RagA/B [26,27]

The Rag GTPases form a heterodimer (A or B with C or D) where the 'active' conformation consists of RagA/B·GTP with RagC/D·GDP which can bind to Raptor and sequester mTORC1 to the lysosomal membrane [24–26]. Activation of Rag GTPases and mTORC1 localisation to the lysosome is regulated predominantly by amino acid sufficiency [24,25] but also glucose availability [28]. Indeed, overexpression of GTP-locked RagA/B renders mTORC1 constitutively active and insensitive to removal of amino acids [24,25]. The nucleotide status of Rag GTPases is regulated by a range of GEF and GTPase activating proteins (GAPs), with the most notable GEF being the Ragulator [26,27] and with GAPs including the GAP activity toward Rags (GATOR) 1 complex (towards RagA/B) [29], the tumour suppressor folliculin (FLCN) in complex with FLCN interacting protein (FNIP) 1/2 (towards RagC/D) [30] and leucyl tRNA synthetase (towards RagD) [31,32].

The recruitment of mTORC1 to the lysosomal membrane is generally considered to bring it into close proximity to its master regulator, Ras homolog enriched in brain (Rheb) which resides on the lysosome, as well as a number of other endomembranes. Although the exact mechanism of how Rheb activates mTOR kinase activity is unknown, the direct interaction between the two is sufficient *in vitro* and *in vivo* to promote the phosphorylation of mTORC1 substrates [33,34]. The most proximal regulator of Rheb is tuberous sclerosis complex (TSC) which consists of TSC1, TSC2 and TBC1D7, where TSC2 has GAP activity towards Rheb and enhances the intrinsic hydrolysis of GTP to GDP, thus inactivating Rheb and inhibiting mTORC1 [35–39]. TSC is a signalling node that integrates inputs from a range of pathways including Ras/ERK, AMPK and PI3K/Akt pathways and is heavily regulated by phosphorylation events [36,40,41]. Thus by providing a platform for both Rags and Rheb small G proteins, the lysosome creates a signalling hub that tightly controls mTORC1 activity.

3.3.1 Sensing of amino acids: inside or out?

Increasing evidence suggests that amino acids can signal to mTORC1 both from the cytoplasm and from within the lumen of the lysosome. The first evidence that amino acid sensing may propagate from within lysosomes came from the identification that the v-ATPase forms a signalling complex with the Ragulator-Rag GTPase. The mechanism via which the v-ATPase may signal amino acid sufficiency is currently not understood; in particular the role of ATPase activity for mTORC1 activation is not clear. While pharmacological inhibition of ATP hydrolysis activity can inhibit mTORC1 [42], others have demonstrated that amino acid starvation may increase ATPase activity [43]. Specifically, amino acid deprivation enhances the interaction between Rag GTPases, Ragulator and v-ATPase which may be a result of conformational changes [42] and potentially increased v-ATPase assembly [43]. Furthermore, others have demonstrated that intraluminal pH of the lysosome is important to support mTORC1 activity [44]. It is unclear at present whether amino acid sensing via the lysosomal lumen involves the direct trafficking of amino acids from the cytoplasm into the lysosome or whether the amino acids sensed here are liberated via the degradative nature of the lysosome. For example, supplementation of methyl esters [45] or radio-labelled amino acids shows they rapidly accumulate in the lysosome [42], while over-expression of the proton-assisted amino acid transporter, PAT1, inhibits mTORC1, presumably by leaching amino acids from the lysosomal lumen [42]. However, other studies in cells and *in vivo* in *Drosophila*, show PAT1 actually supports amino acid-dependent mTORC1 activation [46,47] indicating a careful balance in amino acid distribution or transport is required for proper mTORC1 activity.

3.3.2 Sensing of specific amino acids

Leucine, arginine and glutamine have been widely shown to be the main amino acids contributing to mTORC1 activation [26,31,48–51]. A number of mechanisms via which these amino acids can influence mTORC1 have now been elucidated, demonstrating the complex control of amino acid sensing (Figure 2).

Glutamine contributes to mTORC1 activation via the process of glutaminolysis which produces alpha-ketoglutarate (α-KG) and is supported by leucine availability as a co-factor for glutamate dehydrogenase (GDH) that participates in deamination of glutamine [50]. Interestingly, cell permeable α-KG is sufficient to activate mTORC1 [50] and involves prolyl hydroxylases via a currently unknown mechanism [52]. Another mechanism of glutamine sensing has been identified, interestingly, in Rag knock-out cells via a mechanism dependent on the v-ATPase and involving the Golgi-localised GTPase Arf1 [51].

Recently, cytoplasmic sensors of leucine and arginine have been identified to work via very similar mechanisms. Sestrin (SESN) 2 has long been known to influence cellular growth and metabolism. Recent work has revealed that SESN2 can bind leucine and this is required for leucine-dependent mTORC1 activation [53–56]. (Further crosstalk mechanisms between the sestrin proteins, mTORC1 and AMPK are detailed in Section 5.3). Via a similar mechanism, arginine has recently been shown to bind to a protein called CASTOR1. This interaction prevents CASTOR1-GATOR2 interaction, freeing GATOR2 to inactivate GATOR1, leading to increased Rag-dependent mTORC1 signalling [57].

In addition to sensing via CASTOR1/2 in the cytoplasm and SLC38A9 [58] (see below) via the lysosomal lumen, arginine sensing has also been attributed to controlling the interaction of TSC2 with Rheb at the lysosome [49]. TSC2 recruitment to lysosomes has been linked to various mitogenic signals, including growth factors, amino acids (most notably arginine), intracellular pH, osmotic

stress and mechanical stimulation [49,59–63]. Growth factor and amino acid regulation of TSC2 localisation occurs via a mechanism that involves Rheb [49,59], although Rag GTPases have also been implicated [61]. Interestingly the two reports that focus on Rheb as the main regulator of TSC2 on lysosomes show that TSC interacts most strongly with Rheb in the absence of growth factors and amino acids (arginine) [49], specifically in its GDP-bound state [60] and prevents the interaction of Rheb with mTORC1. This is intriguing as classically, most GAP-substrate interactions are transient and the GAP quickly dissociates from its substrate upon GTP hydrolysis. This raises the very interesting possibility that TSC2 may have additional regulatory roles to that of a GAP. We noted for example that overexpression of GAP-deficient mutants of TSC2 can still inhibit Rheb-dependent mTORC1 activity and conversely wild-type TSC2 is able to inhibit constitutively active Rheb-dependent mTORC1 [49]. It remains to be clarified whether perhaps TSC2 can function as a guanine nucleotide dissociation inhibitor (GDI) as well as GAP towards Rheb. This is a real possibility as no GEF has been identified to activate Rheb but it is generally considered to be highly loaded with GTP.

3.3.3 Amino acid transporters

If amino acids are transported directly into the lysosome, it is currently not clear which transporters may be involved. It is equally not clear whether specific, individual amino acids are sensed within the lysosome or overall amino acid concentration. At present, the only transporter that has been implicated in sensing of a specific amino acid from within the lysosome is SLC38A9 which participates in sensing arginine following starvation and re-feeding. SLC38A9 has been placed as part of the larger sensing platform on lysosomes, by interacting with Ragulator-Rag complex [58,64,65]. Interestingly, knock-down of SLC38A9 prevented starvation-induced re-localisation of mTOR to the cytoplasm [65] however the mechanism of this retention is unknown.

The amino acid transporters, SLC7A5 and SLC3A2 (also known as LAT1 and 4F2hc, respectively) can be recruited to the lysosomal membrane via a protein called LAPTM4b which helps load the lysosome with leucine and activate mTORC1 [66]. These same transporters are important at the plasma membrane in controlling uptake of amino acids. Similarly, SLC1A5 transports glutamine into the cell, effectively loading the cell with glutamine which can then be used by SLC7A5/SLC3A2 to exchange with leucine [67].

As mentioned above, the proton-assisted transporter, PAT1 has been implicated in controlling amino acid-dependent mTORC1 activity and it does this through an interaction with Rag GTPases on lysosomal membranes [46,47]. Interestingly, PAT1 transports amino acids including glycine, proline and alanine which are not potent mTORC1 activators so it not necessarily clear exactly how PAT1 may influence mTORC1. More recently, the localisation of PAT1 to the lysosome has been shown to be negatively regulated by FLCN levels. Overexpressed FLCN leads to decreased PAT1 localisation to the lysosome, thus 'trapping' intra-lysosomal amino acids such as leucine and supporting mTORC1 activity even in amino acid limiting conditions [68]. This is an interesting concept as FLCN is normally recruited to lysosomes during amino acid deprivation however perhaps at endogenous levels, FLCN concentrations are not sufficient to affect PAT1 and intracellular amino acid levels, thus mTORC1 is switched off [69]. Another member of the PAT family, PAT4, localises to the Golgi where it can interact with a complex including mTORC1 and the small GTPase, Rab1 to control serine and glutamine-dependent activation of mTORC1 [70] possibly by promoting the Rheb-mTORC1 interaction [71].

Future perspectives are likely to identify additional amino acid transporters both on the lysosome and plasma membrane that contribute to controlling intracellular concentrations of amino acids and subsequently mTORC1. One interesting avenue for this research is the idea that amino acid transporters can further act as transceptors, functioning via sensing and signalling amino acid availability rather than their transport. For example, the non-hydrolysable amino acid substrate, Me-AIB can activate mTORC1 in cells expressing SLC38A2/SNAT2 [72]. This may also help to explain discrepancies including why SLC38A9 specifically senses arginine [58] when it has a higher transport affinity for other amino acids, such as glutamine [64] and why PAT1 transports amino acids that are not potent mTORC1 activators. It is likely that in addition to specific amino acids, a basal concentration of other amino acids is also required to activate mTORC1 [47]. Indeed we have seen previously that while leucine, arginine and glutamine together can minimally activate mTORC1, a full complement of the other amino acids is required to induce the maximal amino acid-dependent mTORC1 signal [49].

4. Cross-talk between autophagy and mTORC1

Autophagy is tightly regulated in an inverse manner to anabolic processes mediated by mTORC1 activity. This regulation is maintained through several points of cross-talk between the two pathways. Direct cross-talk occurs through phosphorylation events mediated by the key kinase complexes, ULK1 and mTORC1. The ULK1 complex can phosphorylate Raptor within mTORC1 to inhibit mTORC1 activity when autophagy is active [73,74]. Conversely, mTORC1 can phosphorylate several components within the ULK1 complex in order to downregulate autophagy when nutrients are plentiful [75–77]. Additionally, mTORC1 has been shown to regulate the regeneration of lysosomes following activation of autophagy. Although mTORC1 is downregulated during the initial stages of autophagy, it becomes reactivated several hours later, with the reactivation dependent on the release of intracellular nutrients during the autophagic process [5]. This increased mTORC1 activity inhibits further autophagy and permits the generation of proto-lysosomal tubules which extend from autolysosomes before maturing into functional lysosomes [5]. Lysosomal regeneration is dependent on phosphorylation of UV irradiation resistance-associated gene (UVRAG) by mTORC1. This phosphorylation of UVRAG enhances VPS34 lipid kinase activity, which appears to be critical for tubule scission [4]. These feedback mechanisms between mTORC1 and autophagy prevent excessive autophagy and keep anabolic and catabolic processes in check.

Interestingly, feedback from the lysosome itself to mTORC1 signalling has also been documented. In addition to the generation of amino acids during autophagy which permit the reactivation of mTORC1 as described above [5], it is observed that in chondrocytes, pharmacological inhibition of lysosomal function activates mTORC1 signalling independently of autophagy [78]. Similarly, this cross-talk operates in reverse, where inappropriate mTORC1 activity prevents lysosomal activation under starvation [18].

Other interesting nodes of crosstalk between mTORC1 and autophagy are the adaptor protein p62 (also known as SQSTM1) and microphthalmia-associated transcription factor (MITF). p62 is an important mediator of selective autophagy of ubiquitinated proteins [79]. p62 also interacts with the mTORC1 component, Raptor, as well as RagC/D [80], thus providing a signalling nexus for mTORC1 activation on the lysosome. These multiple roles of p62 have recently been reviewed elsewhere [81]. MITF, a basic helix-loop-helix leucine zipper transcription factor, drives transcription of all the subunits of the v-ATPase, thus regulating lysosomal metabolism [82]. A conserved regulatory loop was reported, whereby activation of mTORC1 at the lysosome (which requires the v-ATPase)

functions to negatively regulate MITF activity by sequestering MITF in the cytoplasm. Therefore, mTORC1, v-ATPase and MITF provide a feedback mechanism to maintain cellular homeostasis [82].

In addition to nutrients and mitogenic signals controlling the localisation of mTORC1 and its regulators, they also control the subcellular distribution of lysosomes themselves [83]. In nutrient replete conditions, lysosomes are distributed to the cell periphery, while starvation leads to the perinuclear clustering of lysosomes. Some of the proteins involved in cellular lysosomal positioning have recently come to light. For example, the multisubunit complex, BORC, has been shown to associate with the cytosolic face of lysosomes, where it recruits the small GTPase, Arl8, to promote peripheral movement of lysosomes [84]. Functioning in reverse to mediate the transport of lysosomes towards the perinuclear region upon autophagy induction is the PI(3,5)P$_2$—TRPML1—ALG-2 pathway [85].

This lysosomal localisation directly impacts on mTORC1 activity and genetic manipulation of lysosomal localisation to the periphery activates mTORC1 while perinuclear clustering inhibits it [83]. It is currently not clear however exactly why or how lysosomal positioning can impact on mTORC1 activity. Recently it was shown that maintenance of perinuclear lysosomes may be mediated by the starvation-induced recruitment of FLCN to the lysosome which recruits RILP and interacts with Golgi resident Rab34 to tether lysosomes in this region [86]. Furthermore, it is generally considered that in the presence of nutrients, being in close proximity to the cell periphery promotes activation of mTORC1 via signalling cascades originating from the membrane, however the mechanisms controlling the sequestration of lysosomes in these regions are not known. Amino acid and insulin-dependent increases in PI(3,5)P$_2$ may recruit mTORC1 via Raptor to the plasma membrane [87]. It would be interesting to investigate whether this interaction could also control mTORC1-positive lysosome localisation. Intriguingly, mTOR has been reported to suppress the calcium efflux function of TRPML1 via phosphorylation [88], revealing an additional level of signalling complexity between mTOR and lysosomes.

A further link between autophagy and mTORC1 signalling is AMPK signalling. This functions to inhibit anabolic processes and activate autophagy when cellular energy levels are low, as discussed below.

5.1. AMPK signalling and energy homeostasis

AMPK is central to the cellular response to low energy levels. It functions as a heterotrimeric protein, consisting of a catalytic α subunit, a β subunit and an adenosyl nucleotide binding γ subunit (for review, see [89]). This complex is sensitive to changes in the cellular AMP/ATP and ADP/ATP ratios, where binding of AMP to the γ subunit modulates phosphorylation of Thr172 by the LKB1-STRAD-MO25 complex [90,91]. Once active, AMPK drives processes which will enhance cellular energy stores, such as increasing glucose uptake and promoting autophagy while inhibiting biogenic synthesis [89].

As it is essential for cells to maintain energy and nutrient homeostasis, it has long been appreciated that the mTORC1 and AMPK signalling pathways must be coordinated. Over a decade ago, it was shown that AMPK could inhibit mTORC1 signalling though the phosphorylation of TSC2, the tumour suppressor which functions upstream of mTORC1 [92]. Subsequently, a second level of regulation was uncovered when it was found that AMPK could also directly phosphorylate the mTORC1 component, Raptor, on Ser722 and Ser792. This permits 14-3-3 binding to Raptor, and inhibition of mTORC1 signalling [93]. Treatment with biguanides (such as metformin and phenformin) is closely

associated with AMPK activation [94], so it was interesting that a later report indicated that mTORC1 can also be inhibited by biguanides in a TSC2- or AMPK-independent manner. Intriguingly, biguanides were found to require the Rag GTPases in order to abolish mTORC1 activation [95]. Additionally, AMPK coordinates with the ULK1 complex to regulate autophagy. AMPK phosphorylates ULK1 on multiple residues to positively regulate autophagy [96–99], but interestingly, under starvation, ULK1 can phosphorylate all three AMPK subunits to downregulate AMPK activity [100]. This indicates a finely balanced regulatory signalling network.

New research to better understand the complex crosstalk between the mTORC1 and AMPK signalling pathways and their coordinated control of metabolic homeostasis has revealed that the lysosome may function as a crucial hub for integration of signalling in response to both nutrients and energy.

5.2 AMPK at the lysosome

AMPK was known to be found in both the nucleus and cytoplasm, with its localisation regulated by several factors including environmental stress, cell density and MEK-ERK signalling [101]. Although both AMPK and lysosomes are intrinsically linked to autophagy, no direct association had been reported. The first indication that AMPK may have a specific late endosomal/lysosomal function was when its binding partner, AXIN, was found to interact with LAMTOR1 [102]. Although initially linked to Wnt signalling [103], AXIN was later identified as a scaffold protein for AMPK and its upstream regulator, LKB1, displaying a weak constitutive interaction with both AMPK and LKB1, which is enhanced upon glucose starvation or in the presence of AMP. Through this complex, AXIN mediates AMP-triggered AMPK activation by LKB1 [104]. LAMTOR1, a lysosome localising protein [26] (previously described in Section 3.2) was then subsequently identified through a yeast two-hybrid screen as an AXIN interactor [102]. Starvation was found to enhance AXIN/LKB1-AMPK complex binding to LAMTOR1 and the other members of the Ragulator, while loss of LAMTOR1 expression inhibited AMPK activation. Interestingly, a significant proportion of cellular AMPK was found constitutively localised on the late endosome/lysosome membrane and under starvation, phospho-AMPK was found exclusively on this membrane, indicating that this membrane surface is where LKB1 phosphorylates AMPK [102]. It was then investigated how the AXIN/LKB1 complex was recruited to the Ragulator on the late endosome/lysosome membrane during starvation. It was shown that the energy sensor, v-ATPase, which was already known to regulate the Ragulator in response to glucose starvation [28], was also required for the enhanced AXIN/LKB1-LAMTOR interaction under starvation [102].

The involvement of several mTOR activating factors in AMPK localisation and signalling further highlights the intrinsic crosstalk between these two pathways. Therefore, Zhang *et al* elucidated the precise mechanisms whereby the v-ATPase/Ragulator could govern both AXIN/LKB1 and mTORC1 localisation on the lysosome. Their study showed that AXIN is a key component in this regulatory mechanism. Not only does it translocate with LKB1 to the lysosome to activate AMPK and thus turn on catabolic processes, but it also is important for turning off anabolic processes through facilitating starvation-induced lysosomal dissociation and inhibition of mTORC1. AXIN regulates mTORC1 activity through its ability to impair the GEF activity of Ragulator, leading to dissociation of mTORC1 from the lysosome and the downregulation of mTORC1 signalling [102]. Interestingly, AXIN was found to specifically mediate this effect under energy stress and did not regulate mTORC1 activity under amino acid deprivation. This suggests that although several core components of amino acid signalling and energy sensing are shared, cells can still exquisitely fine-tune their signalling pathways through auxiliary proteins, such as AXIN, to respond appropriately to specific environmental cues.

The key role of the AXIN-Ragulator-v-ATPase axis in controlling both AMPK and mTORC1 is further demonstrated by studies assessing the mechanistic action of metformin (N,N-dimethylbiguanide). Metformin is widely used to treat type 2 diabetes due to its ability to decrease cellular energy levels. A recent study revealed that both AXIN and LAMTOR1 are required for metformin-mediated AMPK activation, with evidence that metformin operates to promote the formation of the v-ATPase-Ragulator-AXIN/LKB1-AMPK complex, thus activating AMPK [105]. In parallel, metformin treatment results in dissociation of mTORC1 from v-ATPase-Ragulator [105]. Two further mechanisms of mTORC1 downregulation by metformin have also been proposed. In hepatocytes, low concentrations of metformin inhibit hepatic mTORC1 signalling, with this inhibition dependent on AMPK and the TSC complex [106]. Additionally, metformin treatment has been shown to prevent nucleocytoplasmic shuttling of RagC. This means RagC remains predominantly GTP bound and cannot activate mTORC1 [107]. The authors propose that disruption of this RagC-mediated mTORC1 activation might be a mechanistic link between kinase activity and cellular energy levels [107].

These mechanistic studies are not the only new links between AMPK signalling and lysosomes. A recent study examining AMPK knockout embryonic stem cells (ESCs) surprisingly revealed that lysosomal gene expression was substantially decreased in the absence of AMPK, with TFEB, a member of the TFE family of transcription factors, the most consistently reduced lysosomal gene [108]. TFEB is known to be regulated at the protein level by energy status; in the presence of nutrients, mTORC1 can phosphorylate TFEB to inhibit TFEB activity, but TFEB becomes hypophosphorylated and localised to the nucleus when energy levels are low [109]. Cells with AMPK knockout did not effectively generate hypophosphorylated TFEB, so TFEB failed to shuttle to the nucleus. As a result, these cells exhibited diminished lysosome function [108].

5.3 Additional crosstalk mechanisms

A further crosstalk mechanism between AMPK, mTORC1 and the lysosome occurs via the sestrin proteins. SESN1 was originally identified in a condition called heterotaxia [110], and the sestrin family was found to play a role in re-establishing cellular antioxidant defences [111]. Subsequently, sestrins were found to play a broader role in cellular growth repression through inhibition of mTORC1. SESN2 was reported to interact with TSC1, TSC2 and AMPKα2. Through these interactions, sestrins were shown to stimulate AMPK activity, enhance TSC2 phosphorylation and decrease Rheb-GTP loading, thus downregulating mTORC1 activity [112]. More recent work has indicated that TSC2 is not essential for mTORC1 downregulation by sestrins [56,113] and sestrins can downregulate mTORC1 independently of AMPK via both the Rags and their upstream regulator, GATOR2 [55,113]. Several studies demonstrated that mTORC1 inhibition by sestrins is GATOR- and Rag-dependent under both normal cell culture conditions or upon amino acid refeeding and that sestrins inhibit mTORC1 activity by preventing localization of mTORC1 to the lysosome [55,56,113]. SESN2 was shown to bind to GATOR2 in an amino acid sensitive manner. Specifically, leucine binding to SESN2 prevents SESN2 from interacting with the GATOR2 complex, thus promoting Rag-dependent activation of mTORC1 [53,54]. Furthermore, there is evidence that sestrins can interact directly with the Rag GTPases and function as a Rag GDI, thus inhibiting mTORC1 through stabilisation of RagA GDP binding [113].

Clearly, elucidating the role of the sestrin family in mTORC1 regulation is still developing and sestrins function at multiple points to regulate the amino acid sensing arm of mTORC1 signalling (Figure 3). Further illustrating this, the SESN2-GATOR2 interaction can be disrupted in the absence of amino acids by inhibition of the v-ATPase which interacts with the Ragulator on the lysosomal surface [56]. Ongoing studies will help reveal the complexities of sestrin-mediated inhibition of mTORC1.

6. Summary

It is vital for cellular health that anabolic and catabolic processes are inversely controlled and tightly regulated. To this end, multiple levels of crosstalk occur between them. As highlighted in this review, recent publications have revealed that the lysosome forms an important integration point for multiple signalling pathways, thus providing a spatial hub for both anabolic and catabolic signalling. Further scrutiny of proteins such as the lysosomal v-ATPase which is already known to be important for mTORC1 signalling (via the Ragulator) and AMPK signalling (via AXIN), along with the identification of new components of mTORC1 and AMPK signalling, will deepen our understanding of the exquisite control of cellular metabolism. In turn this may reveal pathways which could be targeted in disease states where metabolism is dysregulated.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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Figure Legends

Figure 1: Overview of the role of the lysosome

The lysosome is an integral platform in the control of cellular growth and homeostasis. mTORC1 integrates intra- and extracellular signals of energy (via AMPK), oxygen, amino acid (via Rag GTPases, TSC2 and a variety of other proteins/complexes) and nutrient (via TSC2 complex) availability to regulate protein translation, gene transcription, glycolysis, lipogenesis, nucleotide synthesis and inhibit the catabolic process of autophagy. Thus mTORC1 and the lysosome manage the balance of anabolic vs catabolic cellular processes. The lysosome also functions as the ultimate acceptor of material to be degraded via autophagy.

Figure 2: Amino acid sensing by mTORC1

The presence of free amino acids is essential for mTORC1 activation. Leucine, glutamine and arginine are the most potent activators of mTORC1 and a number of different mechanisms have been identified via which they are sensed and signal to mTORC1. These include cytoplasmic sensors, amino acid transporters and v-ATPase on the lysosomal membrane. All mechanisms work to control to the nucleotide status of either Rag GTPases or Rheb, the most proximal regulators of mTORC1.

Figure 3: Crosstalk between mTORC1 and AMPK: Sestrins

Under amino acid starvation, sestrins have been proposed to inhibit mTORC1 signalling at 3 points (1) Interaction with AMPK/TSC1/TSC2 to activate AMPK, block TSC1/2 and reduce Rheb activity (although subsequent studies have not found evidence of sestrin/TSC2 interaction (2) Blocking GATOR2 function, thereby keeping the Rags inactive (3) Stabilising RagA-GDP to keep the Rags inactive. Both (2) and (3) maintain mTORC1 inactive in the cytoplasm. In both panels, dashed lines represent pathways which are switched off under the conditions described due to upstream inhibitory processes.