Non-canonical mTORC1 signaling at the lysosome

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The mechanistic target of rapamycin complex 1 (mTORC1) signaling hub integrates multiple environmental cues to modulate cell growth and metabolism. Over the past decade considerable knowledge has been gained on the mechanisms modulating mTORC1 lysosomal recruitment and activation. However, whether and how mTORC1 is able to elicit selective responses to diverse signals has remained elusive until recently. We discuss emerging evidence for a 'non-canonical' mTORC1 signaling pathway that controls the function of microphthalmia/transcription factor E (MiT-TFE) transcription factors, key regulators of cell metabolism. This signaling pathway is mediated by a specific mechanism of substrate recruitment, and responds to stimuli that appear to converge on the lysosomal surface. We discuss the relevance of this pathway in physiological and disease conditions.

mTOR
mTOR is a 289 kDa serine/threonine protein kinase belonging to the phosphoinositide 3-kinase (PI3K)-related family of protein kinases [1,2]. Since its discovery in the early 1990s, mTOR has attracted much attention owing to its central role in the control of cell metabolism and its intimate association with many conditions including cancer, aging, metabolic disorders, and neurodegenerative diseases. mTOR is the catalytic subunit of two main protein complexes – mTORC1, a master controller of cell metabolism and growth, and mTORC2, which plays an important role in the control of cell proliferation and survival [3]. Whereas limited knowledge is available on mTORC2 upstream signals and activation mechanisms, extensive studies have shown that mTORC1 is a central signaling node that integrates many upstream stimuli, including nutrients (e.g., amino acids, glucose, lipids), metabolic intermediates (e.g., nucleotides, oxygen), and growth factors (e.g., insulin, IGF), thus coordinating an adaptive response of cell metabolism to environmental cues [4–17] (Figure 1). mTORC1 has a dual role in the control of cell metabolism: (i) it promotes protein, lipid, and nucleotide synthesis by phosphorylating several substrates including S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), and (ii) it inhibits catabolic pathways by phosphorylating the autophagy initiation components ULK1 and Atg13, as well as transcription factor EB (TFEB) and other MiT-TFE factors that are master transcriptional regulators of lysosomal biogenesis and autophagy [18–25] (Figure 1).

Seminal work has shown that mTORC1 activity is dependent on its physical recruitment to the lysosomal membrane [7,26]. However, despite tremendous knowledge of the sophisticated mechanisms that modulate mTORC1 lysosomal recruitment and activation, how this kinase complex is able to integrate many upstream stimuli to induce selective downstream responses has only recently started to emerge. Indeed, mTORC1 has long been considered to be a 'binary' on/off kinase that phosphorylates all its substrates indiscriminately. However, recent findings indicate that certain conditions induce selective mTORC1-mediated phosphorylation of specific substrates. In this review we summarize the most recent evidence indicating the existence of a pathway for selective mTORC1 regulation, defined here as non-canonical mTORC1 signaling.
which is based on a newly identified mechanism of mTORC1 substrate recruitment. We describe how this mechanism allows selective modulation of cell metabolism by mTORC1 and discuss the relevance of these findings in physiological and disease conditions.

**Canonical mTORC1 signaling**

Owing to its role as a major degradative organelle, the lysosome is a key source of cellular metabolites, thus functioning as an ideal platform where mTORC1 can sense and integrate nutritional inputs. Recruitment of mTORC1 to the lysosomal surface relies on the activity of Rag GTPase heterodimeric complexes which are composed of RagA or B (RagA/B) bound to RagC or D (RagC/D) [6,7,27]. RagA/B:RagC/D complexes associate with the lysosomal membrane via binding to the pentameric lysosomal complex Ragulator [26,28–31]. Like all GTPases, the Rags can bind either GTP or GDP and this affects their functional activity. To recruit mTORC1 to the lysosomal surface, the Rags must have RagA/B in the GTP-bound state. Furthermore, structural data suggest that only the RagA:GTP/RagC:GDP configuration is compatible with mTORC1 binding [32,33]. However, recent studies have shown that the nucleotide-binding state of RagC/D plays a lesser contribution than RagA/B in mTORC1 lysosomal recruitment. Accordingly, mTORC1 can efficiently phosphorylate its substrates S6K and 4E-BP1 even when RagC/D is in the GTP-bound state [34–37]. This is
consistent with early studies showing that a Rag dimer in which the RagA/B and RagC/D modules are both GTP-bound was still able to efficiently bind to mTORC1 [7]. Thus, RagC/D participates in dimer formation but the RagC/D GTP/GDP-binding state is less relevant for mTORC1 binding than the RagA/B nucleotide-binding state. This indicates that both the RagA/B-GTP:RagC/D-GDP and the RagA/B-GTP:RagC/D-GTP heterodimeric configurations are similarly able to mediate mTORC1 lysosomal recruitment. More recent studies have revealed that the RagC/D nucleotide-binding state is involved in a ‘non-canonical’ mTORC1 substrate recruitment mechanism [37] (discussed below).

The nutrient-dependent transition between the inactive RagA/B (i.e., GDP-bound) and active (i.e., GTP-bound) states is a finely modulated process controlled by complex machinery that can sense both cytosolic and intra-lysosomal nutrients (Figure 2). A crucial effector of this machinery is the trimeric GATOR1 [GTPase-activating protein (GAP) activity towards Rags 1] complex, consisting of the subunits Nprl2, Nprl3, and DEPDC5. This complex acts as a GAP that specifically inhibits RagA/B [38] and exerts its function to the lysosomal surface via its interaction with the tetrameric KICSTOR (KPTN-, ITFG2-, C12orf66-, and SZT2-containing regulator of TOR) complex [39,40]. GATOR1 activity is counteracted by the GATOR2 complex, a pentameric complex consisting of the subunits MIOS, WDR59, WDR24, Seh1L, and SEC13 [38]. Although the exact function of GATOR2 has not yet been defined, this complex is thought to modulate Rag GTPase activity either by acting as a GATOR1 inhibitor or by working in parallel to GATOR1 via an incompletely understood mechanism [39,41–43].

The availability of both cytosolic and intra-lysosomal nutrients is conveyed to the Rags via the action of dedicated sensors. Several cytosolic amino acid sensors such as Sestrin, CASTOR (cellular arginine sensor for mTORC), and SAMTOR (S-adenosylmethionine sensor upstream of mTORC1) sense cytosolic amino acid levels and convey this information to mTORC1 via direct binding to and modulating the GATOR complexes. In particular, Sestrin2 and CASTOR have been shown to specifically to leucine and arginine, respectively, and to transmit their availability via their inhibitory binding to GATOR2, which occurs during starvation [41–44]. By contrast, SAMTOR is a specific sensor of S-adenosylmethionine (SAM), a methionine byproduct [45]. Upon SAM deprivation, SAMTOR inhibits mTORC1 activity via GATOR1 binding. The integral lysosomal membrane protein solute carrier SLC38A9 mediates the sensing of intra-lysosomal amino acid levels [46,47,115]. Furthermore, the lysosomal v-ATPase complex, which is required

Figure 2. Mechanisms of canonical and non-canonical mTORC1 signaling. mTORC1 activation occurs upon its recruitment to the lysosomal surface, which is mediated by the Rag GTPases. mTORC1 activity towards S6K and 4E-BP1 (A) is modulated by the TSC2–Rheb axis, whereas the phosphorylation (P) of TFEB (B) occurs through the FLCN–RagC/D axis.
for the maintenance of the lysosomal proton gradient, also affects amino acid sensing and Rag activity by interacting with the Ragulator–Rags complex [48] (Figure 2). Finally, the Rags also signal availability of other nutrients, such as glucose and cholesterol, via GATOR1-mediated and SLC38A9/Niemann–Pick C1 (NPC1)-mediated mechanisms, respectively [17,49,116].

mTORC1 lysosomal recruitment is a crucial step that allows the activation of mTORC1 by the small GTPase Rheb, an allosteric activator of mTORC1 that localizes at least in part to the lysosomal surface [50,51] (Figure 2). Structural data have shown that Rheb binding to mTOR causes a dramatic conformational change that allows the alignment of residues in the active site, thus enhancing the phosphorylation of S6K and 4E-BP1 [52]. Growth factors are able to activate Rheb by blocking the GAP activity of the trimeric tuberous sclerosis complex (TSC), a Rheb inhibitor, resulting in activation of Rheb through the conversion from its inactive GDP-bound state to the active GTP-bound state [53,54]. Thus, both nutrient-stimulated Rag activity and growth factor-dependent Rheb activation are required for mTORC1 activation and phosphorylation of its substrates, thereby ensuring integration of multiple inputs. Although this ‘dual’ mechanism of mTORC1 activation is required for the phosphorylation of well-known mTORC1 substrates such as S6K and 4E-BP1, recent studies have shown that this mTORC1 activation system does not apply to all substrates because some substrates are phosphorylated by mTORC1 by a different mechanism (discussed further below).

Non-canonical mTORC1 signaling
The RagC/D nucleotide-binding state is regulated by the FLCN:FNIP (folliculin:folliculin-interacting protein) complex, which acts as a GAP that converts RagC/D–GTP into RagC/D–GDP (Figure 2) [34,55,56]. During amino acid starvation, the FLCN:FNIP complex is recruited to the lysosomal surface by binding to GDP-bound RagA/B [34,57]. Nutrient replenishment induces FLCN:FNIP dissociation from the lysosomal surface and stimulates its GAP activity towards RagC/D.

Accumulating evidence has suggested that phosphorylation of MIT-TFE factors, well-known substrates that are phosphorylated and negatively regulated by mTORC1 (Box 1), differs from that of other well-characterized mTORC1 downstream targets. For instance, it was reported that some conditions inhibit the phosphorylation of MIT-TFE factors without impairing the phosphorylation of S6K/4E-BP1 [34–36,58]. Similarly, MIT/TFE factors were found to be dephosphorylated and active in some inherited diseases [59] as well as in several types of cancer, including melanoma, pancreatic, and kidney cancer, despite concomitant hyperphosphorylation of the mTORC1 substrates S6K and 4E-BP1 [60–62]. Finally, the phosphorylation and subcellular localization of MIT-TFE factors, unlike the phosphorylation of S6K and 4E-BP1, were found to be insensitive to growth factor-mediated activation of Rheb, an unprecedented feature for an mTORC1 substrate [35,37].

The mechanistic basis for this differential phosphorylation behavior of MIT-TFE factors compared to S6K and 4E-BP1 has been only recently elucidated and relies on the different mechanisms by which these substrates are recruited by mTORC1. Early evidence and recent structural data have demonstrated that S6K and 4E-BP1 contain a TOR signaling (TOS) motif, a five amino acid region that is directly recognized and bound by mTORC1 via its subunit Raptor, thus allowing substrate recruitment and phosphorylation [52,63,64]. By contrast, MIT-TFE factors lack a TOS motif, and instead contain a Rag-binding site in their N-terminal regions that enables them to interact with the Rags [65] (Figure 3). Notably, the interaction of MIT-TFE factors can occur only when RagA/B is GTP-bound and RagC/D is GDP-bound (i.e., the active configuration) [37]. Such an interaction mediates a novel mTORC1 substrate-recruitment mechanism and explains why TFEB phosphorylation is highly sensitive to stimuli that modulate Rag activity, such as nutrient...
The MiT-TFE transcription factors

MiT-TFE comprises four basic helix-loop-helix (HLH) leucine-zipper (ZIP) transcription factors – TFEB, TFE3, MITF, and TFEC – which share an identical DNA-binding domain and highly similar HLH and ZIP domains that are required for homo/heterodimerization [85,86]. MITF has long been known as a modulator of melanosome biogenesis, whereas TFE3 has been shown to act as a master regulator of lysosomal biogenesis and autophagy through the control of a transcriptional network of genes named CLEAR (coordinated lysosomal expression and regulation) [23,24,97,98]. Other MiT-TFE factors, such as TFEC and MITF, also regulate the CLEAR network and appear to have a cooperative and partially redundant function [25,99–101]. The transcriptional network controlled by MiT-TFE factors varies in different tissues and, in addition to genes involved in lysosomal biogenesis and autophagy, also includes genes involved in lipid degradation, glucose homeostasis, mitochondrial biogenesis, and the control of immune and inflammatory responses [100–104]. The activity of MiT-TFE factors is regulated by mTORC1-mediated phosphorylation, which controls their nucleocytoplasmic shuttling [105]. In rested, non-stressed, and normally fed cells, mTORC1 phosphorylates specific serine residues in MiT-TFE factors, leading to their cytoplasmic retention. Stimuli that inhibit mTORC1 activity, such as starvation, infection, and endoplasmic reticulum (ER) stress, result in MiT-TFE dephosphorylation and nuclear translocation. This regulatory mechanism explains the response of MiT-TFE factors to environmental cues. In addition to Rag1 and Rag2, other kinases are involved in the regulation of TFEB subcellular localization or stability, including PKCβ, GSK3, ERK2, AKT, and the cyclin-dependent kinases CDK4/6 [24,106–109].

However, how the cell integrates the roles of these kinases in the control of MiT-TFE factors remains unclear. Nucleocytoplasmic shuttling of MiT-TFE factors is also influenced by the regulation of their nuclear export, which occurs through a CRM1-mediated mechanism [110–112]. mTORC1 and MiT-TFE factors are involved in a feedback loop in which activation of MiT-TFE factors positively regulates mTORC1 activity via transcriptional induction of RagC/D GTPases [81]. Dysregulation of MiT-TFE transcription factors is an established feature of several human tumors [82]. Approximately 5–20% of melanoma cases are due to amplification of the MITF gene. Chromosomal translocations involving the TFE3 and TFEB genes can cause a specific subtype of renal cell carcinoma (RCC), defined as translocation-RCC (tRCC), and are also found in alveolar soft part sarcoma (ASPS). Most of these translocation events result in significant overexpression of either full-length or truncated forms of TFE3 or TFEB, suggesting that highly increased levels of MiT-TFE factors are tumorogenic. This is consistent with the results obtained in mice in which TFEB overexpression in specific tissues such as kidney and liver results in tumor formation [73,113]. In addition to TFE3 translocations, increased expression and activities of MiT-TFE factors were identified as crucial contributors of tumor growth in PDAC, as well as in invasive basal-like breast carcinomas [60,114]. Thus, the aberrant activation of MiT-TFE factors may be implicated in a significantly larger number of malignancies than was initially expected (discussed in main text).
among GTPases, provides the opportunity to mediate distinct functions by differentially exploiting the RagA/B and RagC/D modules. Accordingly, the activity of RagA/B is strictly required for mTORC1 lysosomal localization, and is thus essential for its activity towards all its substrates [7,37,38]. Conversely, the activity of RagC/D is strictly required for TFEB recruitment and phosphorylation but plays a minor contribution to mTORC1 lysosomal localization and phosphorylation of other substrates such as S6K and 4E-BP1 [37] (Figure 2). This system explains why stimuli that modulate RagA/B activity, such as amino acids, glucose, and cholesterol, equally affect the phosphorylation of all mTORC1 substrates, whereas inputs specifically impinging on RagC/D activity (e.g., lysosomal damage, mitophagy, and xenophagy; discussed below) only influence TFEB phosphorylation without affecting TOS-containing substrates. The physiological and medical relevance of this mechanism is highlighted by recent evidence (discussed below).

**Dysregulation of non-canonical mTORC1 signaling**

*Birt–Hogg-Dubé (BHD) syndrome*

BHD syndrome is an autosomal dominant genetic disorder caused by germline loss-of-function mutations in the gene encoding folliculin (*FLCN*) [66]. The disease belongs to the family of so-called ‘hereditary hamartoma syndromes’ and is characterized by benign cutaneous fibrofolliculomas, bilateral pulmonary cysts, and multiple kidney cysts and tumors, the latter being the most severe manifestation of BHD syndrome. These tumors are caused by loss of heterozygosity as a result of second-hit mutations and occur in about one third of affected individuals. They are associated with distinct histological subtypes, the most frequent being the hybrid oncocytic tumor, followed by chromophobe renal cell carcinoma and renal oncocytoma [67,68].

Since the discovery of *FLCN* gene mutations in patients with BHD syndrome, concerted efforts have been made to elucidate the cellular pathways responsible for the disease manifestations. One obvious candidate factor was mTORC1, which was found to be significantly hyperactive in tumors from BHD patients [69]. Such mTORC1 hyperactivation in BHD syndrome was considered to be a paradox because FLCN was originally thought to be a positive regulator of mTORC1 activity [55,70]. However, recent studies indicate that FLCN is not required for the phosphorylation of mTORC1 substrates S6K and 4E-BP1, whose phosphorylation is generally tested to measure mTORC1 activity [34–37]. FLCN-mediated activation of RagC/D instead plays a crucial role in the phosphorylation of TFEB and the other MIT-TFE factors. Therefore, in the absence of FLCN these transcription factors cannot be phosphorylated by mTORC1 and translocate to the nucleus to induce their transcriptional programs [37,59,70] (Figure 4). Once activated, MIT-TFE factors are able to further promote mTORC1 activity through a negative feedback loop that relies on transcriptional induction of RagC/D GTPases [61]. Such induction in the absence of FLCN results in an increased number of RagA/B-GTP:RagC/D-GTP dimers, leading to enhanced lysosomal recruitment of mTORC1 and increased phosphorylation of S6K and 4E-BP1 but not of TFEB. These findings explain the ‘paradoxical’ hyperactivation of mTORC1 in BHD syndrome in which S6K and 4E-BP1 are hyperphosphorylated whereas TFEB is dephosphorylated as a result of loss of FLCN function. Furthermore, a similar mechanism is involved in several MIT-TFE-dependent types of cancers such as renal cell carcinoma (RCC), pancreatic ductal adenocarcinoma (PDAC), and melanoma (Box 1).

Kidney-specific conditional FLCN knockout (KO) mice develop a severe phenotype characterized by multiple renal cysts and cancerous lesions, leading to renal insufficiency and death before 30 days of age [71,72]. Interestingly, the renal phenotype of FLCN-KO mice is remarkably similar to that observed in transgenic mice with kidney-specific TFEB overexpression, a murine model of RCC caused by chromosomal translocations involving the *TFEB* and *TFE3* genes (Box 1) [73]. These similarities suggest that BHD patients may develop kidney cysts and tumors as a
consequence of MiT-TFE hyperactivation. Genetic evidence in mice has shown that kidney-specific depletion of TFEB is sufficient to completely rescue cyst formation, tumorigenesis, and early lethality [37]. These data identify TFEB as a main driver of the kidney phenotype of BHD syndrome and suggest that other disease manifestations may also be caused by TFEB induction.

Although the mechanism by which aberrant TFEB activity promotes kidney cystogenesis and tumorigenesis has not been fully elucidated, the ability of this transcription factor to induce mTORC1 hyperactivation likely represents a major determinant. TFEB activation in FLCN-KO mouse kidneys results in increased expression of RagC/D, which, in the absence of FLCN, is likely to cause increased mTORC1 activity towards S6K and 4E-BP1 but not TFEB. TFEB depletion completely normalizes RagC/D levels and mTORC1 activity in FLCN-KO mouse kidneys [37]. Whether the upregulation of other TFEB-mediated transcriptional programs (e.g., lysosomal biogenesis, autophagy, lysosomal exocytosis, etc.) also contributes to the kidney phenotype has yet to be determined.

Finally, although the role in BHD pathogenesis of other MiT-TFE factors, such as TFE3 and MITF, remains to be established, it is likely that each MiT/TFE factor plays tissue-specific roles. Accordingly, whereas genetic depletion of TFEB in kidney distal tubular cells (i.e., cadherin 16-positive) is sufficient to rescue the kidney phenotype caused by loss of FLCN, depletion of TFES is able to rescue the phenotypes observed in hematopoietic-lineage or adipocyte-specific FLCN-KO mice [35,36,74], thus suggesting that the expression levels of each transcription factor in different tissues may determine their relative contributions to the disease phenotype.

**TSC**

Similarly to BHD syndrome, TSC is an autosomal dominant genetic disorder characterized by hamartomatous lesions in multiple tissues such as brain, heart, lung, skin, and kidney [75]. The disease is caused by germline loss-of-function mutations in the genes encoding either TSC1 or TSC2 which, together with TBC1D7, form the TSC complex as described above. Loss-of-function of TSC results in constitutive activation of mTORC1 signaling.
As observed in BHD, most TSC symptoms manifest as a consequence of second-hit somatic mutations resulting in TSC1/2 loss of heterozygosity. As a further parallelism with BHD, TSC tumors can present different histological phenotypes such as papillary RCC, chromophobe RCC, and hybrid oncocytoma [76,77]. A recent study showed that, in TSC2-KO cells and tissues, TFEB is dephosphorylated and nuclear, resulting in a significant induction of lysosomal biogenesis and upregulation of several TFEB lysosomal target genes [78]. It is important to emphasize that in TSC, similarly to BHD, TFEB dephosphorylation occurs in the context of mTORC1 hyperactivation. Notably, constitutive TFEB nuclear localization and activity in TSC2-KO cells had been already reported some years ago [79]. Furthermore, the expression of a constitutively active form of RagC in TSC2-KO cells restored the cytoplasmic localization of TFEB, suggesting that loss of TSC may impair RagC/D activity through an unknown mechanism (Figure 4). Most importantly, TFEB depletion inhibited the growth of TSC2-KO fibroblasts subcutaneously injected in mice [78]. Although future studies will be necessary to dissect the mechanisms underlying the activation of TFEB in TSC, it is possible that, in some pathological contexts, ‘canonical’ mTORC1 hyperactivation results in the activation of MiT-TFE factors through an unknown feedback mechanism. These observations provide another example in which TFEB is dephosphorylated in the context of mTORC1 hyperactivation and point to TFEB as a key player in the phenotype of this disease.

Lysosomal damage response to kidney injury
Several endogenous and exogenous factors, including silica, the accumulation of undigested material, and lysosomotropic drugs, induce lysosomal membrane permeabilization, a potentially harmful rupture that may lead to the leakage of digestive enzymes. For this reason, damaged lysosomes need to be either eliminated or repaired by the cell, a process that occurs via sophisticated mechanisms. The elimination of heavily damaged lysosomes is mediated by ‘lysophagy’, a selective form of autophagy [80,81]. Small lysosomal ruptures can be repaired via the activation of the endosomal sorting complexes required for transport (ESCRT) machinery, which is recruited to the sites of damage and allows their closure [82,83]. Interestingly, both pathways require the activation of TFEB [84,85]. Recent evidence has shown that induction of lysosomal damage with the lysosomotropic compound L-leucyl-L-leucine methyl ester (LLOMe) promotes TFEB nuclear translocation, without affecting the phosphorylation of S6K, via selective inhibition of RagC/D activity [86] (Figure 4). RagC/D inhibition in this context depends on the activity of the lysosomal calcium channel TRPLM1, which promotes LC3 lysosomal recruitment and lipidation through a process that is independent from canonical autophagy. Accordingly, following treatment with LLOMe, the ATG conjugation machinery (which includes the ATG12–ATG5–ATG16L1 complex) is recruited to the lysosomal membrane where it mediates non-canonical, single-membrane LC3 lipidation [86]. Depletion of ATG16L1 prevents lysosomal LC3 lipidation and impairs TFEB nuclear translocation in response to LLOMe treatment. By contrast, depletion of mediators that are essential for ‘canonical’ autophagosome formation but dispensable for non-canonical LC3 lipidation, such as ATG9 and FIP200, has no effect [86]. Although the mechanistic details of this LLOMe-elicited process need to be further elucidated, this pathway ensures a specific and efficient response to lysosomal damage via selective activation of TFEB. Kidney-specific depletion of TFEB in mice impairs this response and drastically exacerbates calcium oxalate-induced crystal nephropathy, a lysosomal damage-induced kidney injury [86]. These data represent another example in which selective modulation of mTORC1 activity mediates a physiologically relevant process that allows restoration of lysosomal integrity and tissue homeostasis through TFEB activation.

Lysosomal sequestration of FLCN by GABARAP
Similar to lysosomal damage, other conditions promoting selective autophagy (e.g., mitochondrial damage, pathogen infections), as well as the use of lysosomal ionophores such as TRPML1
activators, have also been shown to promote TFEB nuclear translocation without affecting the phosphorylation of mTORC1 substrates S6K and 4E-BP1 [87]. Also in this case, the mechanism for TFEB activation has been shown to rely on the specific inactivation of RagC/D and on the integrity of the ATG conjugation system, independently of canonical autophagy (Figure 4). However, in contrast to what was shown for LLOMe treatment, conjugation of GABARAP, rather than LC3, is required for RagC/D inactivation and TFEB nuclear translocation in response to TRMP1 activators. Lysosomal lipidation of GABARAP was shown to promote FLCN:FNIP binding, thus serving as a sequestration mechanism that blocks FLCN:FNIP GAP activity and ultimately prevents RagC/D activation [87]. Thus, different physiological and pathological conditions affecting the endolysosomal system converge on ‘non-canonical’ mTORC1 signaling for the selective modulation of MiT-TFE factors to support lysosomal biogenesis, repair, and activity.

Mutations in the homotypic fusion and vacuole protein sorting (HOPS) complex
The HOPS complex is a multi-subunit tethering complex which plays a crucial role in the fusion of lysosomes with late endosomes and autophagosomes [88]. Recent studies reported the presence of loss-of-function mutations in the genes encoding the subunits of the HOPS complex, in particular VPS41 and VPS16, in patients with severe neurological impairment [89,90]. Loss of VPS41 was associated with constitutive nuclear localization of TFE3, even though the phosphorylation of S6K and 4E-BP1 was not altered [89]. These findings strongly suggest that dysfunction of the HOPS complex can specifically affect the non-canonical mTORC1 signaling axis. Future studies will be necessary to understand how the HOPS complex specifically affects mTORC1-mediated TFEB phosphorylation without affecting the phosphorylation of S6K and 4E-BP1. Considering that the loss of HOPS leads to altered lysosomal function and amino acid sensing [91], one likely hypothesis is that VPS16/VPS41 deficiency impairs non-canonical mTORC1 signaling by affecting RagC/D activity, thus impairing the substrate-specific mTORC1 recruitment of MiT-TFE factors. HOPS subunits may also have a more direct role in the regulation of Rag GTPase activity. The yeast homolog of the VPS39 HOPS subunit has been proposed to act as a guanine nucleotide exchange factor (GEF) for the RagA/B homolog Gtr1 [92]. Whether a similar function is exerted by the mammalian HOPS complex in the regulation of Rag GTPases is currently unknown. Further studies will be necessary to demonstrate the involvement of the FLCN–RagC/D axis in conditions associated with loss of HOPS function. The selective activation of the MIT/TFE-dependent transcriptional programs in HOPS-deficient cells may represent a compensatory response mechanism to ameliorate lysosomal function and activity in these cells and points to the relevance of non-canonical mTORC1 signaling in neurological diseases.

Concluding remarks
As a key regulator of cell growth and metabolism, mTORC1 senses a variety of environmental cues. The newly identified non-canonical mTORC1 signaling pathway has revealed how mTORC1 is able to integrate multiple upstream stimuli to mediate specific downstream responses. So far two major classes of mTORC1 substrates, defined by their recruitment mechanisms, have been identified: TOS-containing substrates (e.g., S6K and 4E-BP1) versus Rag-binding substrates (e.g., MIT-TFE factors). Current data indicate that non-canonical mTORC1 signaling appears to be specific for MIT-TFE factors. However, whether other mTORC1 substrates also use a similar recruitment mechanism remains unknown (see Outstanding questions). Furthermore, it is possible that additional, and totally novel, mTORC1 substrate recruitment mechanisms exist. This concept implies that multiple substrates must be tested to determine mTORC1 kinase ‘activity’, considering that the phosphorylation status of different substrates may be discordant. Future proteomic and phosphoproteomic approaches, together with structural studies, will be pivotal for the identification of novel mTORC1 substrates and for a deeper understanding of their mechanism of recruitment.

Outstanding questions
Are there substrates, other than MiT-TFE factors, which are phosphorylated by mTORC1 in a non-canonical manner?
Are there other types of mTORC1 substrate-recognition mechanisms?
Where in the cell does mTORC1 phosphorylate S6K and 4E-BP1?
Are additional disease conditions caused by dysregulation of non-canonical mTORC1 signaling?
Can non-canonical mTORC1 signaling be specifically targeted through pharmacological approaches?
The identification of a non-canonical mTORC1 pathway has clear medical relevance. In this review we have discussed a few pathological conditions in which this pathway is involved. The hallmark of this type of condition is the association between dephosphorylated/nuclear-localized MIT-TFE factors and constitutively active/hyperactive mTORC1.

Dysregulation of mTORC1 signaling is a common feature of many human diseases, including neurodegenerative diseases and cancer [2,93,94]. However, owing to the broad involvement of mTORC1 in the regulation of several basic physiological processes, including cell growth and metabolism, general inhibition of mTORC1 activity may lead to undesired effects. Thus, the generation of novel pharmacological strategies for selective modulation of mTORC1 activity (e. g., by acting on the FLCN–RagC/D axis) may be a promising approach in specific disease conditions, as in the case of BHD syndrome and TSC, and may become relevant in a significantly larger number of human diseases.

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Declaration of interests
A.B. is a cofounder of Casma Therapeutics and advisory board member of Next Generation Diagnostics, Avilar Therapeutics, and Coave.

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Declaration of interests
A.B. is a cofounder of Casma Therapeutics and advisory board member of Next Generation Diagnostics, Avilar Therapeutics, and Coave.

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