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COMMENTARY

SEA you later alli-GATOR – a dynamic regulator of the TORC1 stress response pathway

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ABSTRACT

Cells constantly adapt to various environmental changes and stresses. The way in which nutrient and stress levels in a cell feed back to control metabolism and growth are, unsurprisingly, extremely complex, as responding with great sensitivity and speed to the 'feast or famine, slack or stress' status of its environment is a central goal for any organism. The highly conserved target of rapamycin complex 1 (TORC1) controls eukaryotic cell growth and response to a variety of signals, including nutrients, hormones and stresses, and plays the key role in the regulation of autophagy. A lot of attention has been paid recently to the factors in this pathway functioning upstream of TORC1. In this Commentary, we focus on a major, newly discovered upstream regulator of TORC1 – the multiprotein SEA complex, also known as GATOR. We describe the structural and functional features of the yeast complex and its mammalian homolog, and their involvement in the regulation of the TORC1 pathway and TORC1-independent processes. We will also provide an overview of the consequences of GATOR deregulation in cancer and other diseases.

KEY WORDS: TORC1, Amino acid sensing, SEA complex, GATOR complex, Autophagy

Introduction

Target of rapamycin (TOR) is a serine/threonine kinase, which belongs to the phosphatidylinositol 3-kinase (PI3K)-related family. TOR is highly conserved in eukaryotes and is also called mTOR (mammalian or mechanistic TOR) in various organisms (Hall, 2013). It phosphorylates a large number of targets, and its kinase activity is modulated in response to various stresses (Fig. 1) (Bar-Peled and Sabatini, 2014; Laplante and Sabatini, 2012; Loewith and Hall, 2011). The budding yeast *Saccharomyces cerevisiae* has two TOR-encoding genes (*TOR1* and *TOR2*), whereas almost all other eukaryotes have a single TOR-encoding gene. TOR can form two distinct complexes, known as TOR complex 1 and complex 2 (TORC1 and TORC2, respectively), which respond to somewhat different (although overlapping) stress signals, and together are among the most important hubs in the cellular metabolic and signaling pathway. TORC1 is the target of the SEA complex (Seh1-associated, see below) and controls transcription, ribosome biogenesis, translation, autophagy, glycolysis, lipogenesis and pyrimidine biosynthesis (Fig. 1) (Bar-Peled and Sabatini, 2014; Betz and Hall, 2013; Dibble and Manning, 2013).

TORC1 can be found in several subcellular locations, although it appears that one of its main sites of action is around the yeast vacuole or the mammalian lysosome (which in many ways is the functional equivalent of the vacuole) (Betz and Hall, 2013). Vacuoles and lysosomes are the storage and recycling depots of the cell. Among their many roles, they mediate protein degradation, store amino acids, and sequester small ions and polyphosphates (Li and Kane, 2009; Luzio et al., 2007). These organelles are also key players in autophagy – a process in which cytosol and organelles are sequestered within double-membraned vesicles, which deliver their contents to the vacuole or lysosome for degradation or recycling (Yang and Klionsky, 2009). The lysosome also plays an active role in amino acid sensing through its proton pump, the vacuolar type H^+-ATPase (v-ATPase) (Zoncu et al., 2011), and it is likely that the vacuole plays a similar role. Localization of TORC1 to the vacuole is independent of nutrient availability (Binda et al., 2009), whereas, in mammals, translocation of mTORC1 to the lysosome is stimulated by nutrients (Sancak et al., 2008).

TORC1 integrates signals from many intracellular and extracellular cues – amino acids, growth factors, energy, and oxygen. The transmission of these signals to TORC1 requires the coordinated interaction of many kinases and GTPases, as well as their modulators and substrates. In particular, the pathways upstream of TORC1 are governed by the action of GTPases and their effectors, such as GTPase-activating proteins (GAPs), which stimulate GTP hydrolysis, guanine-nucleotide-exchange factors (GEFs), which promote GDP dissociation, and guanine nucleotide dissociation inhibitors (GDIs), which inhibit GDP dissociation (Fig. 2; Box 1). Recently a new upstream regulator of the TORC1 pathway, the yeast SEA complex, has been identified and shown to be part of this web of GTPase effectors (Algret and Dokudovskaya, 2012; Panchaud et al., 2013b). Importantly, its homolog in mammals, the GATOR complex (GAP towards Rags, see below), was subsequently identified and also shown to play a similar key regulatory role (Bar-Peled et al., 2013; Bar-Peled and Sabatini, 2014).

This Commentary is the first comprehensive review on the SEA/GATOR complex and will focus on the structural and functional features of the yeast complex and its mammalian homolog, and their involvement in the regulation of the TORC1 pathway and TORC1-independent processes. We will also provide an overview of the consequences of GATOR deregulation in cancer and other diseases.

The SEA complex – a newly identified regulator of TORC1

The SEA complex was identified through an unusual route (Algret and Dokudovskaya, 2012; Dokudovskaya and Rout, 2011; Dokudovskaya et al., 2011). On mapping the interactome of yeast nucleoporins (the proteins making up the nuclear pore complex), we found that one nucleoporin, Seh1, appeared to be 'moonlighting' outside of the nuclear pore, in another complex entirely. This complex contains four high-molecular-mass proteins: Yjr138p (also known as Iml1), Yol138p (also known as Rtc1), Ydr128p (also known as Mtc5) and Ybl104p. To reflect their association with Seh1, these proteins were given a common name, Sea (for Seh1-associated), and named Sea1 through Sea4, respectively. Three other protein components complete the full SEA eight-protein

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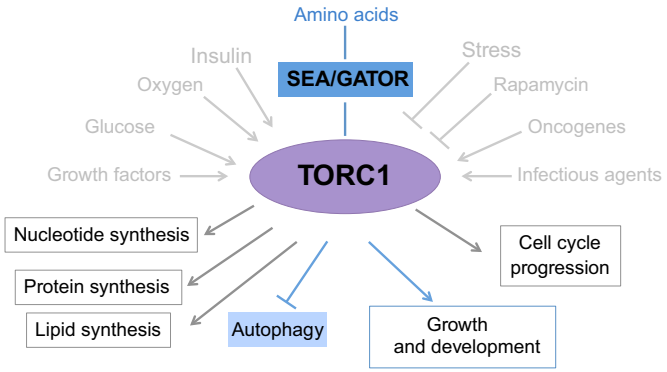

Fig. 1. TORC1 processes numerous signals and controls diverse sets of pathways. TORC1 is activated by growth factors, nutrients, infectious agents and oncogenes, and is inhibited by stress and rapamycin. The axis of amino acid signaling that is controlled by the SEA/GATOR complex is highlighted in blue.

complex: Sec13, Npr2 and Npr3 (Dokudovskaya et al., 2011). Sec13 is also a component of the nuclear pore (Alber et al., 2007; Siniossoglou et al., 2000) and, in addition, pairs up with Sec31 to form the outer coat of coat protein complex II (COPII) vesicles involved in ER trafficking (Fath et al., 2007; Stagg et al., 2006; Stagg et al., 2008). Phylogenetic analyses have demonstrated that these SEA complex subunits are present across various eukaryotic kingdoms, suggesting an origin of these factors before the last common eukaryotic ancestor (LCEA); importantly, homologs for all eight proteins can be clearly found in the genomes of metazoans, including humans (Dokudovskaya et al., 2011). Indeed, affinity capture of Mios, the mammalian ortholog of the SEA complex component Sea4, allowed the co-precipitation of all the mammalian orthologs of the SEA complex subunits (Bar-Peled et al., 2013). The homologs of Npr2 and Npr3 have been also investigated in *Schizosaccharomyces pombe* (Ma et al., 2013), *Caenorhabditis elegans* (Zhu et al., 2013) and *Drosophila melanogaster* (Wei and Lilly, 2014) (Box 2).

The SEA complex consists of two subcomplexes (Table 1), named SEA subcomplex inhibiting TORC1 (SEACIT, see below) and SEA subcomplex activating TORC1 (SEACAT, see below); in mammals these are termed GATOR1 and GATOR2 (Algret et al., 2014; Bar-Peled et al., 2013; Panchaud et al., 2013a; Panchaud et al., 2013b). SEACIT is composed of Sea1, Npr2 and Npr3 (Depdc5, Nprl2 and Nprl3, respectively in GATOR1), and SEACAT contains Sea2, Sea3, Sea4, Seh1 and Sec13 (Wdr24, Wdr59, Mios, Seh1L, Sec13 in GATOR2). In yeast, SEACAT and SEACIT can interact to form the full SEA complex (Algret et al., 2014). In humans, it has been suggested that GATOR1 and GATOR2 exist separately, and perhaps do not normally form a full stoichiometric GATOR complex (Bar-Peled et al., 2013); further work is required to establish whether the two mammalian subcomplexes indeed exist in a stable complex.

The SEA complex – structural features and components

Two characteristics of the SEA complex and its mammalian ortholog immediately stand out. First, they have components that moonlight between different, functionally unrelated complexes. Second, these different complexes are nevertheless related structurally in that they are variations on the theme of vesicle-coating scaffolds. The nuclear pore complex shares its core architecture with that of vesicle-coating complexes, such as COPII (see above), COPI and clathrin (Alber et al., 2007; Devos et al., 2004), and one of the most remarkable structural features of SEACAT is just how closely it resembles membrane-coating assemblies. SEACAT shares common subunits with both COPII (Sec13) and the nuclear pore (Sec13 and Seh1). Another subunit, Sea4, contains N-terminal WD40 repeats arranged into a β-propeller structure followed by an α-solenoid stretch, which is a structure that is characteristic for proteins that form the oligomeric coats in vesicle-coating complexes (such as clathrin and Sec31) (Devos et al., 2004; Dokudovskaya et al., 2011; Field et al., 2011). Furthermore, every protein in SEACAT contains a β-propeller (and Sea3 probably has two β-propellers), a domain common in coating assemblies (Field et al., 2011). Finally, there are two dimers, Seh1–Sea4 and Sec13–Sea3 (Algret et al., 2014; Dokudovskaya et al., 2011), that could be analogs to the Sec13–Sec31 dimer, which forms the structural unit of the COPII complex (Fath et al., 2007).

Sea4 also contains a C-terminal RING domain, which together with its β-propeller and α-solenoid motifs, makes it closely resemble several protein subunits of the homotypic fusion and protein sorting (HOPS) and class C core vacuole and endosome tethering (CORVET) complexes, which have been implicated in tethering of membranes prior to their fusion (Nickerson et al., 2009). As their names suggest, HOPS and CORVET are associated with the vacuoles and endosomes, respectively, and play a role in endosomal and vacuolar assembly and trafficking, and, notably, also in nutrient transport and autophagy (Balderhaar and Ungermann, 2013; Nickerson et al., 2009).

The presence of the same folds and fold arrangements in both the SEA complex and in coating and tethering assemblies, as well as the fact that they contain the same moonlighting components, are the key indicators that these complexes share a common evolutionary origin. The majority of intracellular membranes are likely a result of evolutionary expansion of an ancestral membrane-curving module – termed the 'protocoatomer' complex (Devos et al., 2004; Field et al., 2011). The SEA complex is a member of the coatomer group, and its existence thus provides further evidence that an expansion of the protocoatomer family underpins much of the functional diversity of the endomembrane system.

Just like Sea4 and several of the HOPS and CORVET components, Sea2 and Sea3 also have a C-terminal RING domain (Table 1). Clusters of RING domains are associated with E3 ubiquitin ligase activity, suggesting that SEACAT might have such a role. The RING domains appear to be crucial for maintaining the interactions between Sea2, Sea3 and Sea4, and the rest of the complex. For example, a Sea4 protein that lacks the RING domain can only interact with Seh1, whereas Sea2 or Sea3 without the RING domain are no longer able to interact with any of the SEACAT complex components (Algret et al., 2014). In addition, Sea3 contains an RWD domain, which is enriched in β-sheets and common in proteins that also contain a RING motif and a β-propeller (Doerks et al., 2002). The RWD domain of Sea3 strongly resembles that of ubiquitin-conjugating E2 enzymes (Nameki et al., 2004); however, such an enzymatic activity has never been demonstrated. Given that SEACAT contains three proteins with RING domains, as well as numerous β-propeller domains, which can mediate the recognition of phosphorylated substrate within E3 ligase complexes (Patton et al., 1998), it will be very interesting to investigate whether SEACAT can act as a E3 ubiquitin ligase, and if this is the case, what its targets are (e.g. SEACAT itself or perhaps SEACIT). In this context, it is noteworthy that treatment of yeast cells with the TORC1 inhibitor rapamycin specifically increases ubiquitylation of Npr2. Moreover, all subunits of the SEA complex (except for Sec13) appear to be phosphorylated (Albuquerque et al., 2008; Breitkreutz et al., 2010; Spielewoy et al., 2010) and ubiquitylated (Hitchcock

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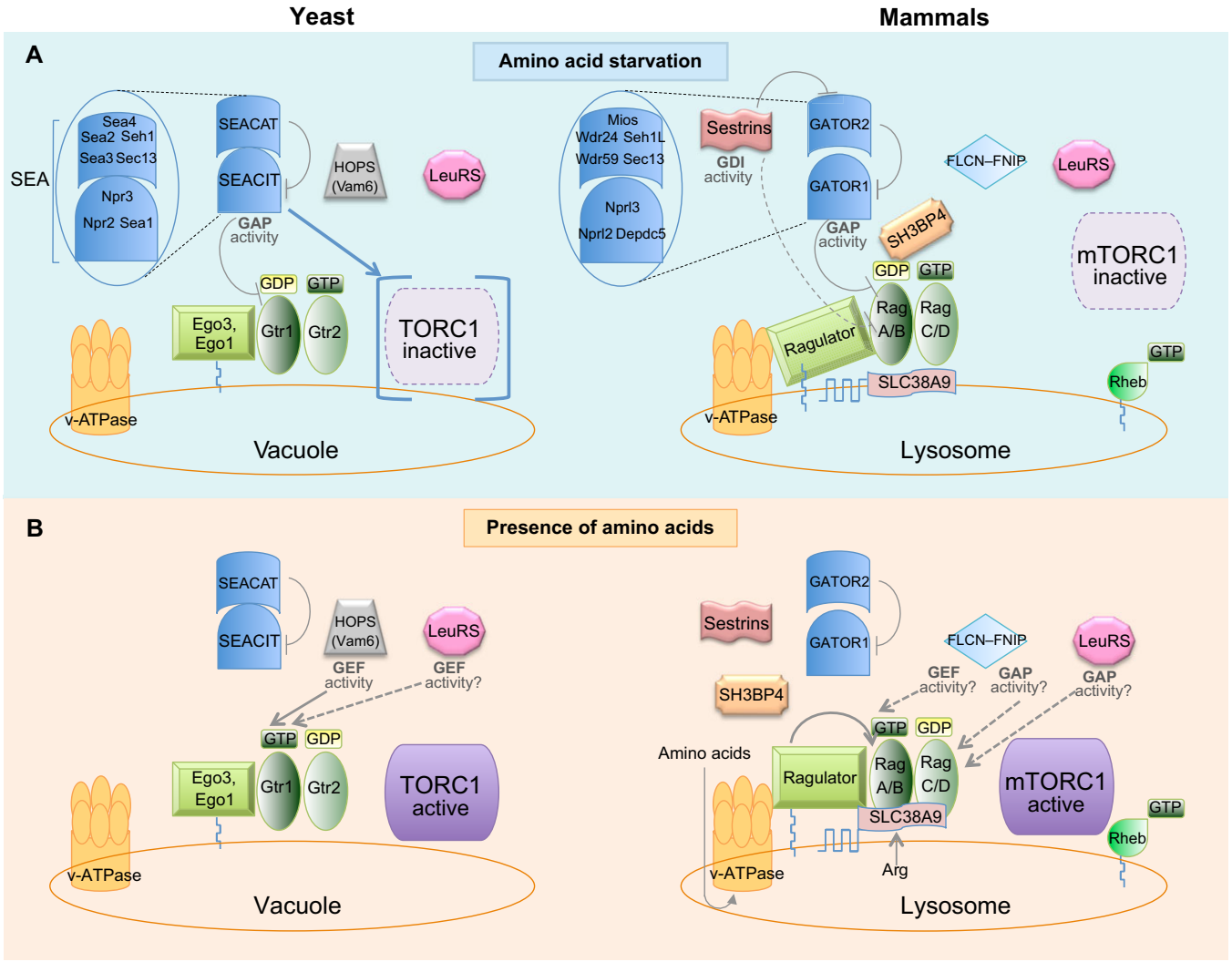

Fig. 2. Schematic representation of amino acid sensing in yeast and mammals at the vacuole or lysosome. Yeast and mammalian orthologs are designated with the same color. Functions of different TORC1 regulators, which are currently controversial in the field, are indicated with '?' and dashed arrows (see also Box 1). (A) TORC1 regulation during amino acid starvation. In yeast, SEACIT is involved in the maintenance of TORC1 at the vacuole membrane (blue arrow and brackets). Mammalian TORC1 is diffused throughout the cytoplasm. Mammalian-specific sestrins inhibit GATOR2. SEACAT (GATOR2) inhibits SEACIT (GATOR1). SEACIT (GATOR1) act as GAP for Gtr1 (RagA or RagB in mammals), preserving it in inactive form. Both yeast and mammalian TORC1 are inactive during amino acid starvation. Schematic representations of the composition of the SEA and GATOR complexes are also shown. (B) Activation of TORC1 upon amino acid stimulation. In yeast, SEA acts in the form of a stoichiometric SEACIT–SEACAT complex (shown on the left), whereas the GATOR subcomplexes GATOR1 and GATOR2 appear to act separately from each other. In mammals, Ragulator and v-ATPase undergo conformational changes that result in GEF activity by Ragulator towards RagA or RagB. Active Ragulator–Rag then promotes the recruitment of mTORC1 to the lysosomal membrane, where mTORC1 becomes fully activated by Rheb-GTP. LeuRS and FLCN–FNIP are also involved in the interaction with Gtrs/Rags (see Box 1), but their exact molecular functions (GEF or GAP, and for which Gtr or Rag) is currently a subject of controversy in the field. In yeast, TORC1 is activated by the EGO complex which is comprised of Gtr1-GTP and Gtr2-GDP.

et al., 2003; Iesmantavicius et al., 2014), suggesting that post-translational modifications of modulators upstream of TORC1 are involved in the regulation of the TORC1 signaling pathway.

The structural profile of the SEACIT subunits is completely different and they contain motifs that are typically found in GAPs and GEFs (Table 1). Npr2 is a possible paralog of Npr3 (Kowalczyk et al., 2012a), and both proteins possess N-terminal longin domains, which are found in GEFs, although a GEF activity has not yet been demonstrated for these two proteins (Levine et al., 2013; Nookala et al., 2012; Zhang et al., 2012). Sea1 is a multidomain protein that carries, at its center, a domain that has been shown to be important for its GAP activity (see below) (Panchaud et al., 2013a). GATOR1 in mammals also exhibits GAP activity (Bar-Peled et al., 2013). SEACIT components also have PEST motifs, which are often found in rapidly degraded proteins (Dokudovskaya et al., 2011). However, the PEST motifs are not well preserved in mammalian orthologs and thus could be a specific feature of the yeast SEA complex.

Sea1 in SEACIT (and Depdc5 in GATOR1) also contains domains that are found in membrane-associated proteins, specifically, its N-terminal Cdc48-like domain, which is immediately followed by a vWA-like domain and a C-terminal DEP domain (Table 1). The Cdc48-like domain is found in the SNARE chaperone Sec18/NSF, the vWA domain of Sce23 in COPII vesicles (again returning to the theme of coating complexes), and the DEP domain is involved in the interactions between regulator of G protein signaling (RGS) proteins and their membrane-bound receptors, the G-protein-coupled receptors (GPCRs) (Ballon et al., 2006). Interestingly, the DEP domain is also found in a Deptor subunit of mTORC1.

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Box 1. Amino acid signaling in yeast and mammals

Amino acid levels are signaled to yeast TORC1 through the EGOC, which consists of a Ego3 dimer, transmembrane Ego1, Gtr1 and Gtr2 (Binda et al., 2009). The mammalian analog of the trimeric yeast Ego3–Ego1 complex is pentameric Ragulator, which is anchored to the lysosome where it interacts with v-ATPase (Bar-Peled et al., 2012; Sancak et al., 2010; Zoncu et al., 2011). Currently there is no data for an interaction between Ego3–Ego1 and v-ATPase in yeast. The small GTPases Gtr1 and Gtr2 and their mammalian analogs RagA and RagB, and RagC and RagD function as heterodimers.

When amino acids are low, the EGOC and Ragulator–Rag is inactive, Gtr1 (RagA or RagB) is loaded with GDP and Gtr2 (RagC or RagD) with GTP. SEACIT (GATOR1) acts as a GAP for Gtr1 (RagA or RagB), preserving it in an inactive form (Bar-Peled et al., 2013; Panchaud et al., 2013a). SEACAT (GATOR2) inhibits SEACIT (GATOR1). Mammalian-specific SH3BP4 interacts with RagB, inhibiting the dissociation of GDP (Kim et al., 2012). Mammalian-specific sestrins might also interact with RagA or RagB and inhibit GDP dissociation, therefore acting as GDIs (Peng et al., 2014). Sestrins interact with and inhibit GATOR2 (Chantranupong et al., 2014; Parmigiani et al., 2014).

After amino acid stimulation, Ragulator and v-ATPase undergo a conformational change that results in Ragulator exerting GEF activity towards RagA or RagB (Bar-Peled et al., 2012). In yeast, GEF activity is mediated by the Vam6 protein from the HOPS complex. In addition, leucyl t-RNA synthetase LeuRS (also known as LRS or LARS) might exhibit GEF activity towards Gtr1 in yeast (Bonfils et al., 2012) or GAP activity towards RagD in mammals (Han et al., 2012). Similarly, a complex between folliculin (FLCN) and folliculin-interacting protein (FNIP) 1 and/or 2 might either stimulate the GEF activity towards RagA or RagB (Petit et al., 2013) or GAP activity towards RagC (Tsun et al., 2013). There are currently no data regarding an involvement of the yeast FLCN and FNIP orthologs Lst7 and Lst4 in the regulation of the TORC1 pathway. GEFs and GAPs promote the conversion of corresponding Gtrs and Rags into Gtr2-GDP (RagC/D-GDP)]. The mammalian-specific transceptor SLC38A9, which is imbedded in the lysosomal membrane and important for TORC1 activation by arginine, interacts with Ragulator–Rag and initiates amino acid signaling (Rebsamen et al., 2015; Wang et al., 2015). Active Ragulator–Rag promotes recruitment of mTORC1 to the lysosomal membrane, where it is then fully activated by another small GTPase, Rheb-GTP. In yeast, where TORC1 localization at the vacuole membrane is independent of nutrients, and the Rheb analog is not involved in the TORC1 signaling, TORC1 is activated by EGOC, containing Gtr1-GTP and Gtr2-GDP. Finally, amino acids can stimulate TORC1 independently of the EGOC and Ragulator-Rag. In cells deficient for both RagA and RagB, mTORC1 can be stimulated by glutamine (but not leucine) and this activation is dependent on v-ATPase (Jewell et al., 2015). In addition, in both yeast and mammals, amino acids can activate TORC1 on Golgi membranes. The small GTPase Ypt1 in yeast (Rab1A in mammals) recruits TORC1 to this organelle. mTORC1 is subsequently activated by Rheb, which is located at the Golgi (Thomas et al., 2014).

Box 2. SEA/GATOR homologs in other organisms

Phylogenetic analysis has identified SEA complex homologs in many organisms (Dokudovskaya et al., 2011), although experimental information about the entire complex is so far only available for *S. cerevisiae* and *H. sapiens*. With regard to the individual components, the majority of data are available for the role of the Npr2 and Npr3 homologs in the TORC1 pathway, which have been investigated in *S. pombe* (Ma et al., 2013), *C. elegans* (Zhu et al., 2013) and *Drosophila* (Wei and Lilly, 2014).

In *S. pombe*, as in *S. cerevisiae*, Npr2 acts as a negative regulator of TORC1 signaling. However, several differences have been reported. For example, depletion of Npr2 in *S. pombe* results in rapamycin sensitivity (Ma et al., 2013), which is not the case in *S. cerevisiae* (Wu and Tu, 2011). In addition, based on genetic interactions, it has been suggested that in *S. pombe*, Npr2 acts upstream of TORC1, but downstream of Gtr1 (Ma et al., 2013). However, this conclusion is difficult to reconcile with the data for *S. cerevisiae* and the human system, where Npr2, as a member of SEACIT or GATOR1, acts upstream of Gtrs or Rags (Bar-Peled et al., 2013; Panchaud et al., 2013a).

In *C. elegans*, a new sphingolipid-TORC1 signaling pathway has been shown to be under the control of Nprl2 and Nprl3 (Zhu et al., 2013). In the absence of sphingolipids, postembryonic growth and development could be initiated either by activating TORC1, or by inhibiting Nprl2 or Nprl3.

In *Drosophila*, Nprl2 and Nprl3, which localize to lysosomes, interact with each other and inhibit TORC1 signaling in the female germline in response to nitrogen starvation (Wei and Lilly, 2014). This inhibition is important for female fertility during protein scarcity. The components of *Drosophila* GATOR2, Mio and Seh1 oppose activity of all three GATOR1 components (Nprl2, Nprl3, Iml1), which prevents TORC1 inhibition and blocks oocyte development and growth (Wei et al., 2014).

Our previous phylogenetic analysis failed to identify Sea1–Sea4, Npr2 and Npr3 in plants (Dokudovskaya et al., 2011). However, it is possible, that functional homologs of these proteins do exist. An *Arabidopsis* Seh1 homolog has been found localized at the prevacuolar compartment and has been suggested to have a role in membrane association of dynamin-related protein 2A, which, in turn, is required for protein trafficking from trans-Golgi network to the central vacuole (Lee et al., 2006).

Recently, a combination of biochemical and computational approaches has revealed the first 3D map of the yeast SEA complex (Fig. 3) (Algret et al., 2014), showing that SEACAT and SEACIT form discrete modules that are connected by interactions between the N-termini of Sea3 and both Npr3 and Sea1. Npr2 is proximal to Sea1, whereas Seh1, Sec13 and the N-termini of Sea4 and Sea2 form a large cluster of β-propeller domains (Fig. 3). Similar arrangements of β-propeller domains have been described at the vertex of the evolutionarily related complexes COPI and COPII (Lee and Goldberg, 2010).

Where in the cells are SEAs and GATORs?

The expression levels of SEA and GATOR subunits are very low, both in yeast and in mammalian cells, making the task of determining their localization challenging. Nevertheless, a combination of subcellular fractionation and fluorescent microscopy of yeast SEA components that have been genomically tagged with GFP allowed us to show that endogenous yeast SEA complex dynamically associates with the vacuole membrane (Dokudovskaya et al., 2011). Although attempts by several groups to localize endogenous Npr3 have been unsuccessful (Dokudovskaya et al., 2011; Neklesa and Davis, 2009), Npr3, Npr2 and Sea1 can be seen to localize to the vacuole membrane upon moderate overexpression (Panchaud et al., 2013a). In these experiments, Sea1 did not require other SEA components to localize to the vacuole membrane; however, in contrast, Npr2 and Npr3 mutually depended on each other and on Sea1 for vacuolar localization (Panchaud et al., 2013a).

Consistent with these findings in yeast, proteomic studies of isolated human lysosomes have found that Depdc5, Wdr24, Mios, Seh1L and Sec13 are associated with the lysosomal membrane (Schröder et al., 2007). Immunofluorescence experiments in *Drosophila* using a GFP-tagged version of its Sea4 homolog Missing Oocyte (Mio), expressed from its native promoter, and overexpressed GFP–Seh1 (also known as Nup44A in *Drosophila*) have shown that these proteins localize to lysosomes and autolysosomes (Wei et al., 2014). Although there are currently no immunofluorescence-microscopy-based data for the localization of the endogenous human proteins, in cell lines that express introduced Depdc5–EGFP, it has been shown to localize to the lysosomal surface (Bar-Peled et al., 2013). Interestingly, overexpressed Nprl3 is found in both the cytoplasm and nucleus, where it colocalizes with

Table 1. Structural features of the SEA/GATOR complex members

Yeast protein (size in amino acids)	Human protein (size in amino acids)	Domains present	Domain boundaries in yeast protein (amino acids)	Domain boundaries in human protein (amino acids)	Reference(s)
SEACIT/GATOR1					
Sea1 (1584)	Depdc5 (1594)	Cdc48-like	101–275	4–170	Dokudovskaya et al., 2011; Algret et al., 2014
		vWA-like	279–473	171–372	Dokudovskaya et al., 2011; Algret et al., 2014
		GAP	877–1178	Not determined	Panchaud et al., 2013
		DEP	1178–1273	1171–1254	Dokudovskaya et al., 2011; Algret et al., 2014
Npr2 (615)	Nprl2 (380)	Longin	9–127	5–121	Zhang et al., 2012
		PEST motif	138–166	Not determined	Dokudovskaya et al., 2011
Npr3 (1146)	Nprl3 (569)	Longin	1–31	4–165	Dokudovskaya et al., 2011; Algret et al., 2014
		PEST motif	140–203	437–481	Dokudovskaya et al., 2011
		Longin	322–438		Zhang et al., 2012
		Longin	531–577		Zhang et al., 2012
SEACAT/GATOR2					
Sea2 (1341)	Wdr24 (790)	β-propeller	127–520	21–530	Dokudovskaya et al., 2011; Algret et al., 2014
		Ring	1280–1341	858–913	Dokudovskaya et al., 2011; Algret et al., 2014
Sea3 (1148)	Wdr59 (974)	β-propeller	54–278	7–432	Dokudovskaya et al., 2011; Algret et al., 2014
		RWD	430–536	381–498	Dokudovskaya et al., 2011; Algret et al., 2014
		Ring	1092–1139	918–973	Dokudovskaya et al., 2011; Algret et al., 2014
Sea4 (1038)	Mios (875)	β-propeller	45–426	3–351	Dokudovskaya et al., 2011; Algret et al., 2014
		SPAH	659–835	487–723	Dokudovskaya et al., 2011; Algret et al., 2014
		Ring	942–1032	797–856	Dokudovskaya et al., 2011; Algret et al., 2014
Seh1 (346)	Seh1L (360)	β-propeller	1–346	1–360	Devos et al., 2004
Sec13 (296)	Sec13 (322)	β-propeller	2–296	1–322	Devos et al., 2004

promyelocytic leukemia (PML) bodies (Lunardi et al., 2009). Nprl2 and Nprl3 overexpressed in *Drosophila* can also be found in the nucleus, in addition to their localization at the lysosome (Wei and Lilly, 2014).

The SEA/GATOR complex – an upstream regulator of the TORC1 pathway

Npr2 and Npr3 regulate TORC1 in response to amino acid starvation

The structural features of the yeast and mammalian SEA complex discussed above together with their localization strongly suggest that they function in the regulation of certain aspects of membrane biology, most probably those that are involved in the processes taking place around the vacuole or lysosome. Since its initial discovery, functional studies of the SEA complex have concentrated on its possible involvement in regulating the TORC1 signaling pathway, and indeed, the first data for such a role came from an elegant genome-wide screen for TORC1 regulators in yeast (Neklesa and Davis, 2009). The screen revealed that, in response to amino acid starvation (but not carbon starvation or rapamycin treatment), cells lacking Npr2 and Npr3 failed to fully activate the TORC1-controlled transcription factors Gln3 or Gat1; they also did not dephosphorylate the TORC1 effector Npr1 and did not repress ribosomal protein gene expression. The authors concluded that both proteins act upstream of TORC1, communicating when amino acids become scarce. However, Npr2 and Npr3 have little effect on TORC1 signaling under conditions of glucose starvation or stress (Hughes Hallett

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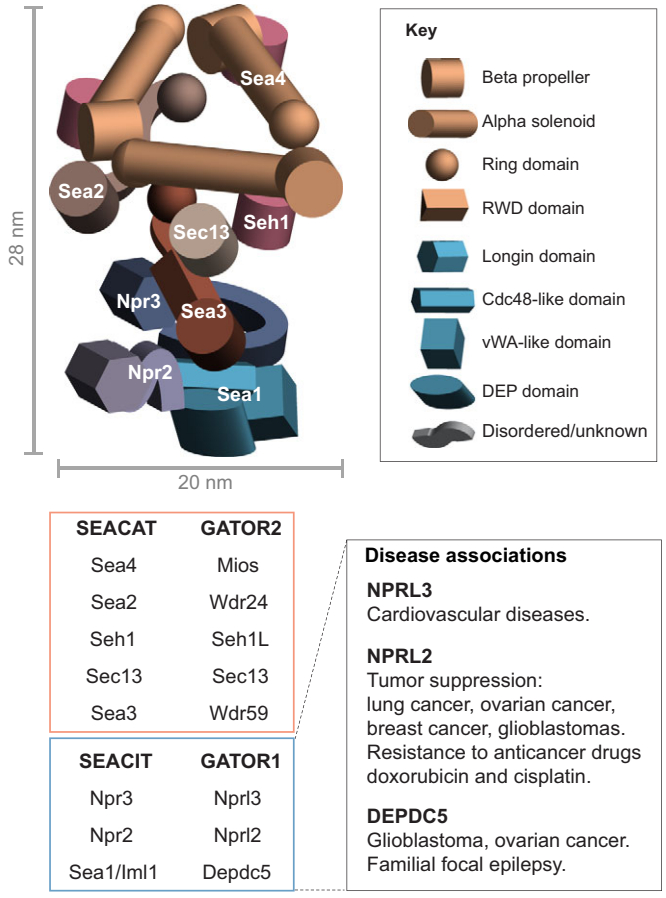

Fig. 3. Molecular architecture of the SEA complex. Schematic representation of the molecular architecture of the SEA complex obtained through integrative modeling. Domains and folds of each SEA member are represented by different geometric forms. Mammalian homologs of SEACAT (GATOR2) and SEACIT (GATOR1) subcomplexes are indicated. All of the components of GATOR1 have been linked to cancer and other diseases; see main text for details.

et al., 2014; Neklesa and Davis, 2009). Taken together, these data suggest that Npr2- and Npr3-mediated signaling through TORC1 diverts energy from protein biosynthesis towards the production of amino acids and other metabolites (Hughes Hallett et al., 2014).

Molecular basis of SEA/GATOR-mediated regulation of TORC1

During the past few years, it has become clear that not only Npr2 and Npr3, but also the entire SEA and GATOR complexes are upstream regulators of the TORC1 pathway and have an essential role in nutrient-mediated and, in particular, amino-acid-mediated signaling. The way in which nutrient and stress levels in a cell are fed back to control metabolism and growth are, unsurprisingly, highly complex – as responding with great sensitivity and speed to the 'feast or famine, soothing or stress' status of its environment is a central goal for any organism, be it yeast or yak.

Recent studies in yeast, *Drosophila* and human cell lines have provided a breakthrough in our understanding of how the communication between SEA/GATOR and TORC1 occurs on the molecular level (Bar-Peled et al., 2013; Panchaud et al., 2013a; Panchaud et al., 2013b). Deletion of either Sea1, Npr2 and Npr3 or their human orthologs results in increased enzymatic activity of TORC1, both in the presence and the absence of amino acids, indicating that these proteins are involved in TORC1 inhibition (Bar-Peled et al., 2013; Panchaud et al., 2013a); hence the subcomplex formed by Sea1, Npr2 and Npr3 was given the name SEA complex inhibiting TORC1 (SEACIT). By contrast, deletions of Sea2, Sea3, Sea4, Seh1 and Sec13 led to inhibition of TORC1 activity, implying that these SEA components are required for TORC1 activation (Panchaud et al., 2013a; Panchaud et al., 2013b), and this subcomplex was named SEA complex activating TORC1 (SEACAT).

TORC1 activity is under control of several GTPases (Fig. 1). The small GTPases that signal amino acid levels to TORC1 are part of the EGO complex (EGOC) in yeast, whose mammalian counterpart is the Ragulator–Rag complex. EGO consists of Ego1–Ego3 (Ragulator in mammals), Gtr1 (RagA or RagB in mammals) and Gtr2 (RagC or RagD in mammals) (RagA–RagD are also known as RRAGA–RRAGD) (Bar-Peled et al., 2012; Binda et al., 2009; Sancak et al., 2010; Sancak et al., 2008). The small GTPases Gtr1 and Gtr2 function as heterodimers and in their active form exist as a Gtr1-GTP–Gtr2-GDP complex. In mammals, availability of amino acids results in loading of RagA or RagB with GTP and of RagC or RagD with GDP, which promotes the interaction of Ragulator–Rag with mTORC1 (Sancak et al., 2010; Sancak et al., 2008), although Rag-independent stimulation of mTORC1 by amino acids has also been discovered very recently (Jewell et al., 2015; Oshiro et al., 2014; Thomas et al., 2014). To regulate their GTP-bound states, and thus their activity, these GTPases must have GAPs (Han et al., 2012; Tsun et al., 2013), GEFs (Bar-Peled et al., 2012; Binda et al., 2009; Bonfils et al., 2012; Petit et al., 2013) and GDIs (Kim et al., 2012; Peng et al., 2014) – and this is where the SEA (GATOR) complex comes in (Bar-Peled et al., 2013; Panchaud et al., 2013a). Indeed, Sea1 in yeast and GATOR1 in humans exhibit GAP activity towards Gtr1 (Rag A and/or Rag B), but it has not been determined which of the GATOR1 proteins is the GAP in the human system (Bar-Peled et al., 2013; Panchaud et al., 2013a). Therefore, the human homolog of SEACIT was named GATOR1 (GTPase activating proteins activity towards TORC1 regulator RagA), and the SEACAT analog was named GATOR2. Some experiments suggest that SEACAT/GATOR2 acts upstream of SEACIT/GATOR1, thus being an 'inhibitor of an inhibitor' (Bar-Peled et al., 2013; Panchaud et al., 2013b). Recently, it has been reported that GATOR2 interacts in an amino-acid-sensitive manner with a family of growth regulators called sestrins (consisting of sestrin-1 to -3) (Chantranupong et al., 2014; Parmigiani et al., 2014). Sestrins also require GATOR1 and the Rags to function as negative regulators upstream of TORC1 (Chantranupong et al., 2014; Parmigiani et al., 2014). In a parallel study, sestrins have been identified to act as GDIs for RagA and RagB (Peng et al., 2014). However, sestrins do not have homologs in yeast and so far it is unknown whether there are any proteins in the yeast TORC1 pathway that have GDI function.

Immunoprecipitation of various SEA components has revealed that they can be co-purified with members of TORC1 (i.e. Tor1, Kog1 and Lst8) (Algret et al., 2014). Interestingly, this proteomics study also revealed that the SEA complex can interact with components of the vacuole protein pump v-ATPase (Algret et al., 2014), which has been described as an essential modulator of amino-acid-mediated mTORC1 signaling (Zoncu et al., 2011). SEA components are also involved in ensuring the proper localization of TORC1, as deletion of either *SEA1*, *NPR2* or *NPR3* during nitrogen starvation causes a dramatic relocalization of Tor1 to the cytoplasm (Algret et al., 2014). Therefore, the response of TORC1 to stresses not only involves changes in its enzymatic activity, but also alterations in its location. Given the structural and evolutionary relationships of the SEA complex with the coating and tethering assemblies (Dokudovskaya et al., 2011), it is conceivable that the SEA complex helps to maintain the localization of TORC1 at the vacuole membrane during nitrogen starvation.

SEA/GATOR in autophagy

A major function of TORC1 is in the regulation of autophagy, which is induced when TORC1 is inhibited, either by rapamycin or by nitrogen starvation. Thus, it is not surprising that SEACIT, which inhibits TORC1, also controls autophagy (Algret et al., 2014; Dokudovskaya et al., 2011; Graef and Nunnari, 2011; Kira et al., 2014; Laxman et al., 2014; Sutter et al., 2013; Wu and Tu, 2011); there are some indications this is also the case in mammals (Kira et al., 2014). So far, the effects of SEACIT have only been described for a specific form of autophagy, non-nitrogen-starvation (NNS)-induced autophagy (Laxman et al., 2013; Sutter et al., 2013; Wu and Tu, 2011). NNS autophagy happens in yeast when cells are switched from a rich to a minimal medium with non-fermentable lactate as a carbon source (Wu and Tu, 2011). NNS autophagy promotes the dephosphorylation of Npr2, which in turn inhibits TORC1 (Sutter et al., 2013). It will be interesting to investigate whether other specific forms of autophagy (i.e. mitophagy or pexophagy) are also subjected to control by the SEA complex and whether such a function is preserved in mammals.

Deletion of *SEA1* or double deletion of *NPR2* and *NPR3* results in the inhibition of vacuolar fusion upon nitrogen starvation (Algret et al., 2014). Autophagic defects are not commonly associated with inhibition of vacuole fusion; however, a recent study has reported that inactivation of TORC1 during nitrogen deprivation, and therefore induction of autophagy, promotes vacuole coalescence (Michaillat et al., 2012). Because deletions of any of the SEACIT members results in increased TORC1 activity during starvation, this leads to increased vacuolar fragmentation and inhibition and/or defects in autophagy. Therefore, signaling upstream of TORC1 controls vacuolar fusion and fission events.

Modulation of nitrogen metabolism by the SEA complex

Nitrogen catabolite repression in yeast is a process that ensures that cells selectively use the preferred nitrogen sources (e.g. glutamate, glutamine) when they are available, whereas in their absence cells can utilize alternative, non-preferred nitrogen sources (e.g. urea, proline) (Ljungdahl and Daignan-Fornier, 2012). The SEACIT component Npr2, which stands for nitrogen permease regulator 2, was originally identified as a protein necessary for yeast growth in poor nitrogen sources (Rousselet et al., 1995). In turn, Npr3 was described as a protein required for sporulation – a 'hard times coming' response that is initiated by the depletion of multiple factors, including nitrogen (Enyenihi and Saunders, 2003). Both Npr2 and Npr3 are involved in controlling nitrogen catabolite repression (Godard et al., 2007).

Nitrogen catabolite repression is ultimately connected to amino acid biosynthesis. The analysis of synthetic genetic interactions for the genes encoding the SEA complex demonstrates that almost the entire set of genes that are responsible for homoserine biosynthesis exhibit very strong genetic interactions with the genes that encode both SEACIT and SEACAT proteins, indicating that the entire SEA complex is involved (Costanzo et al., 2010; Dokudovskaya et al., 2011). The majority of genes encoding for factors that participate in amino acid biosynthesis pathways, including those involved in genetic interactions with SEA component genes, are regulated by the transcriptional activator Gcn4 (Natarajan et al., 2001). Interestingly, the *SEA4* gene has multiple binding sites for Gcn4 in its promoter (Schuldiner et al., 1998), suggesting that this SEACAT component is directly involved in the control of amino acid biosynthesis.

The retrograde signaling pathway is a mechanism by which dysfunctional mitochondria transmit signals to effect changes in nuclear gene expression, which then lead to the reconfiguration of nitrogen and carbohydrate metabolism (Butow and Avadhani, 2004). Mitochondrial dysfunction caused by mitochondrial genomic defects leads to downregulation of TORC1 (Jazwinski and Kriete, 2012; Kawai et al., 2011). Interestingly, the retrograde signaling pathway has been found to be deregulated in *NPR2* and *NPR3* deletion mutants (Neklesa and Davis, 2009). However, links between the SEA complex and mitochondria might be even more direct, as genes encoding SEA components also show synthetic genetic interactions with many mitochondrial genes (Costanzo et al., 2010). Moreover, SEA proteins have been shown to interact with several mitochondrial membrane proteins, including the cytochrome *bc*1 complex, the cytochrome *c* oxidase complex and prohibitins (Algret et al., 2014), and enriched mitochondrial fractions contain SEA proteins (Elbaz-Alon et al., 2014). Taken together, this suggests a close functional and perhaps even physical connection between the SEA complex and mitochondria.

SEA you later – functions of SEA/GATOR components beyond TORC1 regulation

As mentioned above, a peculiarity of the SEA complex is the propensity of a number of its components to moonlight in other processes in the cell. The SEACAT components Seh1 and Sec13 are also found at the core scaffold of the nuclear pore complex, and Sec13 further participates as a component of the COPII vesicle-coating complexes. Of course, as mentioned before, this might reflect the common evolutionary origin of nuclear pore complexes, vesicle-coating complexes, tethering complexes, such as HOPS and CORVET, and the SEA complex as a progenitor membrane-associated coating complex; however, other SEA proteins also appear to have 'double lives'. In *Drosophila*, the Sea4 homolog Mio (Senger et al., 2011) is also localized to the nucleus and is required for the maintenance of the meiotic cycle and oocyte identity (Iida and Lilly, 2004). In addition, a chemical genomic survey in yeast has identified a small group of genes, among them *NPR2* and *NPR3*, that appear to be required for multidrug resistance (Hillenmeyer et al., 2008).

Nprl3 (the human Npr3 homolog) might be involved in some nuclear functions. Overexpression of Nprl3 in human and *Drosophila* cell lines targets a substantial fraction of the protein to the PML nuclear bodies (Lunardi et al., 2009), which have multiple functions, including involvement in DNA repair. In addition, Nprl3 interacts with the transcriptional factor p73, a member of p53 family of proteins that are involved in tumor suppression and embryonic development (Lunardi et al., 2009). Importantly, the *NPRL3* gene is located just upstream of the α-globin gene cluster. Consequently, Nprl3 is widely expressed throughout development and is highly induced in erythroid cells when the α-globin genes are fully activated (Kowalczyk et al., 2012b). Mice in which a promoter of the *NPRL3* gene has been deleted die in late gestation, often with severe cardiac defects, suggesting that perturbation of Nprl3 function might adversely affect the development of the myocardium (Kowalczyk et al., 2012a).

Npr2 has come under particular scrutiny, as mutations in this protein, both in yeast and humans, confer resistance to the anticancer drugs cisplatin and doxorubicin (Schenk et al., 2003; Ueda et al., 2006); the effects of these compounds are mainly mediated by inducing high levels of DNA damage, which eventually lead to cell cycle arrest and apoptosis (Galluzzi et al., 2012; Granados-Principal et al., 2010). Therefore, the possible role of Nprl2 in DNA damage was investigated in non-small-cell-lung cancer cells treated with cisplatin (Jayachandran et al., 2010). Indeed, it was demonstrated that ectopic expression of Nprl2 activates the DNA damage checkpoint pathway in cisplatin-resistant and Nprl2-negative cells, leading to cell cycle arrest in G2/M phase

and induction of apoptosis. It has been suggested that Nprl2 stimulates the phosphorylation of ATM kinase, the key component of the DNA damage signaling pathway, although the underlying molecular mechanisms remain unknown. It is also unclear whether this function of Nprl2 is coupled to or is independent from its role in regulating the TORC1 pathway. Interestingly, in yeast, *NPR2* mutants are synthetically lethal with *CDC45* and *RAD50* mutants, two genes involved in processing of double-strand DNA breaks, suggesting that such a functional interplay exists (Tong et al., 2004).

Deregulation of GATOR in cancer and other diseases

The above described links between Npr2, the DNA damage signaling pathway and resistance to anticancer drugs might reflect a role for deregulation of Nprl2 in oncogenesis. More than a decade ago, Nprl2 was suggested to be a tumor suppressor (Lerman and Minna, 2000). Since then, low levels of Nprl2 expression have been detected in many cancers, including hepatocellular carcinoma (Otani et al., 2009), glioblasomas (Bar-Peled et al., 2013), as well as in lung (Anedchenko et al., 2008; Ji et al., 2002; Li et al., 2004; Ueda et al., 2006), ovarian (Bar-Peled et al., 2013; Li et al., 2004), renal (Li et al., 2004; Tang et al., 2014), colorectal (Liu et al., 2014; Yogurtcu et al., 2012) and breast cancers (Li et al., 2004). In addition, it has been reported that Nprl2 interacts with the kinase Pdk1, a key regulator of cell proliferation and survival (Kurata et al., 2008). Pdk1, which plays a role in cellular transformation and tumor growth, is a well-defined upstream regulator of the TORC1 pathway in mammalian cells – closing the circle of GATOR functions back to the TOR regulatory pathway. Indeed, a growing body of data illustrates the impact of the GATOR–TORC1 regulatory pathway in human diseases (Fig. 3). Of particular note, the first evidence for a genetic link came from inherited focal epilepsies, where loss-of-function mutations in the mammalian *SEA1* gene (*DEPDC5*) have been associated with various forms of this disorder (Baulac, 2014; Dibbens et al., 2013; Ishida et al., 2013; Scheffer et al., 2014). Some of these mutations have been shown to disrupt DEPDC5-dependent inhibition of TORC1 (van Kranenburg et al., 2015). Thus, DEPDC5 is a new factor to be considered in 'mTORopathies' (Crino, 2007; Crino, 2011), a set of conditions associated with multiple neurological disorders and that often exhibit mutations in regulators acting upstream of mTORC1, such as Tsc1 and Tsc2, among many others (Lim and Crino, 2013).

Later, alliGATOR – future directions and perspectives

The TORC1 signaling pathway is one of the most complex networks in the cell and thus a lot of attention has been paid to the factors that function upstream of TORC1. The SEA complex and its mammalian homolog GATOR have emerged as important regulators of TORC1 during amino acid sensing. As these complexes have only been discovered very recently, many details regarding their structure, function and involvement in different human disorders are still unknown.

From a structural point of view it will be important to determine the high-resolution structure of the individual components and the subcomplexes comprising SEA and GATOR. Given that the majority of these components are high-molecular-mass proteins with some disordered regions, solving their structure (as well as the structure of the entire ~1 MDa assembly) using X-ray crystallography or even electron microscopy alone might be a considerable challenge. Instead, integrative approaches to structure determination could be more promising, and indeed this has been successfully demonstrated for the SEA complex (Algret et al., 2014). Nevertheless, further details and information remain to be collected in order to obtain near-to-atomic resolution structures for this assembly. It should be noted that there is practically no structural information for GATOR.

One of the particular interesting outstanding questions is how the two SEA subcomplexes interact with each other and how this interaction is affected during nutrient stresses? What is the molecular function of SEACAT and GATOR2? Is it only a structural platform (and, if so, for which activities), or has it other functions, such as, for example, the ability to act as an E3 ligase (based on its profusion of RING domains)? Given that Npr2, Npr3 and their human orthologs possess longin domains, is it possible that these proteins have GEF activity? If this is the case, how can GAPs (i.e. Sea1) and GEFs (i.e. Npr2 and Npr3), co-exist in one complex? Another important question is how is information regarding amino acid availability is transmitted to SEA/GATOR? And lastly, how do the 'moonlighting' functions of the SEA/GATOR proteins impact upon their roles in the TORC1 pathway?

Furthermore, the emerging realization that SEA/GATOR components are implicated in a number of important genetic and oncogenic diseases puts them in the spotlight for future research. We expect that we will SEA a lot more of GATOR in the future.

Competing interests

The authors declare no competing or financial interests.

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