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Integrated actions of mTOR complexes 1 and 2 for growth and development of *Dictyostelium*

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ABSTRACT Multi-protein complexes mTORC1 and mTORC2 are required for growth and development of eukaryotes. mTORC1 is a nutrient sensor that integrates metabolic signals and energy state to regulate cell growth/proliferation, whereas, mTORC2 primarily regulates developmental processes. *Dictyostelium* proliferate in rich growth media, but initiate development upon nutrient depletion. Both mTOR complexes play essential roles in *Dictyostelium*, where growth and developmental cycles independently require, respectively, mTORC1 or mTORC2. Many protein associations and regulatory pathways for mTORC1 and mTORC2 in *Dictyostelium* have context similarity to mammalian cells and specificity to inhibition by the immunosuppressive drug rapamycin. In *Dictyostelium*, mTORC1 function is inactivated upon starvation-induced development, but development is directly induced through rapamycin-mediated inhibition of mTORC1 activity, even in the absence of nutrient withdrawal. Pharmacologic inhibition of mTORC1, in the absence of nutrient loss, has allowed the identification of a class of essential up-regulated, developmentally-associated signaling genes and down-regulated, growth genes. We also review functional pathway regulations that integrate mTORC1/mTORC2 activities and emphasize complexity of small GTPase regulation of mTORC2 activity. Finally, epistases experiments have suggested novel upstream pathway cross-talk in *Dictyostelium* that requires mTORC1 and mTORC2, but for separate and independent downstream functions.

KEY WORDS: *Rapamycin, cAMP, RNA-seq, adenylyl cyclase, kinases*

Introduction

Rapamycin is an antifungal metabolite synthesized by *Streptomyces hygroscopicus*, discovered in the soil of Rapa Nui (*i.e.* Easter Island). The mechanistic Target Of Rapamycin in eukaryotic cells is the serine/threonine phosphatidylinositol related kinase mTOR (Saxton and Sabatini, 2017; Ben-Sahra and Manning, 2017; Loewith and Hall, 2011; Kim and Guan, 2019). mTOR in protein complex mTORC1, with Raptor and Lst8, promotes growth through phosphorylation of essential growth regulators, including eukaryotic translation initiation factor 4E-BP1 and ribosomal protein S6 kinase (Magnuson *et al.*, 2012; Gingras *et al.*, 1998). Rapamycin, bound to the endogenous cellular protein FKBP12 (12-kDa FK506-binding protein), blocks cell growth through suppression of mTORC1 function (Bjornsti and Houghton, 2004). However, rapamycin does not inhibit mTOR kinase activity; rather, rapamycin/FKBP12 interacts with the FRB domain of mTOR to disrupt mTOR association with Raptor, which blocks substrate recruitment by mTORC1 and active site access (Oshiro *et al.*, 2004; Bjornsti and Houghton, 2004). Thus, phosphorylation levels of mTORC1 targets decline rapidly upon exposure to rapamycin causing cessation of cell growth. As a nutrient sensor, mTORC1 activity fluctuates *in vivo* in direct response to nutrients, energy status, and growth factor stimulation, which collectively balance cell growth. Complex pathway responses integrate signaling through mTORC1, and disruptions in circuitry

Abbreviations used in this paper: GTD, growth-to-development transition; TOR, Target Of Rapamycin.

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signaling can lead to cellular overgrowth and oncogenesis (Inoki *et al.*, 2003; Benvenuto *et al.*, 2000; Sampson, 2003).

The mTOR kinase is part of an additional essential complex, mTORC2, comprised of mTOR, Lst8, Rictor, and Sin1 (Saxton and Sabatini, 2017; Ben-Sahra and Manning, 2017; Loewith and Hall, 2011; Kim and Guan, 2019). Although mTORC1 perceives signals from extracellular nutrients and intracellular metabolites for cell growth, mTORC2 is crucial for multicellular development (Saxton and Sabatini, 2017; Ben-Sahra and Manning, 2017; Loewith and Hall, 2011; Guertin *et al.*, 2006; Kim and Guan, 2019). Still, these distinctions are not absolute, as mTORC2 significantly impacts cell survival (Yu an *et al.*, 2018) and mTORC1 can influence cell fate choice (Patel and Powell, 2017; Chisolm and Weinmann, 2018; Jaiswal and Kimmel, 2019; Zeng and Chi, 2014; Pearce, 2010; Powell and Delgoffe, 2010).

Dictyostelium represents an ideal system for analyzing the different roles of mTOR during various life cycle stages. In the presence of rich nutrients, *Dictyostelium* will rapidly proliferate as individual cells, where mTORC1 remains continuously active (Rosel *et al.*, 2012; Jaiswal and Kimmel, 2019). However, upon nutrient depletion, mTORC1 is inactivated, growth ceases, and *Dictyostelium* inititiate a developmental program leading to multicellularity (McMains *et al.*, 2008; Devreotes *et al.*, 2013; Loomis, 2015; Rosel *et al.*, 2012; Jaiswal and Kimmel, 2019). Central to development is the elaboration of a complex system for cAMP synthesis, secretion, and relay, mediated by plasma membrane associated G protein-coupled, cAMP receptors (CAR1). Stimulated CAR1 directs adenylyl cyclase (AC) activation for cAMP production and chemotaxis toward centers of cAMP accumulation, thus establishing foci for multi-cell aggregation (McMains *et al.*, 2008; Devreotes *et al.*, 2013; Loomis, 2015). Whereas nutrient depletion leads to mTORC1 inhibition (Rosel *et al.*, 2012), cAMP synthesis, chemotaxis, and aggregation require mTORC2 (Lee *et al.*, 2005).

Many mTORC1/2 component pathways have been previously analyzed in *Dictyostelium*, with homologies to other systems, including FKBP12, TSC2, Rheb, Rictor/Pia, Sin1/RIP3 and others (Lee *et al.*, 2005, 1999; Rosel *et al.*, 2012; Chen *et al.*, 1997); for some, gene discoveries in *Dictyostelium* preceded those in mammalian systems. Here, we review common and distinct regulatory modes for mTOR complexes in mammalian cells and *Dictyostelium* and their impact to *in vivo* function for cell growth and multi-cell development. We further discuss where the cellular energy sensor AMP-activated protein kinase (AMPK) interacts with mTORC1 and how the antagonistic actions in response to nutrient sensing define fate choice at the junction of growth and programed development (Jaiswal and Kimmel, 2019). We additionally discuss how directed inhibition of mTORC1 by rapamycin can induce multi-cell development, even in the presence of nutrients, allowing the identification of essential developmental gene targets (Jaiswal and Kimmel, 2019). Finally, we elaborate on previous observations that suggest a cross-talk of a common path requiring either active mTORC1 or active mTORC2 for independent cellular functions that are separately directed toward growth or development (Rosel *et al.*, 2012).

The mTOR complexes of *Dictyostelium*

mTOR complex components

mTORC1 (mechanistic Target of Rapamycin Complex 1) is a primary growth regulator of eukaryotes, comprised of the mTOR kinase, Raptor, and Lst8 (Fig. 1A). Rich nutrients and energy sources stimulate mTORC1 for active cell growth. Amino acid response depends on the Gator-Ragulator-RagA/C pathway (Wolfson and Sabatini, 2017), proteins that are also present in *Dictyostelium* but not yet functionally studied. AMPK, an antagonist of mTORC1 (Inoki *et al.*, 2003; Gwinn *et al.*, 2008; Shaw, 2009), is inhibited with nutrient supported, high intracellular energy status (Hardie, 2011; Fig. 1A).

Primary phosphorylation targets of mTORC1 include S6K and 4E-BP1, where pS6K is a positive factor for growth, but 4E-BP1 is antagonistic and inactivated upon phosphorylation by mTORC1 (Magnuson *et al.*, 2012; Gingras *et al.*, 1998; Fig. 1A). Nutrient depletion reduces mTORC1 activity (and relative levels of pS6K and p4E-BP1), suppressing growth of mammalian cells, *Dictyostelium*, and most other eukaryotes. Cell growth can also be inhibited, even in nutrient-rich media, by the immunosupressant drug rapamycin (Bjornsti and Houghton, 2004). In mammalian cells and in *Dictyostelium*, rapamycin functions in complex with the endogenous cellular protein FKBP12 to disrupt mTOR-Raptor interactions (Oshiro *et al.*, 2004; X-HL and ARK, unpublished; Rosel *et al.*, 2012).

Both positive and negative regulatory inputs into mTORC1 are essential to modulate and balance cell proliferation with nutrient availability (Fig. 1A). The Rheb GTPase, a strong activator of mTORC1, is inhibited by the TSC-GAP complex (Huang *et al.*, 2008; Huang and Manning, 2009), with TSC activity subject to inhibition by the AKT kinase or activation by AMPK (Inoki *et al.*, 2003). Both AKT and AMPK have secondary targets, respectively, for activating or inhibiting mTORC1. AKT inhibits PRAS40, whereas AMPK targets Raptor (Gwinn *et al.*, 2008; Shaw, 2009) to suppress mTORC1 activity (Fig. 1A). Underscoring these very essential growth elements, human syndrome mutations causing activation of AKT (Cowden's) or suppression of AMPK (Peutz-Jeghers) or TSC (Tuberous Sclerosis) lead to cellular overgrowth (van Veelen *et al.*, 2011; Hollander *et al.*, 2011). Indeed, activities of both AMPK and AKT are themselves highly regulated.

Nutrient limitation will cause decline in intracellular ATP energy status, thus activating AMPK (Hardie, 2011), which indirectly inhibits mTORC1 (Fig. 1A). Both starvation and rapamycin promote an increase in AMP/ATP ratios in *Dictyostelium* (Jaiswal and Kimmel, 2019), which leads to AMPK activation. Furthermore, activation of AMPK in growth media using pAICAR, an AMP mimic, or 2-deoxyglucose, a glycolytic inhibitor, will inactivate mTORC1 (Jaiswal and Kimmel, 2019). Thus, mTORC1 and AMPK are antagonistic regulators of each other in *Dictyostelium*, reinforcing their activation/inactivation in nutrient response. Although the TSC2/Rheb pathway is present in *Dictyostelium*, its role is far less critical than in mammalian cells (Rosel *et al.*, 2012; Jaiswal and Kimmel, 2019). Indeed, while AMPK is a strong suppressor of mTORC1 in *Dictyostelium*, its main inhibitory action is *via* phosphorylation of Raptor and not TSC2 (Rosel *et al.*, 2012; Jaiswal and Kimmel, 2019; Fig. 1A).

mTORC2 (mechanistic Target of Rapamycin Complex 2) subunits include the TOR kinase, Lst8, Rictor (Pia in *Dictyostelium*; Chen *et al.*, 1997), and Sin1 (RIP3 in *Dictyostelium*; Lee *et al.*, 1999); other regulatory components are present in mammalian cells but not confirmed for *Dictyostelium* (Fig. 1B). AKT proteins are major regulatory phospho-targets of mTORC2 in both systems (Huang *et al.*, 2008; Huang and Manning, 2009; Liao *et al.*, 2010; Kamimura *et al.*, 2010), and in many, but not all, systems, growth factor activation of PI3K is a central controlling element for mTORC1, where PI3K/

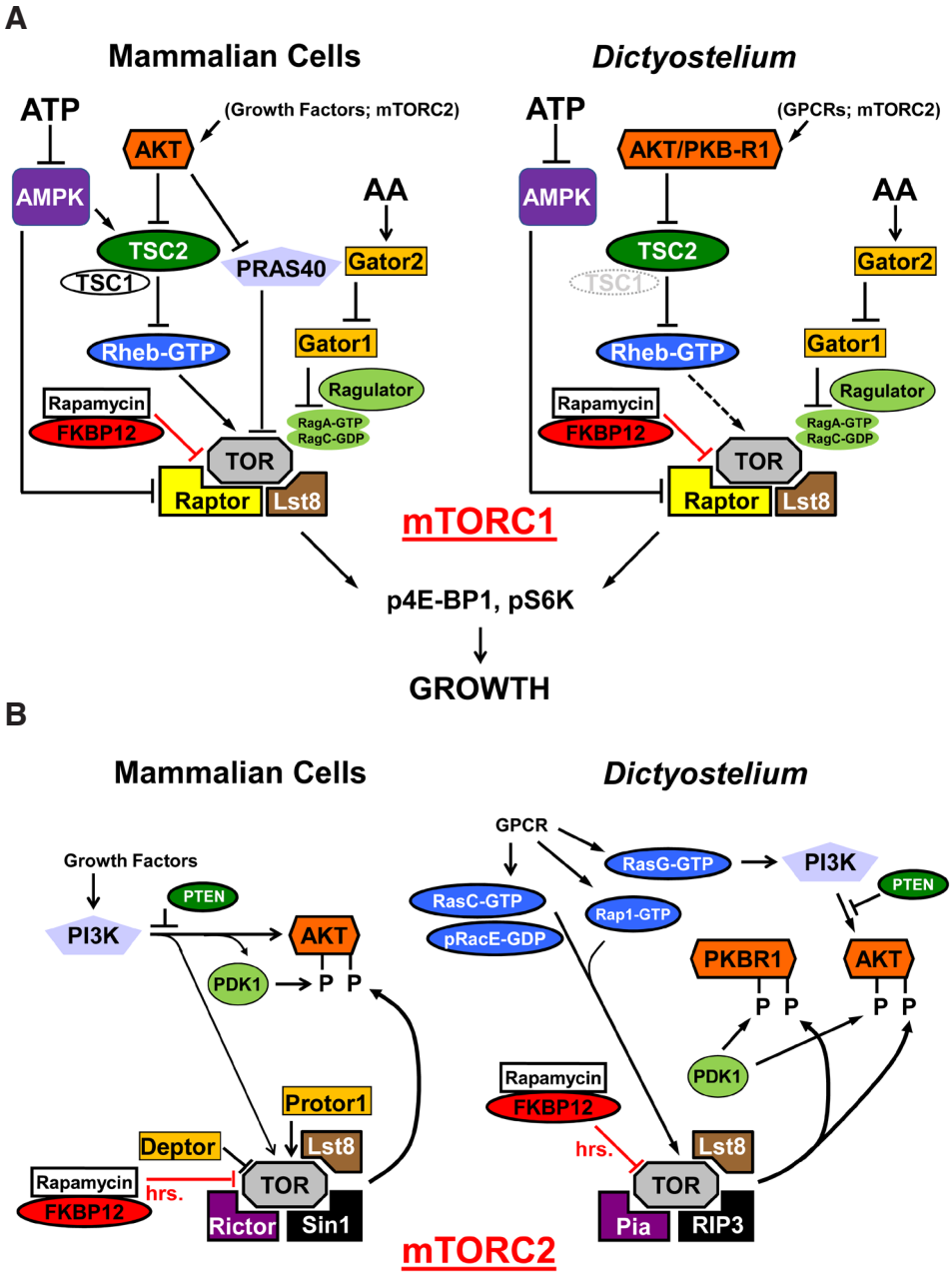
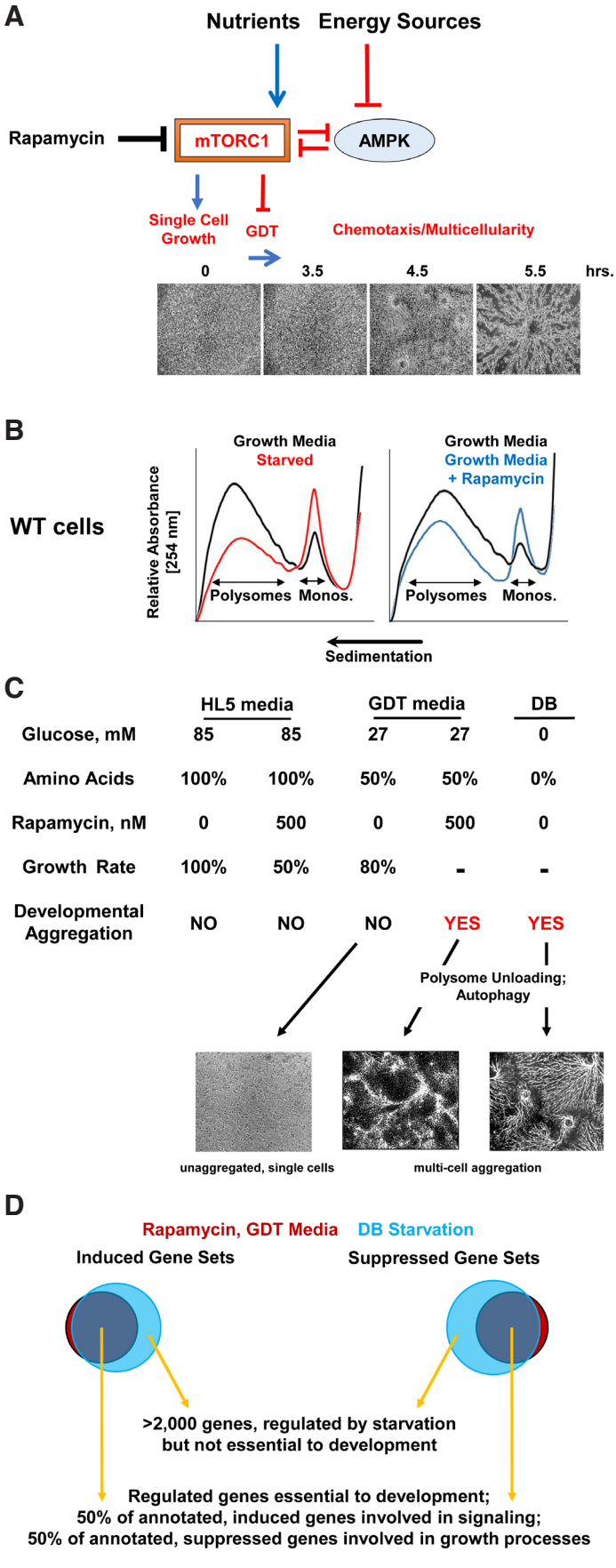


Fig. 1. mTOR complex regulations in mammalian and *Dictyostelium* cells. (A) *The primary proteins of mTORC1 are the mTOR kinase, Raptor, and Lst8. Rich nutrients and energy sources stimulate mTORC1 for phosphorylation of proteins that promote growth. mTORC1 is activated by amino acids through the Gator-Ragulator-RagA/C pathway. Sequence analogs can be found in* Dictyostelium *but they have not been studied functionally. Rheb-GTP is a strong activator of mTORC1 in mammalian cells; growth factor activation of AKT (and PKBR1 in* Dictyostelium*) via PI3K and mTORC2 (see Figures 1B) suppresses Rheb inhibition by TSC2, a Rheb GAP. In* Dictyostelium*, mTORC1 is less dependent on Rheb, where a TSC2/Rheb function in growth is only observed using rapamycin-sensitized cells; a TSC1 component has not been identified in* Dictyostelium*. PRAS40, absent in* Dictyostelium*, is another mTORC1 inhibitor suppressed by AKT. High energy status will also activate mTORC1 by suppressing AMPK, which down-regulates mTORC1 by activating TSC2 and inhibiting Raptor. TSC2 in* Dictyostelium *lacks an AMPK site; AMPK inhibitory function on mTORC1 seems to be through phosphorylation of Raptor. Rapamcyin in complex with the endogenous protein FKBP12 inhibits mTORC1 by rapidly disrupting Raptor interactions in both mammalian cells and* Dictyostelium*. Additional complexity exists including components Deptor, Sestrin, v-ATPase, etc.* **(B)** *The primary proteins of mTORC2 are the mTOR kinase, Lst8, Rictor (Pia in* Dictyostelium*), and Sin1 (RIP3 in* Dictyostelium*). Deptor and Protor1 are reciprocal regulators of mTORC2 in mammalian cells, but have not been identified in* Dictyostelium*. Long-term treatment of mammalian cells and* Dictyostelium *with rapamycin reduces mTORC2 activity. AKT is a major regulatory target of mTORC2. AKT has two primary sites for phosphorylation. The AKT kinase activation domain is phosphorylated by PDK1, while the C-terminal regulatory domain is phosphorylated by mTORC2. Growth factor activation of PI3K promotes the synthesis of PIP_3, which recruits PDK1 and AKT to membranes. Pathway activation in* Dictyostelium *differs. First, there are 2 AKT-like targets of mTORC2, AKT and PKBR1. Although AKT requires PIP_3 for membrane localization, PKBR1 is constitutively associated with membranes, as is PDK1; unlike in mammalian cells, PI3K activity is not required to activate an AKT-like pathway in* Dictyostelium*. Further mTORC2 pathway activations in* Dictyostelium *are through G protein coupled receptors (GPCRs) and several small GTPases, primarily RasC and RacE (similar to RhoA in mammalian cells), but also Rap1, and perhaps Rheb (see Fig. 3).*

PDK1/AKT act upstream of TSC1-2/Rheb and PRAS40 pathways to promote the activity of mTORC1 (Fig. 1A) for cellular growth (Dibble and Cantley, 2015; Janku *et al.*, 2018). Thus, signaling through both mTOR complexes is highly interactive.

AKT has two primary sites for phosphorylation. The AKT kinase activation domain is phosphorylated by PDK1; growth factor activation of PI3K promotes the synthesis of PIP_3, which, in mammalian cells, recruits PDK1 and AKT to membranes. PDK1 phosphorylation is required for AKT activity in mammalian cells (Jacinto *et al.*, 2006). mTORC2 phosphorylates the C-terminal regulatory domain, but unlike the PDK1 site, this phosphorylation determines AKT substrate specificity and is not required for AKT activity in mammalian cells. mTORC2 phosphorylation of AKT is not essential to the TSC1-2/Rheb component, insulating mTORC1 and mTORC2, in mammalian cells, at this level (Jacinto *et al.*, 2006).

Not all mammalian mTOR-associated mechanisms are precisely mirrored in *Dictyostelium* (Rosel *et al.*, 2012; Khanna *et al.*, 2016). First, PDK1 in *Dictyostelium* is regulated independently of PI3K (Liao *et al.*, 2010; Kamimura *et al.*, 2010). In addition, *Dictyostelium* expresses a variant of AKT, PKBR1, that lacks a PIP_3-binding, PH domain, but is anchored to the plasma membrane through myristoylation, and, thus, independently of PI3K/PIP_3 (Meili *et al.*, 2000).



PKBR1 activity requires phosphorylation by PDK1 and mTORC2, but it is fully activatable in cells lacking all PI3K variants (Liao *et al.*, 2010; Kamimura *et al.*, 2010). However, PDK1 phosphorylation of AKT/PKBR1 requires phosphorylation by mTORC2, so absolute AKT/PKBR1 activity is dependent on mTORC2 (Liao *et al.*, 2010). We also note input of several small GTPases in *Dictyostelium* (see below) that function downstream of GPCR signaling with direct positive input to mTORC2 (Khanna *et al.*, 2016; Senoo *et al.*, 2019; Rosel, *et al.,* 2012; Charest *et al.,* 2010; Liao *et al.*, 2013).

Finally, it must be emphasized that in both mammlian cells and in *Dictyostelium*, long-term treatment of cells with rapamycin reduces mTORC2 activity (Sarbassov *et al.*, 2006; Zeng *et al.*, 2007; Rosel *et al.*, 2012; Liao *et al.*, 2010). Nonetheless, although Pia, RIP3, and Lst8 interactions with mTOR are confirmed in *Dictyostelium* (Khanna *et al.*, 2016; Senoo *et al.*, 2019; X-HL and ARK, unpublished), none are immediately sensitive to disruption by rapamycin (X-HL and ARK, unpublished).

mTORC1 regulates the growth-to-development transition

Dictyostelium development is induced upon nutrient withdrawal, which co-ordinately suppresses mTORC1 and activates AMPK (Fig. 2A). Reciprocal actions of mTORC1/AMPK have been suggested to create an essential regulatory switch for developmental induction (Jaiswal and Kimmel, 2019).

During rapid growth of *Dictyostelium*, ~80% of ribosomes are polysomal (Alton and Lodish, 1977). One of the first responses to nutrient withdrawal in *Dictyostelium* is the reduction in polysome density (Alton and Lodish, 1977), where starvation-induced polysome unloading prepares cells for the preferential re-loading of newly synthesized, developmentallly essential mRNAs. Polysome unloading is easily observed in isokinetic sucrose gradients as a large increase in monosome density upon nutrient withdrawal or rapamycin treatment of cells in growth media (Fig. 2B).

Rapamycin treatment of *Dictyostelium* cells in full nutrient media reduces cell growth by only 50%, without inducing development (Rosel *et al.*, 2012; Jaiswal and Kimmel, 2019; Fig. 2C). These media conditions for glucose and amino acids far exceed concentrations in other systems, where it had been previously noted that enhanced nutrient stimulation could partially bypass full inhibitory effects of rapamycin (Kang *et al.*, 2013; Yoon and Roux, 2013).

Fig. 2. Rapamycin inhibition of mTORC1 in the presence of nutrients is sufficient to promote developmental aggregation. (A) *Rich nutrients and energy sources drive mTORC1 activation and promote cell growth. Withdrawal of nutrients by starvation inhibits mTORC1, activates AMPK, and induces Growth-to-Development Transition, GDT. Rapamycin inhibition of mTORC1 in the presence of rich nutrient and energy sources also induces GDT.* **(B)** *In growing WT cells, a majority of ribosomes are loaded on polysomes. A large percentage of these polysomes become unloaded in WT cells inhibited for mTORC1, either by removal of nutrients (starvation) or by treatment with rapamycin in growth media.* **(C)** *WT cells were grown in media as indicated and plated for development, with or without rapamycin. While slight media dilution has minimal effect on growth rate, addition of rapamycin induces multi-cell aggregation, as effectively as by starvation.* **(D)** *Changes in developmentally-induced mRNA transcriptome patterns compared between DB-starved cells or rapamycin-treated cells in GDT media (see Figure 5C). ~50% of starvation-regulated genes are unaffected by rapamycin, suggesting their regulations are not essential to development. Significantly, the rapamycin-regulated genes are significantly classified with GO terms associated with developmental processes and growth suppression.*

When nutrients are slightly titrated (*i.e.* GDT media; Fig. 2C) there is only limited impact to growth rate (Jaiswal and Kimmel, 2019; Fig. 2C). Cells in GDT media grew at an 80% rate compared to growth in full nutrients, but rapidly ceased growth and entered development (with induced autophagy) upon addition of rapamycin (Jaiswal and Kimmel, 2019; PJ and ARK, unpublished; Fig. 2C). Kinetics for developmental aggregation was similar to that of cells in starvation (DB) conditions (Fig. 2C). Thus, mTORC1/AMPK responses present a regulatory switch between growth and development in *Dictyostelium* (Jaiswal and Kimmel, 2019).

Under standard laboratory conditions for development, cells are transferred immediately from nutrient media to 10 mM phosphate (DB starvation) buffer. Within the first hour of response, >4,000 genes exhibit expression changes (Parikh *et al.*, 2010; Rosengarten *et al.*, 2015), which have often been presumed to be markers of developmental progression. However, apart from the obvious absence of nutrient support in starvation buffer, there may be an accompanying response upon exposure to altered extracellular component concentrations. We hypothesize that many of these starvation-induced transciptome effects may not be related to development *per se*, but are secondary to the media shift. To identify developmentally essential gene classes, RNA-seq was performed to compare transcriptome differences between rapamycin/media-developed cells and starvation-developed cells (Jaiswal and Kimmel, 2019).

Although both the starved and rapamycin-treated population sets aggregate similarly, only ~50% of starvation-regulated genes are similarly affected by rapamycin (Jaiswal and Kimmel, 2019; Fig. 2D). Of these, Gene Ontology (GO) analyses show significant bias for growth, in the suppressed set, and for developmental signaling (including autophagy), in the induced set (Jaiswal and Kimmel, 2019; Fig. 2D). The developmental genes associate stongly with the cAMP-signal/response network and include CAR1, Gα2, ACA, PKA, and GtaC (McMains *et al.*, 2008; Devreotes *et al.*, 2013; Jaiswal and Kimmel, 2019). As example, 5 unclassified genes of the induced class were randomly selected for study as cell mutants and none were able to develop under standard conditions (Jaiswal and Kimmel, 2019). In contrast, the starvation-only genes do not show GO enrichment for growth or signaling pathways, and none of 10 random, unclassified genes of the starvation-only induced class were essential for development (Jaiswal and Kimmel, 2019).

mTORC1/AMPK signaling also regulates a developmentally essential pathway involving protein kinase YakA (Jaiswal and Kimmel, 2019; Fig. 3). YakA appears inactive in growing cells, through inhibition by PufA, but is required during starvation to induce PKA and consequently Adenylyl Cyclase A, ACA (Souza et. al., 1998,1999); *yakA*-nulls will not develop, and *pufA*-nulls develop precociously. Both starvation and rapamycin-media treatment lead to the phospho-activation of YakA (Jaiswal and Kimmel, 2019), providing a mechanistic bypass to nutrient withdrawal (Fig. 3). Although additional studies are required, regulation of GDT by mTORC1/AMPK and their immediate genes targets may be strongly dependent upon activated pYakA.

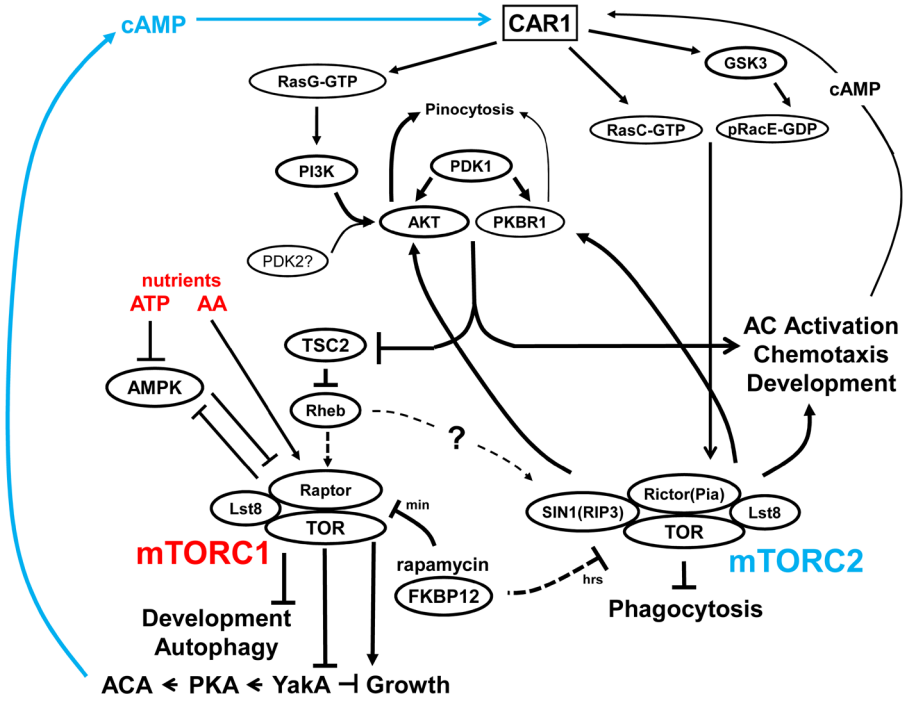


Fig. 3. Interactive components of mTORC1 and mTORC2 in control of *Dictyostelium* growth and development. *Pathway model for interaction of mTORC1 and mTORC2 with differing effects to growth and development. mTORC1 (see Figure1A for component details) is a primary regulator of growth and inhibitor of development and autophagy; nutrients activate mTORC1 directly and indirectly, through inhibition of AMPK. Inhibition of mTORC1 (and activation of AMPK) by starvation or by rapamycin can suppress growth and induce development even in the presence nutrients. One major target, activated downstream of mTORC1 inhibition, by either starvation or rapamycin, is kinase YakA, an inhibitor of growth, but a developmental activator. YakA promotes accumulation of ACA (Adenylyl Cyclase A), with a consequent increase in and secretion of cAMP. mTORC2 (see Figure1B for component details) is a primary regulator of multi-cell formation (*e.g.* *chemotaxis, AC activation) and development, but can suppress certain growth aspects (*e.g.* *phagocytosis). cAMP stimulation of the GPCR cAMP receptor CAR1 rapidly activates mTORC2, with read-out phosphorylations of AKT and PKBR1. This pathway is mediated by several small GTPases, most notably RasC-GTP and pRacE-GDP; Rap1 has an additional positive input (see Figure 1B), as may Rheb-GTP. TSC2 negatively regulates Rheb, which functions during development to regulate phagocytosis and AC in a manner that is dependent upon mTORC2, but is independent of mTORC1.*

The third half: small GTPase control of mTORC2, including Rheb function-dependence upon mTORC2, and not mTORC1

In *Dictyostelium*, the primary pathway for mTORC2 activation is through GPCRs. Most mechanistic understandings of mTORC2 activation have derived from studies of developed cells in response to cAMP (Lee *et al.*, 2005; Charest *et al.*, 2010; Fig. 3), although in growing cells folate receptors also couple to mTORC2 signaling (Liao *et al.*, 2013). CAR1 signaling through Gα2-β/γ rapidly activates multiple Ras GTPases. RasC-GTP (Cai *et al.*, 2010; Charest *et al.*, 2010; Liao *et al.*, 2013) is linked to mTORC2, whereas RasG-GTP is linked to the PI3Ks (Fig. 3). Several other kinases are also acti-

vated by CAR1, including GSK3 (Kim *et al*., 1999). GSK3 has been previously shown to regulate chemotaxis (Kim *et al*., 2011; Kölsch *et al*., 2013), and a new study now links GSK3 effects to mTORC2 (Senoo *et al*., 2019). They show that GSK3-phosphorylated RacE-GDP plays a critical role for RasC activation of mTORC2 (Fig. 3); non-phosphorylated RacE or GTP-loaded RacE are inactive in this function (Senoo *et al*., 2019). Rap1-GTP (Khanna *et al*., 2016) also contributes to mTORC2 activation (see Fig. 1B).

We additionally suggest that Rheb (Rosel *et al*., 2012) is another small GTPase activator of mTORC2 (Fig. 3). mTORC2 is a negative regulator of phagocytosis and TSC2/Rheb interacts with mTORC2 to control phagocytosis (Fig. 3), independently of mTORC1 (Rosel *et al*., 2012). We would suggest that Rheb-GTP may positively regulate mTORC2 for additional dependent functions. In *Dictyostelium*, mTORC2 components Pia, Lst8, and RIP3 are required for cAMP activation of ACA (Lee *et al*., 2005), whereas mTORC1 is not (Jaiswal and Kimmel, 2019), and indeed, a series of epistases experiments have shown that Rheb serves as a positive regulator of AC activation in a manner that is fully dependent upon mTORC2 components Pia, RIP3, and Lst8, but not mTORC1 (DR, TK, and ARK, unpublished). Taken together, data indicate that Rheb-GTP may act upstream of both mTORC1 and mTORC2, but for distinct cellular responses (Fig. 3).

Perspectives: integrated pathways

The mTOR complexes 1 and 2 show highly interactive patterning for the *Dictyostelium* life cycle (Fig. 3). mTORC1 senses nutrients and energy status for cell proliferation, while also suppressing multi-cell development and autophagic degradation of cellular components (Jaiswal and Kimmel, 2019). mTORC1 actvity in *Dictyostelium* has a more minimal dependency on the Rheb pathway than in mammalian cells. However, the strong and rapid antagonistic interplay between mTORC1 and AMPK provides a strong reinforcing mechanism for growth response to rapid nutrient fluctuations. Inactivation of mTORC1, coupled with the activation of AMPK, by starvation or rapamycin, appears to be an essential regulatory switch from growth to development, and mediated through activation of YakA. pYakA provides context for ACA upregulation and mTORC2 activation, in the transitional switch from growth to development.

Activated mTORC2 is central to developmental pathways. mTORC2 interacting with AKT/PKBR1 regulates ACA activation, chemotaxis, and aggregation, but also suppresses large particle nutrient capture, phagocytosis, involving cross-talk with the Rheb pathway. Potentially, mTORC2 activation may promote the Rheb circuit and further signaling through mTORC2. We stress that many essential components (*e.g*. GEFs, GAPs) involving positive, negative, scaffold, feed-forward, and feed-back regulatory inputs could not additionally be included or fully discussed (Charest *et al*., 2010; Liao *et al*., 2013; Devretoes *et al*., 2013).

We have suggested strong cooperative and dependent effects among the mTOR complexes for the *Dictyostelium* life cycle. mTORC1 inhibition directs fate choice for GDT based upon nutrient balance for activity, whereas mTORC2 integrates cell-cell signaling to promote *Dictyostelium* development.

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