Review

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Regulation of mTORC1 by PI3K signaling

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The class I phosphoinositide 3-kinase (PI3K)–mechanistic target of rapamycin (mTOR) complex 1 (mTORC1) signaling network directs cellular metabolism and growth. Activation of mTORC1 [composed of mTOR, regulatory-associated protein of mTOR (Raptor), mammalian lethal with SEC13 protein 8 (mLST8), 40-kDa proline-rich Akt substrate (PRAS40), and DEP domain-containing mTOR-interacting protein (DEPTOR)] depends on the Ras-related GTPases (Rags) and Ras homolog enriched in brain (Rheb) GTPase and requires signals from amino acids, glucose, oxygen, energy (ATP), and growth factors (including cytokines and hormones such as insulin). Here we discuss the signal transduction mechanisms through which growth factor-responsive PI3K signaling activates mTORC1. We focus on how PI3K-dependent activation of Akt and spatial regulation of the tuberous sclerosis complex (TSC) complex (TSC complex) [composed of TSC1, TSC2, and Tre2–Bub2–Cdc16-1 domain family member 7 (TBC1D7)] switches on Rheb at the lysosome, where mTORC1 is activated. Integration of PI3K- and amino acid-dependent signals upstream of mTORC1 at the lysosome is detailed in a working model. A coherent understanding of the PI3K–mTORC1 network is imperative as its dysregulation has been implicated in diverse pathologies including cancer, diabetes, autism, and aging.

Introduction to PI3K and mTORC1 signaling

Signaling networks endow cells with the ability to sense their internal and external environment in an integrated manner and mount coordinated responses involving processes such as growth, proliferation, survival, and differentiation. Cells of multicellular organisms must simultaneously take into account intracellular levels of nutrients and stress, cell–cell contacts, and organismal metabolism and stress conditions. The relay of such information between cells and tissues is mediated in part by secreted ligands including growth factors, hormones, cytokines, and chemokines (referred to here as growth factors for simplicity) that bind receptor tyrosine kinases (RTKs) or G-protein-coupled receptors (GPCRs) at the cell surface and thereby trigger intracellular signaling. One of the key signaling enzymes proximally activated by these receptors is the lipid kinase PI3K [1,2]. Here we exclusively discuss signaling downstream of class I and not class II or III PI3Ks, which are distinct in their structures, regulation, and functions [3]. PI3K, which functions as a heterodimer of catalytic and regulatory subunits, phosphorylates the inositol ring of the membrane phospholipid phosphatidylinositol-4,5-bisphosphate (PI-4,5-P_2) to generate phosphatidylinositol-3,4,5-trisphosphate (PIP_3) at the cytoplasmic face of the plasma membrane [3]. This activity of PI3K is counteracted by phosphatase and tensin homolog deleted on chromosome 10 (PTEN), which converts PIP_3 back to PI-4,5-P_2, and inositol-5-phosphatases including SH2 domain-containing inositol phosphatase (SHIP), which converts PIP_3 to PI-3,4-P_2 [4]. Due to the activity of inositol-5-phosphatases, stimulation of PI3K can also result in the acute production of PI-3,4-P_2. PI3K-generated PIP_3 precipitates signaling cascades by recruiting a subset of pleckstrin homology (PH) domain-containing signaling proteins, such as the protein kinase Akt, to the plasma membrane through a specific interaction between PIP_3 and the PH domain [4]. PI-3,4-P_2 can also robustly bind a subset of PH-domain proteins including Akt and therefore can contribute to PI3K signaling in some contexts [4]. The various upstream events that can lead to activation of PI3K and its effectors are discussed in more detail in several recent reviews [1–4]. Through Akt and its other downstream effectors, PI3K functions as a major regulator of cellular metabolism, survival, growth, proliferation, and motility, and dysregulation of signaling downstream of PI3K has been implicated in numerous and diverse human pathologies [2,5].

One of the key regulators of metabolism and growth activated downstream of PI3K is the protein kinase complex mTORC1. The kinase mTOR functions within two distinct multiprotein complexes designated mTORC1 and mTORC2 that differ in their subunit composition, upstream inputs, and downstream substrates and functions [5,6]. Here we focus on the regulation of mTORC1, which is composed of the core subunits mTOR, Raptor, and mLST8, plus two endogenous inhibitors of the complex, PRAS40 and DEPTOR [5,6]. Through transcriptional, translational, and post-translational mechanisms mediated by its substrates, including ribosomal protein S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein (4E-BP), mTORC1 stimulates the biosynthesis of three major classes of macromolecules: proteins, lipids, and nucleic acids [7]. In addition, mTORC1 can promote the production of energy (ATP), reducing cofactors (NAPDH), and certain macromolecule precursors required for biosynthesis [7]. Finally, mTORC1 signaling inhibits

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the breakdown of lipids via lipolysis and β-oxidation and the bulk degradation of cytoplasmic constituents via autophagy, presumably to prevent futile cycles of synthesis and degradation [8,9]. Cumulatively, this promotion of anabolic activity underlies the effects on cell, tissue, and organismal growth for which the pathway is best known. This central node of metabolic control is intimately linked to pathways that sense secreted growth factors as well as the cellular abundance of amino acids, glucose, oxygen, and energy, which are concomitantly required for full activation of mTORC1 [10]. Growth factors and amino acids have an especially acute effect on mTORC1 activation and it has come to be appreciated that these two inputs act through parallel, largely independent pathways [11–13]. We discuss the molecular events downstream of PI3K that lead to activation of mTORC1, with emphasis on how growth factor-stimulated PI3K signaling and amino acid signaling are integrated at the lysosome, where mTORC1 is activated.

The primary pathway from PI3K to mTORC1: switching on Rheb

A little more than a decade ago, the PI3K–Akt pathway and mTOR pathway were both known to be important, growth factor-sensitive regulators of protein synthesis and cell growth, but whether they functioned within a linear pathway or in parallel remained an unresolved question [14]. Genetic and biochemical studies unified these pathways through identification of two missing links between Akt and mTORC1: the small GTPase Rheb and its negative regulator, the TSC complex [15–32]. These and subsequent studies have shown that regulation of a switch involving Akt, the TSC complex, and Rheb is the primary mechanism through which PI3K signaling activates mTORC1 (Figure 1).

Direct activation of mTORC1 by Rheb

Rheb, which is essential for development in both flies and mice, is a potent activator of mTORC1 [25,33,34]. Two Rheb family members, Rheb1 and Rheb2 (also known as RhebL1), are found in mammals, and although Rheb1 is the essential isoform in mice and appears to be the dominant regulator of mTORC1, both isoforms are ubiquitously expressed [35,36] and can activate mTORC1 [37–39]. By contrast, Rheb does not stimulate mTORC2 kinase activity *in vitro* or signaling *in vivo* [39–41]. Active Rheb can indirectly inhibit PI3K and mTORC2 signaling by inducing various mTORC1-dependent negative feedback loops [1,42]. Like all GTPases, Rheb cycles between GTP- and GDP-bound states that differ in conformation and function. GTP-bound Rheb, but not GDP-bound Rheb, robustly stimulates mTORC1 activity [39,43] through what is likely to be a direct interaction with the kinase domain of mTOR, mLST8, and perhaps Raptor [39,43–45]. This role appears to be unique to Rheb among small GTPases [28,31]. If recombinant Rheb is purified from bacteria [39] or mammalian cells [43,45] and loaded with GTP, its subsequent addition to *in vitro* mTORC1 kinase assays is sufficient to stimulate mTORC1 activity toward its physiological substrates. Likewise, mTORC1 exhibits *in vitro* kinase activity only if copurified from cells with Rheb mutants that are highly GTP bound, but not those that are nucleotide deficient [44]. Because of the unique role played by Rheb, it is required for activation of mTORC1 in response to both amino acids and growth factors [13].

The evidence that Rheb directly activates mTORC1 is quite strong, but two indirect mechanisms have also been suggested. First, active Rheb has been proposed to competitively bind a putative endogenous inhibitor of mTORC1 known as FK506-binding protein 38 (FKBP38), thereby relieving an inhibitory interaction between FKBP38 and the FKBP12–rapamycin binding (FRB)

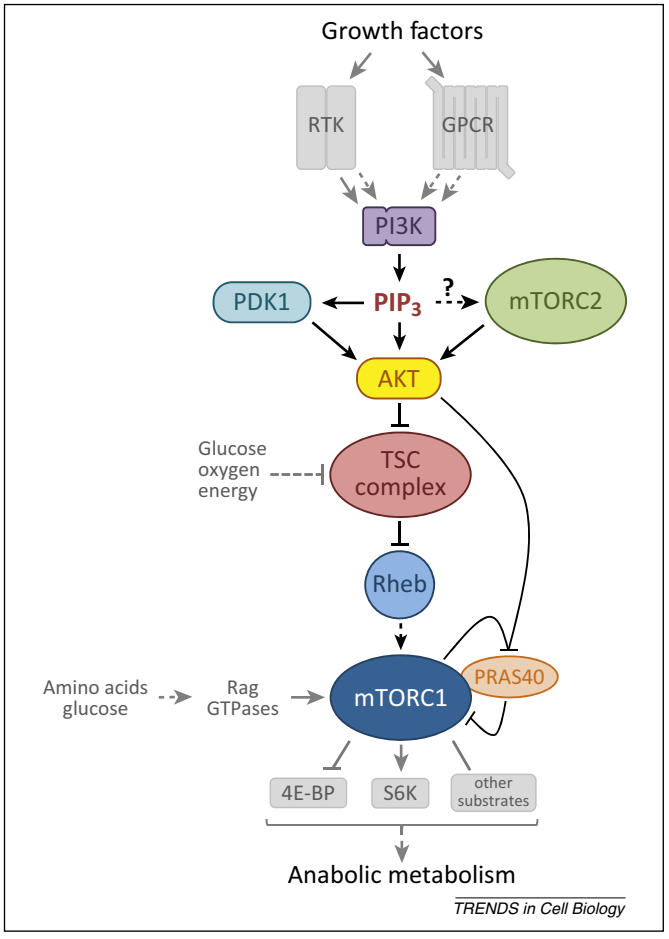

Figure 1. The pathways through which class I phosphoinositide 3-kinase (PI3K) activates mechanistic target of rapamycin complex 1 (mTORC1). Growth factors (including hormones, cytokines, and chemokines) activate receptor tyrosine kinases (RTKs) or G-protein-coupled receptors (GPCRs), which, through various mechanisms, activate PI3K. PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP_3), which specifically binds Akt and 3-phosphoinositide-dependent kinase 1 (PDK1) promoting the phosphorylation and activation of Akt by PDK1. Phosphorylation of Akt by mTORC2 boosts its activity several-fold and mTORC2 activation is at least partially PI3K dependent. Akt inhibits the tuberous sclerosis complex (TSC) complex (TSC complex), the specific GTPase activating protein (GAP) for the small GTPase Ras homolog enriched in brain (Rheb), through multisite phosphorylation of the TSC2 subunit. This relieves inhibition of Rheb, allowing it to become activated and stimulate mTORC1 kinase activity. Once mTORC1 is activated by Rheb, the simultaneous phosphorylation of its inhibitory subunit 40-kDa proline-rich Akt substrate (PRAS40) by Akt and mTORC1 itself causes PRAS40 to dissociate from mTORC1. This is thought to increase substrate access to the complex. Glucose, oxygen, and energy levels are also sensed upstream of the TSC complex. Amino acids (and glucose) are sensed upstream of mTORC1 via pathways that regulate the Rag GTPases, which do not directly activate mTORC1 but serve to bring it in proximity to Rheb in cells. mTORC1 directly phosphorylates numerous substrates including ribosomal protein S6 kinase (S6K) and eukaryotic translation initiation factor 4E-binding protein (4E-BP), which mediate its control of anabolic metabolism, cellular growth, and proliferation.