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G-Protein Coupled Receptor Signaling and Mammalian Target of Rapamycin Complex 1 Regulation

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ABSTRACT

The mammalian target of rapamycin (mTOR) senses upstream stimuli to regulate numerous cellular functions such as metabolism, growth, and autophagy. Increased activation of mTOR complex 1 (mTORC1) is typically observed in human disease and continues to be an important therapeutic target. Understanding the upstream regulators of mTORC1 will provide a crucial link in targeting hyperactivated mTORC1 in human disease. In this mini-review, we will discuss the regulation of mTORC1 by

upstream stimuli, with a specific focus on G-protein coupled receptor signaling to mTORC1.

SIGNIFICANCE STATEMENT

mTORC1 is a master regulator of many cellular processes and is often hyperactivated in human disease. Therefore, understanding the molecular underpinnings of G-protein coupled receptor signaling to mTORC1 will undoubtedly be beneficial for human disease.

Introduction

Over the span of 20 years since the mammalian target of rapamycin (mTOR) was discovered, numerous findings have highlighted the importance of mTOR as the central node in a network of signaling pathways that control cell growth, metabolism, and autophagy (Brown et al., 1994; Sabatini et al., 1994; Sabers et al., 1995). mTOR was discovered following the identification of its inhibitor, rapamycin, which was thought to be an antifungal and immunosuppressant agent. Genetic screens in yeast by several groups found that the prolyl-isomerase FK506-binding protein 12 and rapamycin form a complex and inhibit mTOR (Heitman et al., 1991; Stan et al., 1994; Zheng et al., 1995). Aberrant mTOR activation has been implicated in several human pathologies. mTOR dysregulation can result in the overgrowth of cancers

and pathologies associated with aging and metabolic disease. Rapamycin and analogs of rapamycin (rapalogs) are currently used to treat mTORC1 hyperactivated diseases in the clinic (Tian et al., 2019). The complete mechanistic insight of mTORC1 signaling is still being elucidated, and a new generation of drugs are being investigated to better target mTOR (Popova and Jücker, 2021). mTORC1 activation occurs in response to different extracellular and intracellular stimuli. This mini-review will provide a brief overview of mTORC1 and discuss the regulation of mTORC1 by upstream stimuli. We will specifically focus on G protein-coupled receptors (GPCRs) with respect to mTORC1 regulation and the recent advances in the field.

mTORC1

The evolutionarily conserved Ser/Thr protein kinase mTOR belongs to the phosphatidylinositol 3 kinase-related kinase family (Hay and Sonenberg, 2004). mTOR is the catalytic component of two distinct protein complexes referred to as mTORC1 and mTORC2. This mini-review will not discuss mTORC2; refer to Liu and Sabatini, 2020. There are three

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ABBREVIATIONS: AC, adenylate cyclase; AKAP, A-kinase anchoring protein; AKT, PI3K-Rac- α Ser/Thr-protein kinase; ArfGAP1, ADP-ribosylation factor GTPase-activating protein 1; ERK, extracellular signal-regulated kinase; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GPCR, G-protein coupled receptor; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; PDE, phosphodiesterase; PKA, protein kinase A; Raptor, regulatory protein associated with mTOR; Rheb, Ras homolog enriched in brain; S6K1, p70 ribosomal S6 kinase 1; TSC, tuberous sclerosis complex..

main core components of mTORC1: mTOR, regulatory protein associated with mTOR (Raptor), and mammalian lethal with Sec13 protein 8 (Hara et al., 2002; Kim et al., 2002; Kim et al., 2003). The Raptor subunit of mTORC1 facilitates mTOR substrate recognition and phosphorylation (Nojima et al., 2003; Schalm et al., 2003). mTORC1 activity is positively regulated by mammalian lethal with SEC13 protein 8. Additional research in the future may identify new mTORC1 components. mTORC1 controls cellular events like translation, growth factor signaling, autophagy, lysosome biogenesis, and lipid synthesis (Melick and Jewell, 2020; Saxton and Sabatini, 2017). Protein translation is regulated by mTORC1 mainly through phosphorylation of mTORC1 substrates like p70 ribosomal S6 kinase 1 (S6K1) and eIF4E-binding protein 1 (4EBP1). Similarly, growth factor signaling and autophagy are regulated by mTORC1 through the phosphorylation of mTORC1 substrates growth factor receptor-bound protein 10 and Unc-51 like autophagy activating kinase 1, respectively. Lysosomes are important for cellular recycling and function as the central hub for mTORC1 signaling. mTORC1 also supports lysosomal biogenesis through phosphorylation of transcription factor EB. Furthermore, mTORC1 regulates lipid synthesis through Lipin1 and S6K1 phosphorylation, and positively regulates sterol-responsive element-binding protein. Among the different substrates of mTORC1, the phosphorylation sites on S6K1 (Thr 389), 4EBP1 (Ser 65, Thr 37, and Thr46), and Unc-51 like autophagy activating kinase 1 (ULK1) (Ser 758) are commonly accepted as markers of mTORC1 activity. Other reviews have discussed mTORC1 substrates and downstream signaling in more detail (Liu and Sabatini, 2020; Saxton and Sabatini, 2017).

Upstream Stimuli that Regulate mTORC1 Activity

Growth Factor Signaling. mTORC1 is regulated by several growth factors (Fig. 1). Growth factor signaling converges on tuberous sclerosis complex (TSC), which inhibits small G-protein Ras homolog enriched in brain (Rheb) (Inoki et al., 2003a; Tee et al., 2003). TSC is a GTPase-activating protein (GAP) and interacts with Rheb to hydrolyze Rheb-GTP (active state) to Rheb-GDP (inactive state). Rheb binds to and allosterically activates mTORC1 kinase activity via a conformational change (Long et al., 2005; Yang et al., 2017). Growth factors signaling through PI3K-Rac-alpha Ser/Threonine kinase (AKT) (Potter et al., 2002), extracellular signal-regulated kinase (ERK) (Ma et al., 2005), p90 ribosomal S6 kinase 1 (Roux et al., 2004), I κ B kinase β (Lee et al., 2007), or mitogen-activated protein kinase 2 (Li et al., 2003) phosphorylate and inhibit TSC2, resulting in mTORC1 activation. Low energy status, hypoxia, and DNA damage enhance the TSC GAP to inhibit mTORC1 (Liu and Sabatini, 2020). Hypoxia leads to TSC2 activation through DNA damage and development 1 (Brugarolas et al., 2004; DeYoung et al., 2008). Similarly, nutrient deprivation can trigger AMP-activated protein kinase to phosphorylate and activate the GAP activity of TSC (Corradetti et al., 2004; Inoki et al., 2003b). DNA damage induces phosphorylation of tumor suppressor protein 53 and leads to an AMP-activated protein kinase-dependent TSC activation, resulting in mTORC1 inhibition (Feng et al., 2007).

Amino Acid Signaling. Amino acids promote mTORC1 lysosomal localization and its subsequent activation (Rogala et al., 2019; Sancak et al., 2010; Sancak et al., 2008). Amino acid regulation of mTORC1 does not rely on TSC signaling. Multiple groups have shown that amino acid and growth factors are two distinct pathways in terms of mTORC1 activation (Demetriades et al., 2014; Hara et al., 1998; Menon et al., 2014; Sancak et al., 2008; Smith et al., 2005a). In TSC knockout cells, amino acid starvation still inhibits mTORC1 (Smith et al., 2005a). The discovery of Rag GTPases coupled to mTORC1 at the lysosome has shed some important insight into amino acid signaling to mTORC1 (Kim et al., 2008; Sancak et al., 2008). The Rag GTPase family consists of RagA, RagB, RagC, and RagD (Sekiguchi et al., 2001). Four possible distinct complexes can occur, where RagA or RagB can heterodimerize with either RagC or RagD. Amino acid promotes RagA/B loading with GTP, RagC/D loading with GDP, and Rag-mTORC1 binding on the lysosomal surface. Rheb GTPase associates and directly activates mTORC1 (Long et al., 2005). The Ragulator anchors the Rag GTPase-mTORC1 complex to the surface of the lysosome leading to mTORC1 activation (Sancak et al., 2010). Recently, a Rag GTPase-independent pathway was identified, where Asn and Gln signal to mTORC1. In this Rag GTPase-independent pathway, it was found that the vacuolar ATPase, lysosomal function, and a small GTPase called ADP-ribosylation factor 1 are required for the activation of mTORC1 (Jewell et al., 2015). Additional components involved in this pathway are yet to be discovered. Our recent study found that mTORC1 is activated by 10 out of 20 standard amino acids, at different concentrations and time frames (Meng et al., 2020). Met, His, Arg, Ala, Leu, Thr, Val, and Ser activated mTORC1 through the Rag-dependent pathway (Fig. 2A), whereas Asn and Gln work through a Rag-independent pathway (Fig. 2B). Cytosolic arginine sensor for mTORC1 (CASTOR1), solute carrier family 38 member 9 (SLC38A9), Sestrin2 (SESN2), and S-adenosylmethionine sensor upstream of mTORC1 (SAMTOR) are the identified sensors for Rag-dependent pathway (Liu and Sabatini, 2020). Sensors for amino acids such as Ala, His, Ser, Thr, and Val have not yet been identified. Further details on amino acid sensors can be found in other reviews (Kim and Guan, 2019; Liu and Sabatini, 2020). The subcellular location or interacting proteins of mTORC1 under amino acid-deficient conditions are not completely understood. We recently identified the ADP-ribosylation factor GTPase-activating protein 1 (ArfGAP1) as a crucial regulator of mTORC1 (Meng et al., 2021). ArfGAP1 interacts with mTORC1 in the absence of amino acids and inhibits mTORC1 lysosomal localization and activation. Interestingly, ArfGAP1 represses mTORC1 lysosomal recruitment and cell growth independently of its GTPase-activating function. As lysosomal localization is critical for mTORC1 activation, future studies on understanding where mTORC1 is localized when amino acids are not present will be crucial. Also, understanding what factors regulate the trafficking of mTORC1 to the lysosome will be beneficial.

GPCR-G α_s Signaling. GPCRs are seven transmembrane domain receptors that make up the largest family of membrane proteins. Classically, after a relevant ligand or agonist binds to a GPCR, the activation of downstream GPCR signaling cascades is initiated (Hilger et al., 2018). GPCRs are coupled with G-proteins α , β , and γ . GDP-bound G α and G $\beta\gamma$ are

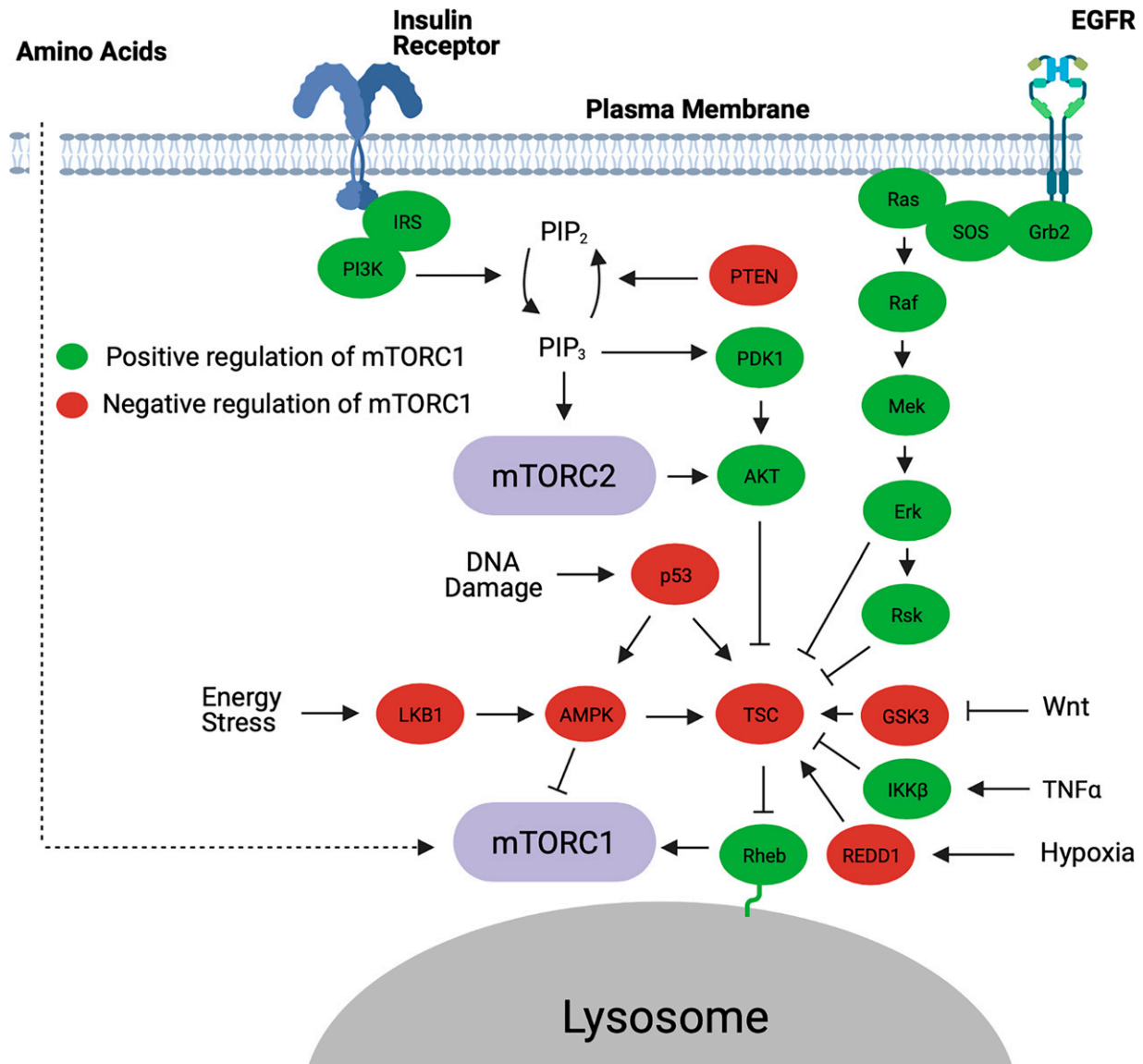


Fig. 1. mTORC1 regulation by upstream stimuli. All positive regulators (green) and negative regulators (red) mTORC1 converge on TSC regulation. Growth factors control the binding of insulin receptor substrate proteins, which activate phosphoinositide 3-kinase (PI3K) and lead to phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP₂) to phosphatidylinositol 3,4,5-triphosphate (PIP₃). Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) negatively regulates this activation. PIP₃ subsequently activates phosphoinositide-dependent kinase 1/2 (PDK1/2), which in turn activates plasma membrane-localized AKT. mTORC2 can also phosphorylate AKT. AKT activates mTORC1 through phosphorylation of TSC, which activates Rheb. TSC is inhibited through ERK by the Ras-Raf-Mek-Erk signaling cascade. Tumor necrosis factor α (TNF α) and hypoxia secondarily regulate mTORC1 through I κ B kinase β (IKK β) and DNA damage response 1 (REDD1), respectively. Wingless-type (Wnt) signaling inhibits TSC by inhibiting glycogen synthase kinase 3 (GSK3). Cellular stress and DNA damage trigger AMP-activated protein kinase activation through p53 and liver kinase B1 (LKB1), respectively, to inhibit mTORC1.

attached to the plasma membrane in the absence of a ligand (Li et al., 2002). However, when a ligand/agonist binds to the GPCR, it is activated and acts as a guanine nucleotide exchange factor (GEF) promoting G α GTP-bound to dissociate from the G $\beta\gamma$ complex (Fig. 3). The intrinsic GTPase activity of G α hydrolyzes GTP, after which G α reassociates with the G $\beta\gamma$ dimer until the next round of activation (Hanlon and Andrew, 2015; Vogler et al., 2008). G $\beta\gamma$ dimer recruits GPCR kinases as a negative feedback loop to phosphorylate and subsequently inhibit it. The phosphorylation leads to the GPCR receptor internalization following β -arrestin binding. Traditionally, GPCR signaling was thought to take place at the cell surface, which then leads to receptor endocytosis (Mohan et al., 2012), but intracellular GPCR signaling also

exists (Eichel and von Zastrow, 2018). G α consists of four different protein families: G α_s , G $\alpha_{i/o}$, G $\alpha_{q/11}$, and G $\alpha_{12/13}$ (Wettshureck and Offermanns, 2005). Among them, G α_s and G $\alpha_{i/o}$ regulate adenylate cyclase (AC), where G α_s activates AC and G $\alpha_{i/o}$ inhibits AC. The GTP-bound G α_s binds to and activates AC, which catalyzes the conversion of ATP to cAMP, ultimately increasing the intracellular cAMP levels (Sassone-Corsi, 2012). In mammals, there are nine different isoforms of AC (AC1–9) and a soluble AC. AC1 and AC8 are tissue-specific to neuronal cells, whereas AC5 is tissue-specific to heart and striatum (Defer et al., 2000). G α_s proteins stimulate AC by interacting with the cytoplasmic catalytic domains, C1 and C2 (Wittpoth et al., 1999). cAMP acts as a secondary messenger and serves to regulate multiple physiologic processes.

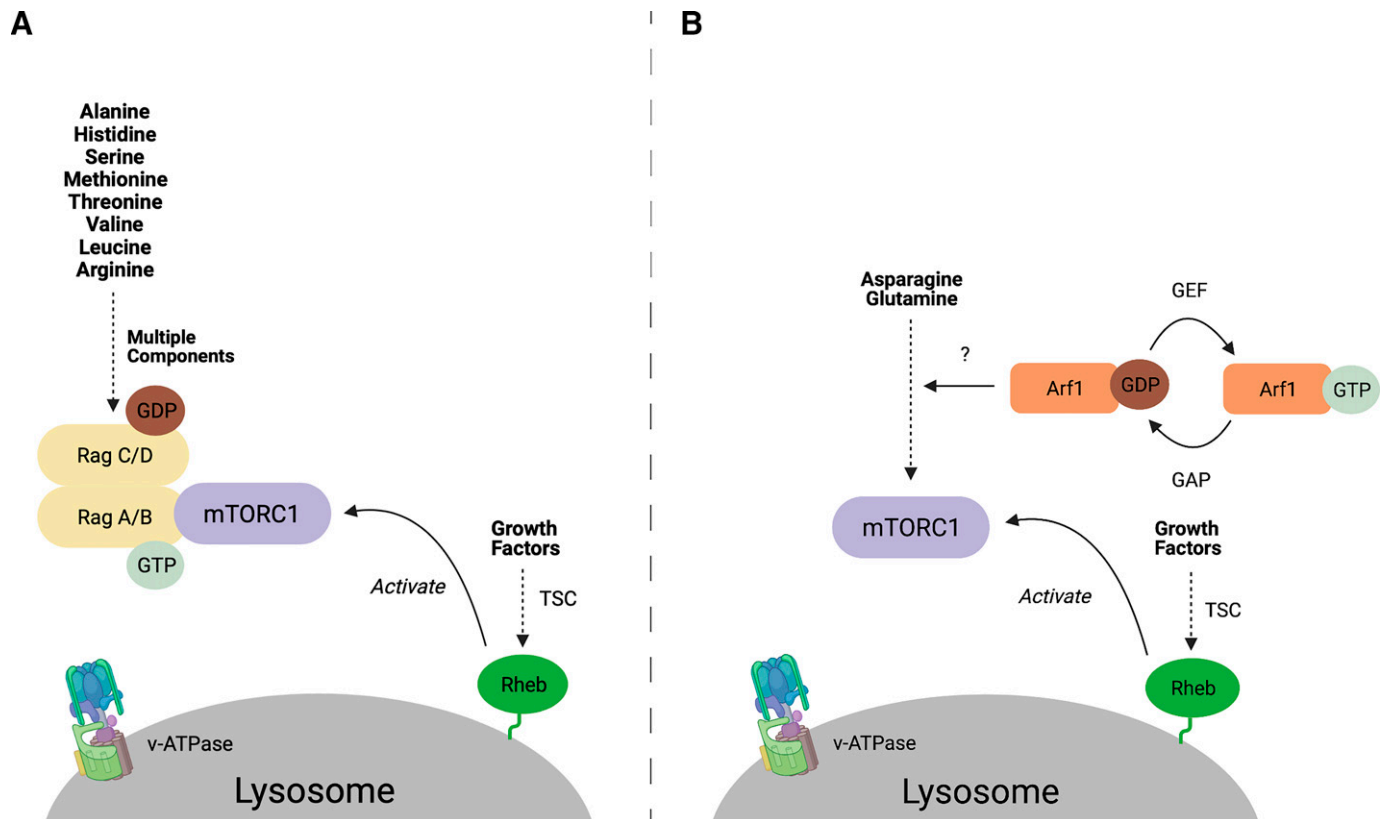


Fig. 2. mTORC1 senses two different amino acid signaling cascades. (A) The Rag-dependent signaling pathway is stimulated by amino acids such as Ala, His, Ser, Met, Thr, Val, Leu, and Arg and converges on the Rag GTPases. Following activation, RagA or RagB is GTP bound and heterodimerizes with RagC or RagD. Rag GTPase heterodimer directly interacts with the Raptor subunit of the mTORC1 at the lysosome. Finally, Rheb activates the mTORC1. (B) The Rag-independent signaling pathway is stimulated by the addition of amino acids such as Gln and Asn. The small GTPase adenosine diphosphate ribosylation factor 1 (Arf1), vacuolar H⁺-ATPase (v-ATPase), and Rheb are necessary components of the Rag GTPase independent pathway. The cycling of Arf1 to a GTP-bound state through guanine nucleotide exchange factor (GEF) and GDP-bound state through GTPase activating protein (GAP) promotes mTORC1 activation and lysosomal localization. v-ATPase is present at the lysosome and required for amino acid signaling to mTORC1 for both pathways.

One of the major targets of cAMP is protein kinase A (PKA) (Beebe, 1994). Other targets include guanine exchange proteins activated by cAMP, cyclic nucleotide-gated channels, and Popeye domain-containing proteins (Zaccolo and Pozzan, 2003; Zaccolo et al., 2021). $G\alpha_{q/11}$ binds and activates phospholipase C to convert phosphatidylinositol 4,5-bisphosphate to inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (Kadamur and Ross, 2013). $G\alpha_{12/13}$ targets Rho GEFs to regulate actin cytoskeleton (Suzuki et al., 2009) such as neurite retraction (Katoh et al., 1998). More details on GPCR signaling are described in other reviews (Hanlon and Andrew, 2015; Pavlos and Friedman, 2017).

GPCR Signaling and mTORC1 Regulation. PKA is a holoenzyme that contains two catalytic and two regulatory subunits (Wong and Scott, 2004). The regulatory subunits of PKA are RI α , RI β , RII α , and RII β . The regulatory subunit RI localizes in the cytoplasm, and RII is found at the membranes of organelles (Døskeland et al., 1993; Ilouz et al., 2012). Increased cAMP levels activate PKA by binding to the regulatory subunits and result in the release of the catalytic subunit (Kapiloff et al., 2014; Taylor et al., 2021). The catalytic subunit of PKA phosphorylates several downstream targets, including cAMP-response-element-binding protein (Beebe, 1994). Previously, RI α has also been shown to interact with and activate mTOR through an unknown mechanism (Mavrikakis et al., 2006; 2007). A recent study

from our laboratory uncovered a link between GPCR- $G\alpha_s$ signaling in the regulation of mTORC1 activity through PKA activation (Jewell et al., 2019). $G\alpha_s$ -coupled GPCRs such as adrenergic, glucagon, and vasopressin receptors through their respective ligand leads to increased cAMP-PKA signaling, resulting in Raptor Ser 791 phosphorylation and mTORC1 inhibition. cAMP-PKA-mediated mTORC1 inhibition was seen in breast, prostate, and pancreatic cancer, nonsmall cell lung carcinoma, near-haploid human cell line, osteosarcoma epithelial cell, human embryonic kidney, and mouse embryonic fibroblast cells (Jewell et al., 2019; Xie et al., 2011). This phenomenon was consistently seen in vivo where mice injected with epinephrine had decreased mTORC1 activity in the liver and brain (Jewell et al., 2019). Additionally, the pharmacological induction of cAMP levels in mouse embryonic fibroblasts and human embryonic kidney 293 cells have also been reported to inhibit mTORC1 (Xie et al., 2011). Furthermore, studies in 3T3-L1 adipocytes (Mullins et al., 2014; Scott and Lawrence, 1998; Soliman et al., 2010), rat hepatocytes (Mothe-Satney et al., 2004), thyroid carcinoma cells (Rocha et al., 2008), lymphoblasts (Monfar et al., 1995), perfused rat liver (Baum et al., 2009), and smooth muscle cells (Scott et al., 1996) reported that high cAMP levels inhibit mTORC1. There are additional examples of regulation of GPCR signaling and mTORC1 activity from other groups (Table 1). GPCRs can

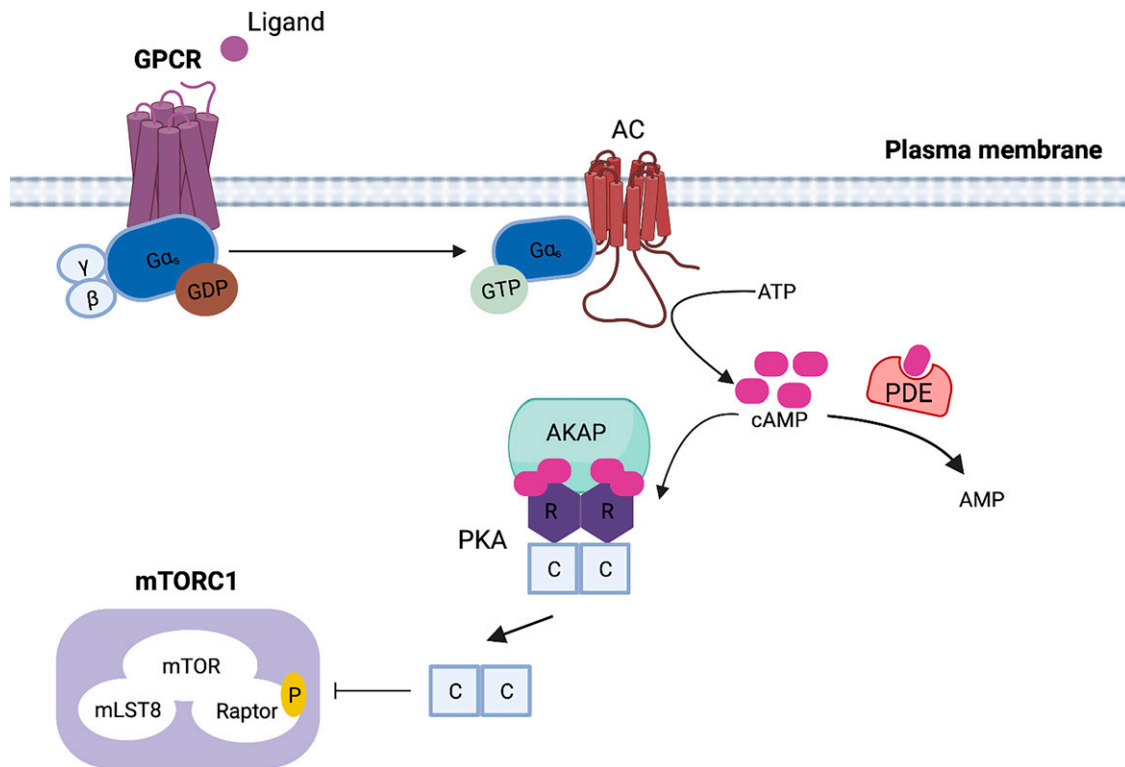


Fig. 3. $G\alpha_s$ -coupled GPCR inhibition of mTORC1. Ligand binding activates GPCRs, resulting in the $G\alpha$ protein switching from inactive GDP-bound state to active GTP-bound state, leading to a $G\alpha$ subunit dissociation following conformational change from the $G\beta\gamma$ complex. The GTP-bound $G\alpha_s$ subunit can activate AC, which converts ATP to cAMP. Elevated cAMP activates PKA by binding to its regulatory subunit and releasing PKA's catalytic subunits. AKAPs act as a scaffolding protein that assists in PKA localization to distinct compartments in the cell to facilitate signaling cascades. PKA mediated phosphorylation of Raptor at Ser 971 to inhibit mTORC1. Phosphodiesterase (PDE) negatively regulates the cAMP signaling by hydrolyzing cAMP to AMP.

potentially be great therapeutic targets due to their broad expression throughout different tissue and cell types (Bohme and Beck-Sickinger, 2009; Insel et al., 2012). Most research has focused on the upstream stimuli that activate mTORC1. However, not much is known about the signaling pathways that negatively regulate mTORC1. Therefore, it is critical to delineate the mechanistic detail of how GPCRs coupled to $G\alpha_s$ proteins regulate mTORC1.

Several studies have reported that GPCRs couple to $G\alpha_s$, increase cAMP, and inhibit mTORC1, but there are also reports of GPCR- $G\alpha_s$ signaling enhancing mTORC1 activity (Arvisais et al., 2006; Wang et al., 2014). *BRAF*-mutated thyroid carcinoma cell lines following GPCR stimulation showed an increase in mTORC1 activity, but the wild-type cells showed a decrease in mTORC1 activity (Rocha et al., 2008). Similarly, another study has revealed that PKA directly phosphorylates mTOR and Raptor, activating mTORC1 in 3T3-L1 adipocytes (Liu et al., 2016). mTORC1 activation in response to GPCR signaling might be the result of the differences in cell types and their response to altered cAMP signaling. Additionally, GPCR signaling to mTORC1 may have different biologic outcomes in different tissues and cell types. There is also evidence of direct interaction between a GPCR and mTORC1 machinery. Lysosome-localized GPCR-like protein G-protein coupled receptor protein 137B was shown to positively regulate mTORC1 lysosomal translocation and RagA-GTP loading (Gan et al., 2019). Future work on the crosstalk between GPCR signaling and mTORC1 may clarify how cAMP levels regulate mTORC1.

A-Kinase Anchoring Proteins and mTORC1 Regulation

A-kinase anchoring proteins (AKAPs) are scaffolding proteins that bind PKA through regulatory subunit domains (Carnegie et al., 2009). AKAPs anchor PKA holoenzymes to distinct subcellular locations maintaining distinct cAMP signaling pathways (Kritzer et al., 2012). Disruption of AKAPs has also been linked to several diseases. For example, AKAP1, AKAP12, and AKAP13 have been reported to play a role in cancer (Bucko and Scott, 2021). As AKAPs are tissue specific and compartmentalized, they have the potential to become useful therapeutic targets in addition to biomarkers for specific human diseases (Esseltine and Scott, 2013; Wong and Scott, 2004). We recently showed that AKAP8L interacts with mTORC1 in the cytoplasm. AKAP8L loss leads to reduced mTORC1-mediated phenotypes such as cell growth, cellular proliferation, and translation (Melick et al., 2020). However, AKAP8L did not inhibit mTORC1 through PKA. We found that AKAP13 (also known as AKAP-Lbc) is an important mTORC1 binding partner and inhibits mTORC1 through PKA. AKAP13 scaffolds PKA next to mTORC1, leading to Raptor Ser791 phosphorylation and mTORC1 inhibition. Additionally, AKAP13 plays a role in the mTORC1-mediated processes of cell proliferation, cell size, and lung tumorigenesis (Zhang et al., 2021). Previous studies found that AKAP13 promotes ERK signaling (Smith et al., 2010) and displays GEF activity for RhoA, promoting activation of p38 α (Pérez López et al., 2013). ERK signaling is known to activate mTORC1 by inhibiting TSC (Ma et al., 2005), and p38 α has been previously reported to negatively alter TSC

TABLE 1

GPCRs that Regulate mTORC1 Activity

5-Hydroxytryptamine (5-HT); α -2 adrenergic receptor (α 2-AR); angiotensin II type I receptor (AT1R); Arg vasopressin receptor (AVP); β -1/ β -2 adrenergic receptor (β 1/ β 2-AR); calcium-sensing receptor (CaSR); C-X-C chemokine receptor 4 (CXCR4); dopamine D1 receptor (D1R); dopamine D2 receptor (D2R); glucagon receptor (GCGR); glucagon-like peptide 1 (GLP-1); GPCR 137B (GPR137B); 5-hydroxytryptamine receptor 6 (HTR6); kappa opioid receptor (KOR); metabotropic glutamate receptor (mGluR); muscarinic acetylcholine receptor M4 (M₄ mAChR); orexin 1/2 receptor (OX1/2R); prostaglandin E₂/F₂- α receptor (PGE₂/PGF_{2 α}); purinergic Receptor (P2Y₁₂); taste receptor type 1 member 1/3 (T1R1/T1R3); thyroid stimulating hormone receptor (TSH); vasopressin V1 receptor (V₁); opioid receptor mu 1; rat adrenal gland cells (PC-12); cell lines: lung adenocarcinoma (A549); bovine steroidogenic luteal cells (bLCs); esophageal squamous cell carcinoma (ESCC); Chinese hamster ovary cells (CHO); human pharyngeal squamous carcinoma (FaDu); haploid 1 cells (HAP1); human embryonic kidney 293T cells (HEK-293T); human pancreatic cancer (PANC-1); Henrietta Lacks cervical adenocarcinoma cells (HeLa); human primary fibroblasts (Hs68); mouse embryonic fibroblast (MEF); mouse insulinoma 6 (MIN6); mouse neuro2A neuroblastoma with FmK6H construct (N2A-FmK6H); mouse embryonic hypothalamus N41 cells (N41); and Rat pancreatic hybrid cells of NEDH and RINm5F (BRIN-BD11).

GPCR	G-Protein	Model/Cell Line	mTORC1 Activity	Reference
α 2-AR	G α (i/o)	PC-12FaDuCervical cancer cell lines (HeLa, Caski, C-33A and SiHa)	Increase;Decrease;Decrease	(Wu and Wong, 2005)(Choi et al., 2018)(Wang et al., 2020)
AVP	G α (s)	Primary hepatocytes	Decrease	(Jewell et al., 2019)
β 1/ β 2-AR	G α (s)	Various cell lines, mouse brain & liver3T3-L1 adipocytes, mouse brown adipose tissueHuman TSC \pm fibroblasts	DecreaseIncreaseIncrease	(Jewell et al., 2019)(Liu et al., 2016)(Le et al., 2018)
CXCR4	G α (i/o)	Gefitinib resistant A549	Increase	(Jung et al., 2013)
GCGR	G α (s)	Primary hepatocytesMouse pancreatic α -cellsRat hepatocytes,H4IIE, HepG2	Decrease	(Jewell et al., 2019)(Solloway et al., 2015)(Welles et al., 2020)
GLP-1	G α (s)	BRIN-BD11	Increase	(Carlessi et al., 2017)
GPR137B	unknown	MEF, HEK-293T/E, HAP1, HeLa, Hs68, zebrafish	Increase	(Gan et al., 2019)
HTR6	G α (s)	Mouse prefrontal cortexMouse hippocampal tissueRat dorsal spinal cord	Increase	(Meffre et al., 2012)(Teng et al., 2019)(Martin et al., 2020)
KOR	G α (i/o)	CD-1 male mice, N2A-FmK6H cells	Increase	(Liu et al., 2019)
M ₄ mAChR	G α (i/o)	PC-12	Increase	(Wu and Wong, 2006)
mGluR	G α (i/o)	Primary neuronal	Increase	(Ma et al., 2010)
OX1/2R	G α (s)	HEK-293T, N41, MEF	Increase	(Wang et al., 2014)
PGE ₂	G α (s)	PANC-1	Increase	(Chang et al., 2015)
PGF _{2α}	G α (s)	bLCs	Increase	(Arvisais et al., 2006)
P2Y ₁₂	G α (i/o)	Human platelets	Increase	(Moore et al., 2014)
T1R1/T1R3	G α (i/o)	MIN6	Increase	(Wauson et al., 2012)
TSH	G α (s)	Rat thyroid, CHO	Increase	(Suh et al., 2003)
V ₁	G α (s)	Rat mesangial cells	Increase	(Ghosh et al., 2001)
GPRC6A	G α i, G α q	HEK293A, liver, PC-3	Increase	(Ye et al., 2019)
BRS-3	G α q	3T3 fibroblast cell	Increase	(Dong et al., 2020)
CaSR	G α (i/o)	cystic kidney epithelial cells	Increase	(Di Mise et al., 2018)
US28	G α q	glioblastoma cells	Increase	(de Wit et al., 2016)
5-HT7	G α ₁₂	endothelial cells	Increase	(Banskota et al., 2016)
AT1R	G α q	ESCC	Increase	(Li et al., 2016a)
HTR2B	G α (i/o)	PDAC cells	Increase	(Jiang et al., 2017)
D1R	G α q	nucleus accumbens of mice	Increase	(Sutton and Caron, 2015)
OPRM1	G α (i/o)	HEK293 cells	Increase	(Wang et al., 2014)
DRD4	G α (i/o)	GBM stem cells	Decrease	(Dolma et al., 2016)
DRD3	G α (i/o)	HEK293T, HeLa, COS-7 cells	Decrease	(Barroso-Chinea et al., 2020; Li et al., 2016b)
D2R	G α (i/o)	mesencephalic neurons	Decrease	(Fasano et al., 2008)
CNR2	G α (i/o)	Neural progenitor cell	Increase	(Palazuelos et al., 2012)
CNR1	G α (i/o)	Glial cells	Increase	(Díaz-Alonso et al., 2015)

function by promoting TSC and 14-3-3 binding (Li et al., 2003; Shumway et al., 2003). However, the pharmacological inhibition of ERK signaling did not change Raptor Ser 791 phosphorylation, and p38 α depletion did not alter mTORC1 activity (Zhang et al., 2021). Similarly, it was found that

TSC knockout cells also did not affect Raptor Ser791 phosphorylation (Jewell et al., 2019), indicating that AKAP13-mediated regulation of mTORC1 may be independent of ERK and p38 α signaling. Moreover, it has been shown that a mitochondrial-associated AKAP, AKAP1, promotes mTORC1 activation through the

TABLE 2
Phosphodiesterase with Affinity to cAMP and Their Inhibitors

PDE Inhibitors	Target	Substrate	Reference
Rolipram	PDE4	cAMP	(Kim et al., 2017)
Apremilast	PDE4	cAMP	(Schett et al., 2010)
Crisaborole	PDE4	cAMP	(Paller et al., 2016)
Roflumilast	PDE4	cAMP	(Baye, 2012)
Cilomilast	PDE4	cAMP	(Compton et al., 2001)
Theophylline	NonselectivePDE4	cAMP	(Barnes, 2013)
Dipyridamole	NonselectivePDE6, PDE8	cAMP	(Gillespie and Beavo, 1989)
Zaprinast	NonselectivePDE6	cAMP	(Zhang et al., 2005)

PDE, phosphodiesterase.

interaction and suppression of Sestrin2 (Rinaldi et al., 2017). Additional research on AKAPs will be important to characterize the PKA signaling to mTORC1.

Phosphodiesterase and mTORC1 regulation. Phosphodiesterases (PDEs) degrade cAMP to negatively regulate $G\alpha_s$ signaling (Sassone-Corsi, 2012). Among 11 characterized PDE family members, PDE4, PDE6, and PDE8 are the main phosphodiesterases that hydrolyze cAMP (Bolger, 2021). In recent years, several pharmacological inhibitors for cAMP substrate-specific PDEs have been identified (Table 2). A direct role of PDEs in cAMP-mediated mTORC1 regulation is not known yet. However, some studies indicate that PDEs might play a role in mTORC1 regulation. PDE4 isoform B inhibition increased cAMP signaling and inhibited growth in diffuse large B-cell lymphoma (Smith et al., 2005b). As mTORC1 is associated with growth, PDE4B may be involved in mTORC1 regulation. Similarly, PDE3, which hydrolyzes both cAMP and cGMP, has been found to regulate mTORC1 activation and β -cell proliferation. cGMP can also promote mTORC1 activation through PKG-mediated Raptor phosphorylation at Ser 791 (Liu et al., 2018). Further research on the role of PDE-mediated cAMP and cGMP hydrolyzation and mTORC1 regulation will be important. Rapamycin and rapalogs are the only compounds that have been shown to have clinical efficacy in targeting mTORC1. However, studies show that prolonged treatment with these inhibitors may have an off-target effect on mTORC2 and exacerbate insulin resistance in mice (Fraenkel et al., 2008; Lamming et al., 2012). Therefore, PDE inhibitors might provide an alternative to negatively regulate mTORC1 by increasing cAMP signaling. Currently, there are multiple US Food and Drug Administration-approved drugs that inhibit PDEs that include sildenafil, vardenafil, tadalafil, avanafil, apremilast, crisaborole, and roflumilast. As PDE inhibitors are already in use in the clinic, combining PDE inhibitors with GPCR-targeting drugs (agonists) could provide therapeutic benefits in targeting mTORC1-hyperactivated diseases.

Conclusions

mTORC1 dysregulation can result in several human diseases such as cancer, metabolic disorders, and neurodegeneration. Exploring amino acid sensors upstream of mTORC1 and delineating specific amino acid pathways could result in novel clinical treatment options. Furthermore, As GPCRs are the most common FDA-approved drug target on the market today (Sriram and Insel, 2018), we believe that understanding how mTORC1 is regulated by GPCRs could have an immediate impact on mTORC1-mediated human diseases.

$G\alpha_s$ -coupled GPCRs agonists in combination with clinically available PDE inhibitors could present novel ways to target mTORC1, although a more comprehensive understanding of the relationship between GPCR signaling and how it regulates mTORC1 will need to be investigated in the future. Determining or identifying other components involved in this pathway that regulate mTORC1 like phosphodiesterase could also provide more potential therapeutic targets.

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Wrote or contributed to the writing of the manuscript: Melick, Lama-Sherpa, Curukovic, Jewell.

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