



REVIEW ARTICLE OPEN

mTOR signaling networks: mechanistic insights and translational frontiers in disease therapeutics

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The mammalian target of rapamycin (mTOR) pathway is a central regulator of cellular growth, metabolism, and homeostasis, integrating a wide array of intracellular and extracellular cues, including nutrient availability, growth factors, and cellular stress, to coordinate anabolic and catabolic processes such as protein, lipid, and nucleotide synthesis; autophagy; and proteasomal degradation. The dysregulation of this signaling hub has broad implications for health and disease. To commemorate the 50th anniversary of the discovery of rapamycin, we provide a comprehensive synthesis of five decades of mTOR research. This review traces the historical trajectory from the early characterization of the biological effects of rapamycin to the elucidation of its molecular target and downstream pathways. We integrate fundamental and emerging insights into the roles of mTOR across nearly all domains of cell biology and development, with a particular focus on the expanding landscape of therapeutic interventions targeting this pathway. Special emphasis is placed on the crosstalk between mTOR signaling and mitochondrial regulation, highlighting the mechanisms by which these two metabolic hubs co-regulate cellular adaptation, survival, and disease progression. The dynamic interplay between mTOR and mitochondrial networks governs key aspects of bioenergetics, redox balance, and cell fate decisions and is increasingly implicated in pathophysiological contexts ranging from cancer and aging to neurodegenerative and immune disorders.

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INTRODUCTION

The year 2025 marks two major anniversaries that forever changed the fields of metabolism and cell signaling: the 50th anniversary of the discovery of rapamycin in 1975^{1,2} and the 60th anniversary of the Medical Expedition to Easter Island (METEI) in 1964–1965,³ during which soil samples, containing *Streptomyces hygroscopicus*, the bacterium that produces rapamycin, were collected. Although fewer than a dozen papers were published on rapamycin in the first ten years following its discovery, the subsequent decades witnessed a significant expansion of the field (Fig. 1). This growth was driven by the identification of the immunosuppressive⁴ and anticancer properties of rapamycin,⁵ as well as the discovery of its molecular target, a protein named “target of rapamycin” (TOR), first in yeast^{6,7} and three years later, its homolog in mammals, subsequently referred to as mammalian TOR (mTOR).^{8–11}

Today, research on TOR/mTOR spans virtually all key aspects of cellular function and development. mTOR represents a major focus in clinical and pharmaceutical research because of the deregulation of mTOR pathway in numerous human diseases, including neurodegenerative disorders and cancer.

This review summarizes five decades of TOR/mTOR research (hereafter referred to as mTOR unless describing the work in yeast or *Drosophila*), covering molecular mechanisms and highlighting recent successes in targeting components of the mTOR pathway in clinical trials. Special emphasis will be placed on the interplay between mTOR signaling and mitochondrial regulation,

illustrating how these two metabolic hubs collaborate to sustain cellular proliferation and survival.

HISTORY OF MTOR RESEARCH

The discovery of the “wonder drug” rapamycin is perhaps the most famous outcome of the METEI, organized by the Canadian scientist Stanley Skorina, initially to evaluate the global health of Easter Island’s population and environmental challenges on the island, with the indigenous name Rapa Nui. One of METEI’s members, microbiologist Georges Nogrády, divided the island into 64 squares and collected soil samples from each. Upon returning to Canada, he generously distributed these samples to many colleagues, both nationally and internationally.^{3,12} Some of the soil samples reached the bacteriologist Claude Vézina and the biochemist Surendra (Suren) Sehgal at Ayerst Research Laboratories in Canada. Between 1975 and 1979, they published a series of articles describing the isolation of a *Streptomyces hygroscopicus* strain from these soil samples, from which they extracted a compound with antifungal properties^{1,2,13,14} and immunosuppressive potential.⁴ The compound was named rapamycin to acknowledge its origin from Rapa Nui Island. In 1984, rapamycin was found to exhibit strong anticancer activity against many solid tumors.⁵ Importantly, rapamycin was cytostatic, in contrast to most anticancer therapies at the time, which were cytotoxic. The structure of rapamycin was solved by X-ray crystallography in 1978,¹⁵ allowing it to be classified as a macrolide. Another

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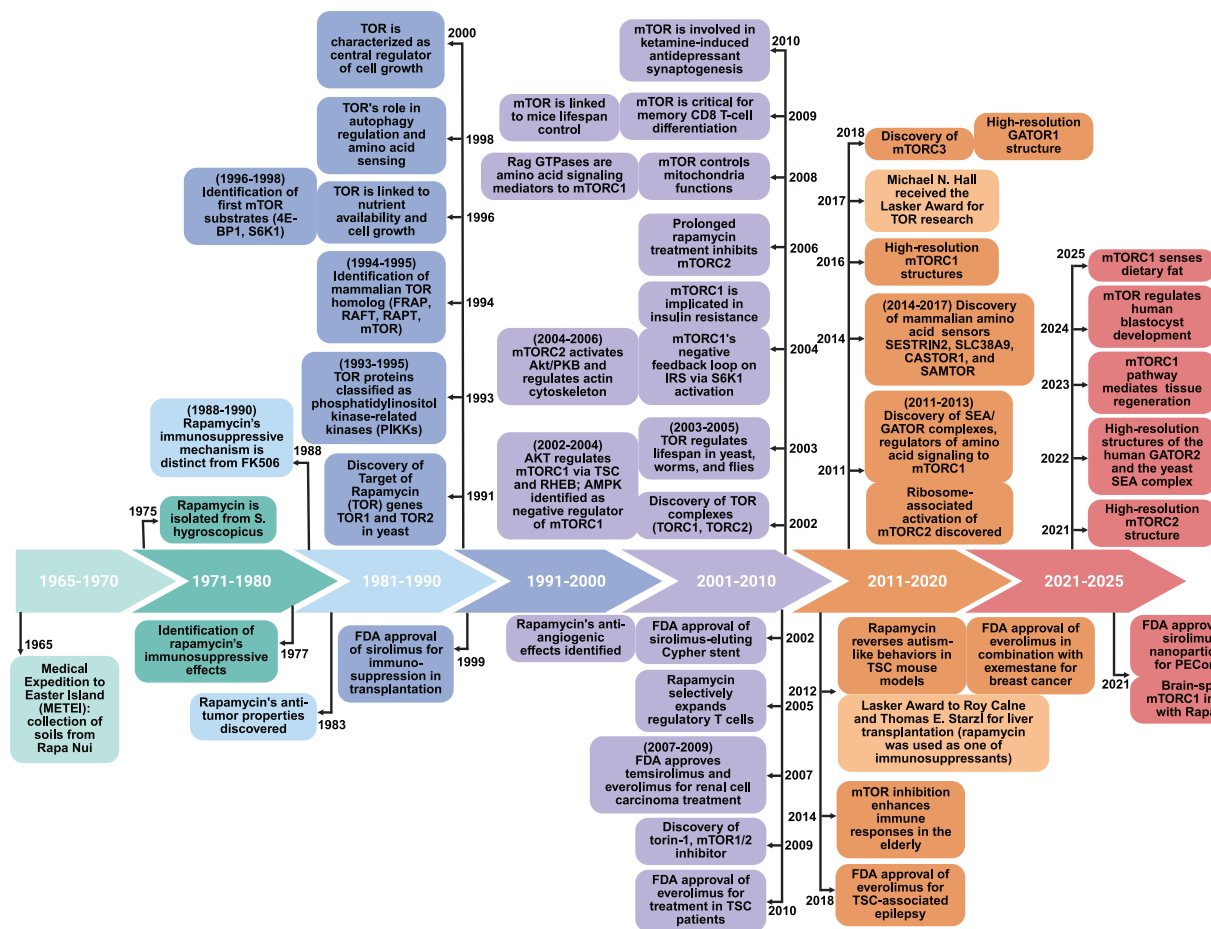


Fig. 1 Historical overview of mTOR research and its clinical application. The timeline illustrates key discoveries in the mTOR signaling pathway (upper panel) and parallel progress in the identification and clinical application of mTOR-targeting agents (lower panel). See the text for details

macrolide, FK506, was already known for its immunosuppressive properties, being successful as a transplant drug (tacrolimus), but its mechanism of action differed from that of rapamycin.¹⁶ Efforts to understand these differences ultimately led to the discovery of the molecular target of rapamycin.

In 1991, a group led by Michael Hall identified two genes, *TOR1* and *TOR2*, that mediate rapamycin sensitivity in the yeast *Saccharomyces cerevisiae*.⁶ Unlike the two other immunosuppressants, FK506 and cyclosporin A, whose immunophilin complexes inhibit calcineurin, the rapamycin-FKBP12 complex does not interfere with calcineurin but instead directly binds to and inhibits a protein that was named “target of rapamycin” (TOR). About the same time, the groups of George Livi¹⁷ and John J. Siekierka¹⁸ independently isolated FKBP12 (designated RBP1 or FKB1, respectively), demonstrating its role as a mediator of rapamycin and FK506 sensitivity in yeast¹⁹ and identifying the yeast TOR gene.²⁰

The discovery of the mammalian homolog of TOR followed approximately three years later, by four laboratories led by Stuart L. Schreiber,⁸ Sol H. Snyder,⁹ Vivian Berlin,¹⁰ and Robert T. Abraham.¹¹ Although each group assigned a different name to the protein (FRAP,⁸ RAFT1,⁹ or RAPT1¹⁰), the term mTOR¹¹ for “mammalian target of rapamycin” ultimately became the accepted standard in mammals and other eukaryotes.

Since the mid-1990s, research on TOR/mTOR has accelerated tremendously (Fig. 1), with over 5000 papers published annually over the past decade on TOR, rapamycin, its derivatives and clinical applications. This growing body of work has advanced

along two main fronts: first, elucidating the molecular mechanisms of mTOR signaling pathways²¹; second, developing therapeutic strategies to modulate mTOR activity in a wide range of human diseases²² (Fig. 1). In the sections below, we review the major achievements in both areas.

STRUCTURES OF MTOR AND ITS COMPLEXES

In yeast, there are two *TOR* genes, *TOR1* and *TOR2*,^{6,23} whereas mammalian cells contain only one *mTOR* gene. The Tor1 and Tor2 proteins share 67% amino acid identity and 85% similarity. The overall amino acid identity between *S. cerevisiae* Tor1/Tor2 and human mTOR is approximately 40–45%.

Tor/mTOR is a serine/threonine kinase with a molecular weight of approximately 289 kDa and is classified within the phosphoinositide 3-kinase-related kinase (PIKK) family owing to structural similarities in its catalytic domain.^{24,25} The N-terminal region of mTOR contains approximately 20 HEAT (huntingtin, elongation factor 3, protein phosphatase 2A, TOR1) repeats, which are involved in mediating protein–protein interactions. Its C-terminus is composed of conserved domains, including the FAT domain (shared by the FRAP, ATM, and TRRAP proteins) and the FKBP12-rapamycin binding (FRB) domain, which facilitates binding of the FKBP12-rapamycin complex, the kinase catalytic domain, and the FATC domain located at the extreme C-terminus. The FAT and FATC domains modulate the activity of the kinase domain.

In yeast, Tor1 is a part of TOR complex 1 (TORC1) or TOR complex 2 (TORC2), whereas the Tor2 protein is a component of

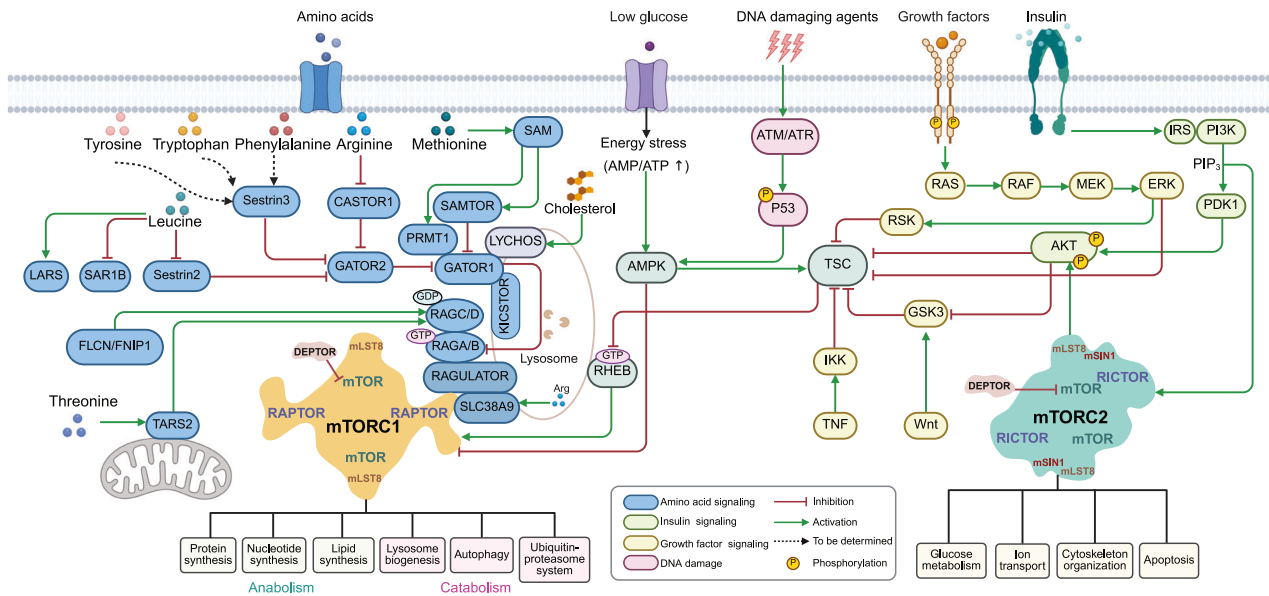


Fig. 2 Overview of the mTORC1 and mTORC2 signaling pathways. The mTORC1 and mTORC2 complexes are depicted as profiles of their respective dimeric structures. Various stimuli, upstream regulators, and downstream effectors are illustrated. Amino acids, glucose, growth factors and DNA damaging agents modulate mTORC1 activity to control anabolic and catabolic processes. Insulin activates PI3K to generate PIP₃, which activates mTORC2. The precise targets of mTORC1 and mTORC2 and their phosphorylation sites are listed in Table 1

TOR complex 2.²⁶ In mammals, the mTOR protein is a part of three protein complexes: mTORC1, mTORC2, and the newly identified and currently poorly characterized mTORC3,^{27–29} of which only mTORC1 is sensitive to acute rapamycin treatment.

The mTORC1 core consists of mTOR, mLST8 and regulatory associated protein of mTOR (RAPTOR),³⁰ whereas the mTORC2 core contains mTOR, mLST8, mSIN1, and rapamycin-insensitive companion of mTOR (RICTOR)³¹ (Figs. 2, 10). mLST8 interacts with the kinase domain of mTOR; RAPTOR interacts with HEAT repeats.²⁸ mTOR complexes associate with other proteins that are generally not considered part of the core, such as DEPTOR, an endogenous inhibitor of both mTOR complexes, and PRAS40, an endogenous inhibitor of mTORC1. In mammals, mTORC2 also interacts with the PROTOR protein, which has two isoforms: PROTOR1 (PRR5) and PROTOR2 (PRR5L). The functions of these isoforms are not well defined.

mTORC1 adopts a rhomboid configuration, measuring approximately 280 × 210 × 130 Å,³² which results from the dimerization of a heterodimer consisting of mTOR, mLST8, and RAPTOR. Within this complex, RAPTOR interacts with the N-terminal region of mTOR, playing a critical role in complex assembly, subcellular localization, stability, and substrates recruitment. PRAS40 is located near the central core and acts as a negative regulator of mTORC1 signaling. mLST8 associates with the kinase domain of mTOR and contributes to maintaining its enzymatic stability. DEPTOR is another inhibitory partner within the complex. Furthermore, the rapamycin-FKBP12 complex binds to the FRB domain of mTOR, effectively blocking substrate access to the catalytic site.

The mTORC2 complex is also organized as a dimer composed of two heterotetrameric units, showing a hollow rhombohedral fold with dimensions of ~220 × 200 × 130 (Å).^{32,33} Like mTORC1, the mTOR-mLST8 heterodimer forms the structural core of mTORC2. The two mTOR molecules interact directly, establishing a central scaffold that facilitates the attachment of additional subunits. mLST8, mSIN1, and RICTOR each bind symmetrically to the mTOR dimer in two copies. RICTOR plays a key role in mTORC2 assembly, substrate specificity, and structural stability by forming extensive interactions with mTOR. Importantly, the position of RICTOR within mTORC2 explains the insensitivity of the complex during acute treatment with rapamycin because the FRB domain of mTOR in

mTORC2 is sterically hindered by RICTOR, preventing the FKBP12–rapamycin complex from binding.

mSIN1 connects RICTOR to mLST8 through its N-terminal region and serves as a scaffold that mediates interactions with serum and glucocorticoid-regulated kinase 1 (SGK1) while also modulating mTORC2 kinase activity in a negative manner. DEPTOR functions as an inhibitor by binding to mTOR. PROTOR-1, which is associated with RICTOR, contributes to the regulation of SGK1 phosphorylation driven by mTORC2.

mTOR can also interact via its FRB domain and LST8 binding element with the E26 transformation-specific transcription factor ETV7 within the cytoplasm. This interaction results in the formation of mTORC3, a complex that exhibits dual mTORC1 and mTORC2-like signaling activities yet functions independently of the typical components associated with mTORC1 or mTORC2.²⁹ To date, the mTORC3 complex has been described by only one research group laboratory and will not be discussed further in this review.

Both mTORC1 and mTORC2 localize to multiple subcellular compartments, but mTORC1 is present mainly at the lysosomal membrane³⁴ and partially at the Golgi,³⁵ while mTORC2 can be found at the plasma membrane, outer mitochondrial membrane, and endosomal vesicles.^{36,37}

Currently, approximately 60 direct substrates of mTORC1 and approximately 30 substrates of mTORC2 have been identified, most of which regulate transcription, translation, and autophagy³⁸ (Table 1). Notably, the mTORC1 substrates include ribosomal protein S6 kinase 1 (S6K1) and eIF4E binding protein (4E-BP1), which play key roles in translation^{22,39–41}; the transcription factors EB and E3 (TFEB/TFE3), which regulate lysosomal gene expression^{42–45}; and Unc-51-like kinase 1 (ULK1),⁴⁶ a crucial initiator of autophagy. mTORC2 signaling controls various cellular functions through its regulation of AGC family kinases, such as AKT, protein kinase C (PKC), and serum and glucocorticoid-induced kinases (SGKs).²⁸

REGULATION OF NUTRIENT AND STRESS SIGNALING UPSTREAM OF MTORC1

To ensure a precise and timely response to both extra and intracellular cues, mTORC1 integrates upstream signals through two major axes regulated by small GTPases — RHEB (Ras homolog

4 enriched in the brain) and RAGs (Ras-related small GTP-binding proteins). RAGs function as heterodimers: RAGA or RAGB bind to RAGC or RAGD. Amino acid availability is primarily conveyed to mTORC1 via the GATOR1/GATOR2-RAG signaling axis,^{28,47} whereas growth factors and energy activate mTORC1 through the TSC-RHEB axis (Fig. 2).^{28,48,49}

In amino acid sufficiency, RAGA/B is bound to GTP, whereas RAGC/D is bound to GDP.^{50,51} RAGs promote the recruitment of mTORC1 to the lysosomal membrane, where mTORC1 is fully

activated by RHEB and bound to GTP. Conversely, when amino acids are low, RAGs are inactive, and RAGA/B is loaded with GDP, while RAGC/D is bound to GTP.

RAGs are not functionally redundant.^{52,53} Thus, RAGD preferentially promotes mTORC1 phosphorylation of TFE3, which is linked to a stronger association of RAGD with the lysosome than RAGC, whereas TFE3 is preferentially recruited by RAGC.⁵³ In contrast, both RAGC and RAGD equally regulate the nonlysosomal mTORC1 substrates S6K1 and 4E-BP1.⁵² Thus, S6K and 4E-BP1

Table 1. Direct substrates and phosphorylation sites of mTOR complexes

mTORC1				
Function	Proteins	Verified sites	Candidate sites	Ref
Autophagy	AMBRA1	S52		38
	ATG13	S258 (UniProt: S259)		531
	ATG14	S3, S223, T233, S383, S440		38
	DAP1	S3, S51		38
	NRBF2	S113, S120		38
	p62	S349 ^a		270
	PACER	S157		38
	TRPML1	S572, S576		38
	ULK1	S757		46
	UVRAG	S498, S550, S571		552
Growth factor signaling	VAMP8	T48		38
	WIP12	S395		38
	GRB10	S474, S476		533
	LST2	S670		534
	IRS1	S422, S423, S636, S639		38
	4E-BP1	T37, T46, S65, T70		535
	eEF2K	S78, S396		38
	eIF2 β	S2, S67		38
	eIF4E	S209		536
	IMP2	S162, S164		537
Translation	LARP1	S689, T692, S697 (S774)	S148, S151, S247, S250, T438, S440, S444, T449, S471, S550, S554, T768, S770, S772, S774, S776, T779, S784, T788, S791, S979, T994	167,168
	S6K1	T389, S371		38
	ACINUS	S240, S243		38
	AR	S96		38
	BACH2	S510 ^a , S536 ^a		38
	ER α	S104, S106		38
	HSF1	S326		38
	JMJD1C	T505		38
	KAP1	S824		38
	MAF1	S60, S68, S75		174
Transcription	P300	S2271, S2279, S2291, S2315		38
	SEN3	S25, S26, S141, T142, T145		38
	STAT3	S727		301
	TFEB	S122, S142, S211		42–44
	TFE3	S321		45
	OTUD5	S323, S332		38
	USP20	S132, S134		38
	p53	S15		538
	AMPK α 1	S347		539
	AMPK α 2	S377, S345		539
Lipid & glucose metabolism	AMPK β 1	S182		539
	AMPK β 2	S184		539
	AMPK γ 2		S113, S143, S162, S196	539
	AS160	S666		38
	CRTC2	S136		38
	LIPIN1	S106, S472		162
	PASK	T640, T642, S949, S953, S956		38
	JSCU	S14		38
	MFN2	S200		540
	SIRT1	S47		38
Mitochondrial metabolism	AMPK α 1	S356 ^a		38
	DEPTOR	S293, T295, S299		541
	PRAS40	S183, S212, S221		38
	PIP4K γ	S324, S328		38
	RAG-C	S21		38
	RAPTOR	S859, S863		38
	ZNRF2	S145		38
	CLIP1	C-terminal SR-rich region (multiple sites)	S304, S308, S312, S315, S316, S322	38
	Unkempt	S606, S611	S255, S336, S359, S608	542
	TAU	S214, T231, S356		38
mTOR regulation				
Cytoskeleton	CLIP1	C-terminal SR-rich region (multiple sites)	S304, S308, S312, S315, S316, S322	38
	Unkempt	S606, S611	S255, S336, S359, S608	542
	TAU	S214, T231, S356		38

mTORC2				
Function	Proteins	Verified sites	Candidate sites	Ref
Apoptosis	AKT1	T450, S473, S477, T479	T443	194,196
	p53	S15		538
	PKCδ	S664		38
	SGK1	S422	S397, T390	543
	FLNA	S2152		38
Cytoskeleton	PKCα*		T631, T638, S657	38
	PKCβII	T634	T641, S660	38
	PKCγ*		T648, T655, T674	38
	PKCε*		T703, T710, S729	38
	PKCζ	T560	T553	38
	PKCι*		T557, T564, S577	38
	PKN1*		T925, S916, T909	38
Growth factor signaling	IGF1R	Y1131, Y1136 (UniProt Y1161, Y1166)		544
	INSR	Y1146, Y1151 (UniProt Y1185, Y1190)		544
	mTOR	S2481		38
Metabolism	ACLY	S455		38
	GFAT1	S243		38
	xCT	S26		38
Ion homeostasis	OSR1	S339		38
	DEPTOR	S293, T295, S299		541
Hippo pathway	AMOTL2	S760		38
	MST1	S438		38
	YAP	S436		38
	DUSP10	S224, S230		38
Ubiquitination	FBXW8	S86		38
	OTUD5	S503		38
mRNA regulation	IGF2BP1	S181		38

Notes: Phosphorylation sites leading to the inhibition of a corresponding protein are indicated in red, those leading to activation are indicated in green, and sites with unknown effects are indicated in blue. Only human phosphorylation sites are listed. * refers to sites not verified experimentally.³⁸ # Sites reported in Battaglini et al.,³⁸ where the residue numbering does not match the original primary publication because Battaglini et al.³⁸ used UniProt-based annotation instead of the numbering applied in the original study. S - serine, T - threonine, Y - tyrosine.^{38,42–46,167,168,174,182,194,196, 270,301,531–544}

were named “canonical” (nonlysosomal, RHEB-TSC-dependent), whereas TFE3/TFEB are “noncanonical” (lysosomal, RHEB-TSC-independent) mTORC1 substrates.⁵⁴ The RAGA and RAGB also differ from each other. Moreover, RAGB has two isoforms: a “short” isoform, which is prevalent in most tissues, and a “long” isoform, which is expressed primarily in neurons. Both isoforms, unlike RAGA, alter the dynamics of mTORC1 activity, allowing it to persist even under amino acid-depleted conditions.⁵⁵

GTPases are dependent on their regulators, GTPase-activating proteins (GAPs), which stimulate GTP hydrolysis, and guanine nucleotide exchange factors (GEFs). The GATOR1 complex acts as a GAP for RAGA, thereby transforming RAGA to its inactive, GDP-bound form, which leads to mTORC1 suppression.⁴⁷ A complex between folliculin (FLCN) and folliculin-interacting protein (FNIP) is also a GAP, but for RAGC/D. FLCN/FNIP transforms RAGC/D to the active GDP conformation, leading to mTORC1 activation.⁵⁶ Finally, growth factors activate RHEB GTPase by blocking the GAP activity of the trimeric TSC complex.⁵⁷ A lipid modification of RHEB at the C-terminus known as farnesylation is essential for its lysosomal targeting and subsequent activation of mTORC1.⁵⁸

GAPs act alongside GEFs, such as RAGULATOR for RAGs⁵⁹ and ATP6AP1 and lysosomal EGFR for RHEB.^{60,61} Pentameric RAGULATOR is not only the GEF for RAGA, RAGB,⁵⁹ and RAGC⁶² but also anchors RAGs to the lysosome.

Amino acid sensing

Amino acid signaling to mTORC1 is arguably the most extensively studied regulatory network upstream of mTORC1 (Fig. 2). Although not fully understood, this signaling axis provides a compelling illustration of the fundamental principles by which the mTORC1 pathway senses and responds to environmental cues.

Multiple amino acid sensors signal amino acid availability to mTORC1. Thus, leucine sensors SESTRIN2⁶³ and SAR1B,⁶⁴ the arginine sensor CASTOR1/2,⁶⁵ and the mitochondrion-localized threonine sensor TARS2⁶⁶ detect free amino acids in the cytoplasm, whereas the lysosomal complex RAGULATOR⁵⁹ senses

the efflux of multiple amino acids from the lysosomal lumen (Fig. 2). SLC38A9, which is also located on the lysosome, can transport multiple amino acids from the lysosome lumen to the cytosol but has been demonstrated to function as a direct sensor only for arginine.^{67–69}

Amino acid sensors function either through the GATOR2 complex (SESTRIN2,⁶³ SAR1B,⁶⁴ CASTOR1/2⁶⁵) or the GATOR1 complex, with SAMTOR and PRMT1 acting as sensors for the metabolite of methionine, S-adenosylmethionine (SAM).^{70–72}

The GATOR1 complex consists of three proteins: DEPDC5, NPRL2 and NPRL3. NPRL2 and NPRL3 form a heterodimer through interactions of their N-terminal longin domains (NLDs) and C-terminal regions, contributing to the stability of the complex.⁷³ Arg78 of NPRL2 serves as the arginine finger responsible for the GAP activity of GATOR1.⁷⁴ DEPDC5 contains domains with similarities to motifs found in membrane-associated proteins.^{73,75} The GATOR2 complex, which is composed of five proteins (WDR24, WDR59, MIOS, SEH1L and SEC13), is a coatomer-like structure.⁷⁶ It shares structural features with other coating assemblies, such as the nuclear pore complex and coated vesicles, including shared components (SEH1 and SEC13).⁷⁵ WDR24, WDR59, and MIOS also contain ring domains, which are critical for maintaining GATOR2 integrity. WDR24 exhibits intrinsic E3-ligase activity and mediates the ubiquitination of NPRL2 upon amino acid stimulation.⁷⁷

In amino acid sufficiency, GATOR2 interacts with and inhibits GATOR1, thereby activating mTORC1.^{47,76,78} Amino acid deprivation leads to GATOR2 inhibition and GATOR1 activation, collectively resulting in mTORC1 inhibition. Thus, GATOR2 functions as an mTORC1 activator, whereas GATOR1 acts as an mTORC1 inhibitor.

In yeast, the GATOR2 and GATOR1 homologs, SEACAT and SEACIT complexes, respectively, can form a stable SEA complex.^{79,80} In mammalian cells, the GATOR1 and GATOR2 complexes exhibit weak direct interactions and rely on the tetrameric KICSTOR complex and the KICSTOR-interacting VWCE protein to anchor GATOR1 to lysosomes under conditions of amino acid or glucose deprivation.^{81,82} However,

KICSTOR has only a limited role in positioning GATOR2 at the lysosomal surface. To coordinate the lysosomal localization of both GATOR complexes, cells depend on interleukin enhancer-binding factor 3 (ILF3).⁸³

Leucine can be sensed via at least three sensors: SESTRIN2, SAR1B and leucyl-tRNA synthetase 1 (LARS1). SESTRIN2 and SAR1B interact with and inhibit GATOR2 in the absence of leucine, and this inhibition is lifted when leucine is present.^{64,84,85} (Fig. 2). SESTRIN2 binds the WDR24/SEH1L subunits of GATOR2, whereas SAR1B directly interacts with the MIOS subunit. Interestingly, LARS1 is not only a canonical leucine sensor that catalyzes the ligation of leucine to its cognate tRNA. LARS1 also functions as a GAP for RAGD, thereby promoting mTORC1 activation.⁸⁶

When arginine is low, CASTOR1/2 binds to and inhibits GATOR2, suppressing mTORC1.⁶⁵ The three aromatic amino acids tyrosine, tryptophan, and phenylalanine (YWF) signal to mTORC1 via interactions between SESTRIN3 and GATOR2; however, whether SESTRIN3 is a direct sensor of YWF remains to be determined.⁸⁷

Unlike other amino acid sensors that inhibit GATOR2 in the absence of their respective ligands, SAMTOR interacts with and activates GATOR1 in the absence of methionine or SAM.^{70,71} (Fig. 2). Binding of SAMTOR to GATOR1 blocks the interaction of PRMT1 with GATOR1. When SAMTOR is bound to the SAM, it dissociates from GATOR1-KICSTOR. This allows SAM-bound PRMT1 to methylate NPRL2 at Arg78, thereby inhibiting the GAP activity of GATOR1 and promoting mTORC1 activation.⁷²

GATOR2, GATOR1, and KICSTOR, together with RAGs and RAGULATOR, constitute key lysosome-localized signaling hub for nutrient sensing through mTORC1. Targeting RAGULATOR to the mitochondrial membrane is sufficient to relocate the RAGs and GATOR complexes to mitochondria, but this relocation alone does not enable nutrient-dependent recruitment of mTORC1 to the organelle.⁸⁸

Nevertheless, RAG-independent activation of mTORC1 by amino acids has been described in both yeast and human cells.^{89–92} For example, in RAGA/B-deficient cells, mTORC1 can still be stimulated by asparagine and glutamine via ARF1 GTPase.⁸⁹ Amino acids released from lysosomal protein breakdown can activate mTORC1 through a mechanism that involves the homotypic fusion and protein sorting (HOPS) complex, independent of RAG GTPases.⁹³ Additionally, mTORC1 activity is suppressed during amino acid deprivation because RAP1 GTPases promote lysosome repositioning to the perinuclear region and reduce lysosomal abundance.⁹⁴ Finally, mTORC1 can also be activated at the Golgi membrane, where it is recruited by the RAB1A GTPase⁹¹ and subsequently activated by Golgi-localized RHEB.⁹⁵ Non-farnesylated RHEB also regulates mTORC1 in the nucleus.⁹⁶ Non-lysosomal mTORC1 appears to be under the control of exogenous amino acids and phosphorylates non-lysosomal, “canonical” substrates S6K1 and 4E-BP1.⁹⁷ At the same time, a fraction of mTORC1 localizes to the lysosome, which locally supplies endogenous amino acids through basal lysosomal proteolysis. This lysosomal, RAG-dependent mTORC1 acts on the “noncanonical substrates” TFEB and TFEB.⁹⁷

While core components of the mTORC1 nutrient-sensing pathway (RAGs, RAGULATOR, and GATOR1/GATOR2 complexes) are conserved across metazoans,⁷⁵ many direct amino acid sensors are not.²⁸ Even conserved proteins may function differently across species.⁹⁸ For example, *D. melanogaster* lacks arginine sensors but retains SESTRIN and SAMTOR. Nevertheless, *Drosophila* Sestrin binds leucine with significantly lower affinity than its human homolog does. Furthermore, SAM is not sensed by dSamtor but by the Umet protein, which binds to GATOR2,⁹⁹ demonstrating that flies and vertebrates independently evolved unrelated, mechanistically distinct sensors that converge upon the same metabolite. In addition, GATOR2 in *Drosophila* has a TORC1-independent role in the regulation of the lysosomal autophagic endomembrane system.¹⁰⁰ *C. elegans* has SESTRIN and SLC38A9

homologs but no clear SAMTOR counterpart. This patchy distribution may reflect species-specific metabolic requirements. In support of this, *S. cerevisiae* seems to regulate TORC1 on the basis of overall nitrogen and carbon availability rather than individual amino acid levels. Indeed, *S. cerevisiae*, which can synthesize all amino acids de novo, lacks specialized amino acid sensors but maintains the general amino acid sensors Gcn2, FLCN/FNIP (Lst4/Lst7) and GATOR1/2 (SEACIT/SEACAT), RAGs (Gtr1/Gtr2) and RAGULATOR (Ego).¹⁰¹ In addition, *S. cerevisiae* lacks the genes encoding components of the TSC complex, and its RHEB homolog, Rhb1, does not participate in TORC1 regulation. Even more intriguing, plants do not seem to have specific amino acid sensors, and GATOR/RAG/RAGULATOR or their equivalents have not yet been identified in plants so far. These observations suggest that nutrient sensors may have evolved in response to organism-specific nutritional environments, highlighting both the evolutionary conservation and divergence of the mechanisms that control TORC1 signaling in different organisms.

Together, these findings underscore the versatility and complexity of mTORC1 regulation across multiple cellular compartments. While the lysosome remains the central platform for amino acid and nutrient sensing via the RAG–RAGULATOR–GATOR–KICSTOR axis, alternative mechanisms allow mTORC1 to integrate diverse metabolic cues from other organelles, such as mitochondria, the Golgi, and even the nucleus. These parallel and sometimes redundant pathways highlight the robustness of mTORC1 as a metabolic rheostat capable of maintaining cellular homeostasis under varying nutrient conditions.

Growth factor sensing

Growth factors are key extracellular signals that regulate mTORC1 (Fig. 2). Receptor tyrosine kinases (RTKs), insulin and insulin-like growth factor 1 (IGF-1), WNT and tumor necrosis factor (TNF), inflammatory cytokines, and RAS activate multiple signaling pathways that converge on a major upstream inhibitor of mTORC1, the TSC complex, which is composed of TSC1, TSC2, and TBC1D7.¹⁰² TSC is a GAP that converts RHEB from its GTP-bound active form to a GDP-bound inactive state.^{103,104}

Phosphorylation of TSC components by various kinases leads to TSC complex inhibition and subsequent activation of mTORC1 (Fig. 2). For example, insulin and IGF1 stimulation promote AKT-mediated phosphorylation of TSC2 at multiple sites.¹⁰⁵ As a result, the GAP function of the TSC complex toward RHEB is diminished, leading to an accumulation of active GTP-bound RHEB, which directly stimulates mTORC1 at the lysosomal surface.^{103,106}

Upon insulin stimulation, mTORC1 activates S6K1, which in turn phosphorylates insulin receptor substrate 1 (IRS1) on multiple serine residues, which marks IRS1 for degradation or functional inhibition, thereby dampening further PI3K–AKT signaling upstream of mTORC1.^{107,108} This feedback mechanism acts as a regulatory brake, preventing prolonged or excessive activation of mTORC1 in response to sustained insulin signaling.

In RTK-mediated RAS signaling, the mTORC1 pathway is activated via MAPK/ERK and its effector p90RSK through the RTK → RAS → MAPK/ERK → p90RSK cascade, further resulting in the phosphorylation of TSC2.¹⁰⁹ WNT and TNFα activate mTORC1 by repressing TSC1.^{110,111} (Fig. 2).

Beyond TSC and RHEB regulation, growth factors can stimulate the activation of mTORC1 through alternative routes (Fig. 2). For example, insulin promotes the phosphorylation of PRAS40 by AKT, which is associated with the sequestration of PRAS40 by the cytosolic anchor protein 14-3-3. This sequestration effectively removes the inhibitory effects of PRAS40 on mTORC1, thereby restoring the kinase activity of mTORC1.^{112,113} Finally, the GATOR1 complex appears to be necessary for rapid and robust mTORC1 inhibition following growth factor withdrawal.¹¹⁴

Glucose sensing

Glucose is a major energy source for cells, supplying ATP through glycolysis and oxidative phosphorylation. Under low-glucose conditions, ATP levels drop and the cellular energy status shifts, leading to an increased AMP/ATP ratio. This triggers the activation of AMP-activated protein kinase (AMPK), a key energy sensor that phosphorylates and stimulates the TSC complex, leading to the inhibition of RHEB and subsequent mTORC1 suppression (Fig. 2). AMPK can also phosphorylate RAPTOR, impairing its interaction with other mTORC1 components, thus dissociating mTORC1 from its activators.^{115,116} In addition, under glucose deprivation, AMPK directly phosphorylates WDR24, subsequently disrupting the integrity of the GATOR2 complex and leading to mTORC1 inactivation.¹¹⁷ Glucose starvation also induces the phosphorylation of the leucine sensor LARS1 by ULK1 at residues important for Leu binding, thereby reducing the affinity of LARS1 for leucine.¹¹⁸ This leads to mTORC1 suppression, the inhibition of protein synthesis and the activation of autophagy, highlighting a point of convergence between glucose and amino acid sensing in the regulation of mTORC1.

When glucose is abundant, AMPK activity is reduced, relieving the inhibition of mTORC1 and enabling its activation to support cell growth and proliferation.¹¹⁹ Metabolic products of glucose, such as DHAP or FBP, can activate mTORC1 either via the GATOR2 complex¹²⁰ or via RAGULATOR¹²¹ respectively. In addition, galectin-3, a lipopolysaccharide sensor, promotes the interaction between RAGA, RAGC and RAGULATOR, activating mTORC1.¹²²

Lipid sensing

While amino acids are the primary nutrients sensed at the lysosome, recent studies have demonstrated that lipids can also be sensed by this organelle (Fig. 2). Thus, the GPCR-like protein LYCHOS detects cholesterol sufficiency and activates mTORC1 in a RAG-dependent manner by sequestering GATOR1.¹²³ Additionally, the arginine sensor SLC38A9 signals cholesterol independently of its arginine-sensing function through a conserved cholesterol-interacting motif, promoting the SLC38A9-RAGA interaction and mTORC1 activation.¹²⁴ In contrast, the Niemann–Pick C1 (NPC1) protein, which regulates cholesterol export from the lysosome, binds to SLC38A9 and inhibits mTORC1 signaling through its sterol transport function.¹²⁴

Another example concerns omega-6 linoleic acid (ω -6LA), a polyunsaturated essential fatty acid that must be obtained through dietary sources. ω -6 LA is a direct activator of mTORC1.¹²⁵ ω -6 LA action on mTORC1 depends on the lipid chaperone fatty acid-binding protein 5 (FABP5). In the presence of ω -6LA, FABP5 binds to RAPTOR, enhancing mTORC1 complex assembly and increasing the phosphorylation of S6K1 and 4E-BP1. ω -6 LA activates mTORC1 even in the absence of amino acids and growth factors, which implies that lipids can provide divergent regulatory signals for mTORC1 activation. Phosphatidic acid (PA), oleic acid, and palmitate also activate mTORC1¹²⁶ but do not require FABP5. Instead, mTORC1 and mTORC2 are activated in response to exogenously supplied fatty acids via the de novo synthesis of PA, a central metabolite for membrane phospholipid biosynthesis.

DNA damage response

DNA damage, which can be induced by ultraviolet (UV) light, ionizing radiation, and reactive oxygen and nitrogen species, suppresses mTORC1¹²⁷ (Fig. 2). To counteract the threats posed by DNA damage, cells have evolved the DNA damage response (DDR), which detects DNA lesions, signals their presence, and facilitates their repair. The crosstalk between the DDR and the mTORC1 pathway constitutes a critical mechanism by which cells handle genotoxic and metabolic stresses.¹²⁸

Several molecules signal DNA damage in mammalian cells, including ataxia telangiectasia mutated (ATM), ataxia telangiectasia and Rad3 related (ATR) and DNA protein kinase (DNA-PK),

which, like mTOR, are members of the PIKK kinase family¹²⁸ (Fig. 2). DNA damage activates ATM, which promotes the phosphorylation of CHK2 and p53, which are required for G1/S arrest initiation and maintenance.¹²⁹ In response to DNA damage, p53 is activated by ATM/ATR kinases through phosphorylation and translocates to the nucleus, where it regulates multiple targets, including the AMPK regulatory subunit β and TSC2.^{130,131} Under oxidative stress or hypoxic conditions, ATM activation suppresses mTORC1 signaling by stimulating the AMPK pathway, which in turn enhances TSC activity.^{128,132} ROS-induced DNA damage also stimulates poly(ADP-ribose) polymerase (PARP), thereby activating AMPK and inhibiting mTORC1 through TSC.^{133,134} Inhibition of mTORC1 by rapamycin disrupts a negative feedback loop mediated by S6K1, which normally suppresses PI3K/AKT signaling through the inhibitory phosphorylation of IRS1. Loss of this feedback enhances IRS1 activity, leading to increased PI3K signaling and subsequent AKT phosphorylation. Under conditions of DNA damage or stress, DNA-PK can further contribute to AKT activation.¹³⁵ AKT then further activates mTORC1 by phosphorylating TSC2 and PRAS40.^{136,137} This compensatory activation of AKT limits the efficacy of mTORC1-targeted therapies and underscores the rationale for using dual PI3K/mTOR or mTORC1/mTORC2 inhibitors to prevent feedback-driven survival signaling.

In summary, the PI3K–AKT pathway, along with p53 signaling, connects the DDR and mTORC1. These intricate regulatory mechanisms highlight the multifaceted nature of mTORC1 as a critical sensor and regulator of cellular stress responses.

UPSTREAM REGULATION OF MTORC2

mTORC2 is mainly controlled by insulin and growth factors (Fig. 2). Upon insulin or growth factor stimulation, increased levels of PtdIns(3,4,5)P₃ compete with the mTOR kinase domain for binding to the pleckstrin homology (PH) domain of SIN1. This interaction relieves the SIN1–PH-mediated autoinhibition of mTORC2 activity. Additionally, the binding of PtdIns(3,4,5)P₃ to SIN1-PH facilitates the recruitment of the mTORC2 complex to the plasma membrane, where it can phosphorylate membrane-associated AKT.^{138,139} In addition, insulin induces mTORC2 activation by assembling a supercomplex with the GTPases KRAS4B and RHOA, termed KARATE, which controls the translocation of the insulin-responsive glucose transporter GLUT4 to the plasma membrane during insulin stimulation in adipocytes.¹⁴⁰ Importantly, increased growth factor-mediated PI3K signaling also enhances mTORC2 association with ribosomes, which promotes cotranslational phosphorylation of the main mTORC2 substrate, AKT.^{141,142}

mTORC2 activation can also be triggered by G protein-coupled receptors (GPCRs), which increase intracellular cAMP levels and facilitate glucose uptake through mTORC2-mediated pathways.^{143,144} Additionally, mTORC2 appears to integrate signals from both the glucagon receptor (GCGR) of a GPCR type and the insulin receptor, contributing to improved insulin sensitivity. While prolonged GCGR activation is typically associated with elevated blood glucose levels and impaired glucose tolerance, short-term stimulation enhances glucose utilization and insulin responsiveness through hepatic RICTOR/mTORC2 and AKT signaling.¹⁴⁵ These findings suggest that mTORC2 plays a supportive role in glucose metabolism upon GPCR activation and that this role may be functionally distinct from classical insulin/PI3K/AKT signaling.

Although mTORC1 is the primary complex that responds to amino acid availability, reintroducing amino acids to cells deprived of both serum and amino acids increases the phosphorylation of AKT by mTORC2¹⁴⁶ (Fig. 2). Amino acid mixtures with naturally alkaline pH, but not those adjusted to physiological pH, also stimulate AKT phosphorylation,¹⁴⁷ suggesting that mTORC2 may respond to intracellular pH changes rather than directly to amino acid concentrations. For example, elevated intracellular alkalinity after treatment with ammonium chloride or ammonium

hydroxide also stimulates mTORC2 signaling.¹⁴⁸ In this context, calcium mobilization and subsequent activation of CaMKK β activate AMPK, which might modulate mTORC2 signaling, possibly through direct phosphorylation of mTOR.¹⁴⁹ In addition, cystine activates mTORC2 by promoting SIN1 phosphorylation.¹⁵⁰

Finally, mTORC2 activity can also be modulated by glucose. Glucose starvation directly activates mTORC2 via AMPK phosphorylation of mTOR and RICTOR,¹⁴⁹ leading to increased AKT phosphorylation.¹⁵¹ On the other hand, glucose can activate mTORC2 through acetylation of RICTOR.¹⁵²

DOWNSTREAM OF MTORC1

Posttranscriptional regulation

mTORC1 impacts mRNA maturation by influencing alternative splicing and m⁶A RNA modification.^{153–157} S6K1 phosphorylates SRPK2, enabling the nuclear translocation of this kinase, which in turn phosphorylates serine/arginine-rich (SR) splicing factors. Nuclear SRPK2 enhances interactions between the splicing factors SRSF1 and U1-70K and supports the formation of a complex involving SREBP1, FAM120A, and RNA Pol II, thereby coordinating nutrient-dependent transcription and splicing of lipogenic genes.¹⁵⁵ In addition, S6K1 phosphorylates SKAR at exon junction complexes, increasing the efficiency of translation elongation of spliced transcripts.¹⁵⁸ When mTORC1 is inhibited, nuclear SRPK2 levels decrease, causing intron retention and nonsense-mediated decay of target mRNAs.¹⁵⁵

In *C. elegans*, mTORC1 regulates mRNA splicing and the production of protein-coding mRNA isoforms by increasing the expression and activity of the SR protein RSP-6 (SRSF3/7 in mammals) and other splicing factors, independent of S6K1. Mutations in other *C. elegans* splicing factors, RNP-6 (PUF60 in mammals) and RBM39, which are involved in 3' splice site recognition, are associated with changes in lifespan through mTOR signaling.¹⁵⁹

mTORC1 also regulates m⁶A mRNA methylation, which is critical for cell growth and cancer progression.¹⁶⁰ Via the S6K1–eIF4A/B axis, mTORC1 enhances the translation of WTAP, a component of the METTL3–METTL14–WTAP m⁶A writer complex, particularly owing to WTAP's structured 5' UTR.¹⁵⁴ m⁶A modifications promote mRNA destabilization of MXD2 transcript, thereby increasing c-Myc activity.^{154,156} mTORC1 also increases c-Myc protein translation by stimulating eIF4A helicase activity.¹⁶¹ Additionally, mTORC1 promotes the degradation of c-Myc antagonists, including MAD1 (via phosphorylation by S6K1) and MXD2 (via m⁶A modification), further enhancing c-Myc transcriptional activity.^{154,162}

Protein synthesis

mTORC1 controls protein synthesis through the phosphorylation of eIF4E binding protein and S6K1 kinase.⁴¹ mTORC1 phosphorylates S6K1 at Thr389, activating it, while phosphorylation of 4E-BP1 at multiple sites inhibits its repressor function (Fig. 2, Table 1). Ribosomal protein S6 is the best-known substrate of S6K1, which also phosphorylates many other proteins, including the translation factors eIF4B, eEF2K, and cap-binding protein 80 kDa (CBP80).¹⁶³ S6K1 substrates promote mRNA translation initiation through interaction with eIF4B, which is crucial for the 5' cap binding of the eIF4F complex. 4E-BP1 blocks translation by binding to eIF4E, thereby preventing the formation of the eIF4F complex. mTORC1 phosphorylates 4E-BP1 at several sites, causing it to release eIF4E, which then permits 5' cap-dependent mRNA translation.¹⁶⁴

mTORC1 regulates the translation of 5'-terminal oligopyrimidine (TOP) motif-containing mRNAs, which encode components of the protein synthesis machinery. LARP1, a key repressor of TOP mRNA translation, binds both the 5' cap and adjacent TOP motif to block eIF4F complex assembly.^{165,166} Upon mTORC1 activation, LARP1 is

phosphorylated, relieving this repression^{167,168} and facilitating the translation of TOP mRNAs via interaction with RAPTOR.¹⁶⁶ mTORC1 also modulates translation through 3' UTR shortening, which enhances polysome formation and protein output, especially in genes related to protein folding and degradation.^{169,170}

mTORC1 also controls translation by regulating ribosome biogenesis, promoting rRNA transcription of RNA Pol I via TIF1A¹⁷¹ and translation of ribosomal proteins, whose mRNAs contain TOP motifs.^{165,172} mTORC1 also phosphorylates and inhibits MAF1, a negative regulator of Pol III, enhancing tRNA and 5S rRNA synthesis, which is critical for ribosome function.^{173–175} Finally, mTORC1 controls ribosome concentration, affecting phase separation in the cytosol,¹⁷⁶ providing new insights into how cells balance efficient biosynthesis with the risk of molecular overcrowding.

Nucleotide synthesis

To support DNA replication and RNA production in dividing cells, mTORC1 regulates the availability of one-carbon units necessary for nucleotide biosynthesis. mTORC1 activates the transcription factor ATF4, which in turn upregulates MTHFD2, a mitochondrial enzyme involved in the tetrahydrofolate cycle that contributes to de novo purine synthesis.¹⁷⁷ Additionally, through S6K1, mTORC1 facilitates the phosphorylation and activation of the CAD complex, which is the rate-limiting step in pyrimidine biosynthesis.^{178,179} In cells with hyperactive mTORC1 signaling, disrupting guanylate nucleotide synthesis can lead to DNA damage, as limited nucleotide resources are preferentially allocated to rRNA synthesis to support elevated ribosome production and protein synthesis.¹⁸⁰

Lipid synthesis

mTORC1 plays a significant role in regulating de novo lipid synthesis through the activation of the sterol regulatory element-binding protein (SREBP) transcription factor, which modulates the expression of metabolic genes involved in fatty acid and cholesterol biosynthesis.¹⁸¹ SREBP is active when sterol levels are low. mTORC1 can activate SREBP through S6K1, which phosphorylates and activates SREBP cleavage-activating protein (SCAP). This leads to the translocation of the active form of SREBP to the nucleus and the upregulation of genes involved in lipid synthesis. mTORC1 also phosphorylates and inactivates LIPIN-1, which is a negative regulator of SREBP activity.¹⁸² When mTORC1 is suppressed, LIPIN-1 represses SREBP. mTORC1 activation leads to LIPIN-1 phosphorylation and inactivation, followed by derepression of SREBP and upregulation of lipid biosynthesis.

Autophagy

mTORC1 is a negative regulator of autophagy, a catabolic process by which the cytosol and organelles are sequestered within double membrane-bound vesicles that deliver their contents to the lysosome for degradation and recycling¹⁸³ (Figs. 2, 7). mTORC1 is essential not only for general autophagy regulation but also for specialized forms such as mitophagy, ribophagy, and lipophagy, where it modulates the selective degradation of mitochondria, ribosomes, and lipid droplets, respectively.

mTORC1 is active and autophagy is suppressed under optimal growth conditions, e.g., a sufficient number of amino acids and glucose. To inhibit autophagy initiation, mTORC1 associates with a ULK protein complex composed of ULK1, ULK2, ATG13, ATG101 and the scaffold protein FIP200. RAPTOR interaction with ULK1/2 brings the ULK complex into proximity with mTORC1, allowing mTORC1 to phosphorylate ULK1/2 and ATG13. This phosphorylation prevents kinase activity of the ULK complex, thereby suppressing autophagosome formation.¹⁸⁴

Under nutrient deprivation conditions or during DNA damage, mTORC1 is suppressed and detaches from the ULK1 complex. This dissociation results in the dephosphorylation of ULK1 and ATG13, which activates the kinase function of ULK1. ULK1 then

phosphorylates both ATG13 and FIP200, triggering the recruitment of the BECLIN-1–VPS34 complex to the pre-autophagosomal membrane and facilitating autophagosome formation.

Another mechanism that involves mTORC1 during autophagy is modulated by the autophagy receptor Sequestosome 1 (SQSTM1)/p62, which interacts with RAPTOR and RAGC, thereby recruiting and activating mTORC1 on the lysosomal surface.¹⁸⁵ Recently, the WD-repeat domain protein MORG1 was shown to play a role in maintaining basal autophagy by suppressing mTORC1 signaling via interference with RAG GTPases.¹⁸⁶ Specifically, MORG1 associates with the active RAG GTPase complex, thereby preventing the RAG-dependent recruitment of mTORC1 to lysosomes. Loss of MORG1 leads to increased mTORC1 activity and reduced autophagic flux. Although p62/SQSTM1 can bind MORG1, the latter is not degraded through autophagy. Upon amino acid refeeding after deprivation, p62/SQSTM1 binds to MORG1, which disrupts the ability of MORG1 to inhibit RAGs, allowing mTORC1 activation.

Autophagy can also be induced when cellular AMP levels are high, activating AMPK and resulting in mTORC1 suppression (Fig. 7). In addition, during glucose starvation, AMPK phosphorylates ULK1 at different sets of serines than does mTORC1, which activates ULK1 and initiates autophagosome formation.¹¹⁹ Moreover, the phosphorylation of ULK1 at Ser555 by AMPK is required for proper mitochondrial homeostasis. Loss of this phosphorylation leads to the accumulation of mitochondria with aberrant morphology, which cannot be eliminated by mitophagy.¹⁸⁷

mTORC1 also regulates autophagy at the transcriptional level by modulating the localization of TFEB, which regulates the expression of autophagy and lysosomal genes. mTORC1 phosphorylates TFEB, thereby inhibiting TFEB activity when nutrients are present.^{43,188} Upon mTORC1 inhibition, TFEB becomes dephosphorylated and translocates to the nucleus, where it activates the transcription of genes involved in autophagy and lysosomal biogenesis.

Ubiquitin–proteasome system

The ubiquitin–proteasome system (UPS) is responsible for targeted protein degradation. The activation of mTORC1, either through the loss of TSC1 or TSC2 or in response to growth factors, promotes the expression of proteasome subunits by increasing the activity of the transcription factor nuclear respiratory factor 1 (NRF1). NRF1 drives proteasome gene transcription in response to proteotoxic stress,¹⁸⁹ demonstrating that mTORC1 signaling supports proteasome biogenesis during periods of high anabolic demand. Additionally, mTORC1 can influence substrate degradation by regulating the phosphorylation status of UPS-targeted proteins. For example, phosphorylation of LIPIN1 by mTORC1 allows its recognition by the E3 ligase β -TRCP1, leading to its ubiquitination and proteasomal degradation.¹⁹⁰ Paradoxically, other studies have demonstrated that mTOR inhibition, rather than activation, can induce overall protein degradation via both the UPS and autophagy.¹⁹¹ If mTORC1 activation promotes proteasome biogenesis, why would mTORC1 inhibition also increase degradation?

These discrepancies might be explained and reconciled by recent studies, which demonstrated that after amino acid deprivation, the proteasome moves from its predominant location in the nucleus to the cytoplasm, promoting protein degradation in the cytoplasm and thereby supplying the amino acids necessary for cell survival.^{87,192} This relocation is mediated via the mTORC1 pathway and the lack of three aromatic amino acids, tyrosine, tryptophan, and phenylalanine (YWF), which transmit their signals upstream of mTORC1 by disrupting SESTRIN3 binding to the GATOR2 complex (Fig. 2). Active mTORC1 then phosphorylates p62 and TFEB, influencing proteasomal function and autophagy. When mTORC1 is activated by YWF and the proteasome remains confined to the nucleus, this proteolytic response is suppressed, resulting in cell death. Interestingly, the modulation of the signaling cascade governed by YWF is also applicable to non-

starved cells by using a relatively high concentration of YWF to achieve a surplus relative to all other amino acids.¹⁹² Therefore, proteasome localization, which is regulated by mTORC1 and YWF signaling, could explain why both the activation and inhibition of mTORC1 can lead to increased protein degradation under different conditions and via different mechanisms.

DOWNSTREAM OF MTORC2

AKT, a key substrate of mTORC2, regulates glucose metabolism by inhibiting glycogen synthase kinase 3 β (GSK3 β) and the Forkhead Box O (FOXO) family of transcription factors.¹⁹³ mTORC2 phosphorylates AKT at Ser473 in response to the presence of growth factors, which enhances PI3K activation.^{194,195} mTORC2 also mediates Thr450 phosphorylation of AKT, which is independent of PI3K, occurs during AKT translation and contributes to AKT stability.^{141,196} Phosphorylation at Thr450 is dependent on glucose due to the sensitivity of mTORC2 to ATP levels.¹⁹⁷ AKT also mediates the connection between mTORC2 and mTORC1 by phosphorylating and inhibiting TSC2. Furthermore, mTORC2 contributes to cytoskeleton rearrangements and cell mobility through PKC, a key regulator of the cytoskeletal network.¹⁹⁸ mTORC2 also activates SGK1,¹⁹⁹ which augments the expression and activity of Na⁺/K⁺ ATPase (sodium-potassium pump) and glucose and amino acid carriers in the plasma membrane and promotes the upregulation of Na⁺, Ca²⁺, K⁺, and Cl[−] channels.

Thus, through its key effectors, mTORC2 coordinates glucose metabolism, cytoskeletal dynamics, membrane transport, and ion channel regulation.

REGULATION OF MITOCHONDRIAL HOMEOSTASIS BY MTORC1 AND MTORC2

Both mTORC1 and mTORC2 are essential for mitochondrial biogenesis, dynamics, the phosphorylation of mitochondrial proteins, mitochondrial genome repair, the cellular response to mitochondrial ROS, and the regulation of mitophagy.^{200,201}

mTORC1 (more so than mTORC2) plays a critical role in maintaining mitochondrial mass by balancing two opposing processes: promoting mitochondrial biogenesis and facilitating degradation. Mitochondrial biogenesis is enhanced by mTOR-mediated activity in the transcription and translation of nuclear-encoded mitochondrial RNAs and proteins as well as fusion and fission of preexisting organelles. On the other hand, mTOR also participates in mitochondrial quality control and coordinates the degradation of defective organelles via autophagy and mitophagy.

Mitochondria cannot be synthesized *de novo*. New organelles are formed from preexisting ones through fusion and fission.^{202,203} Mitochondrial biogenesis also requires the replication, transcription and translation of mitochondrial DNA (mtDNA)-encoding genes, as well as the import of lipids and nuclear-encoded proteins.²⁰⁴ Because human mtDNA contains only 37 genes, the majority of proteins essential for mitochondrial function are nuclear encoded. After transcription and translation, these proteins are targeted to the mitochondria and imported into various subcompartments.²⁰⁵ Mitonuclear feedback is achieved through bidirectional communication: anterograde signaling, where information flows from the nucleus to the mitochondria, and retrograde signaling (RTG), where the mitochondria transmit signals to the nucleus in response to mitochondrial dysfunction.^{206,207} mTORC1 plays a central role in mitonuclear communication by modulating various extra- and intracellular stimuli, that regulate mitochondrial biogenesis and function.

mTORC1 in mitochondrial biogenesis

mTORC1 regulates the transcription of mitochondrial genes by acting on the master regulator of mitochondrial biogenesis, peroxisome proliferator-activated receptor gamma coactivator

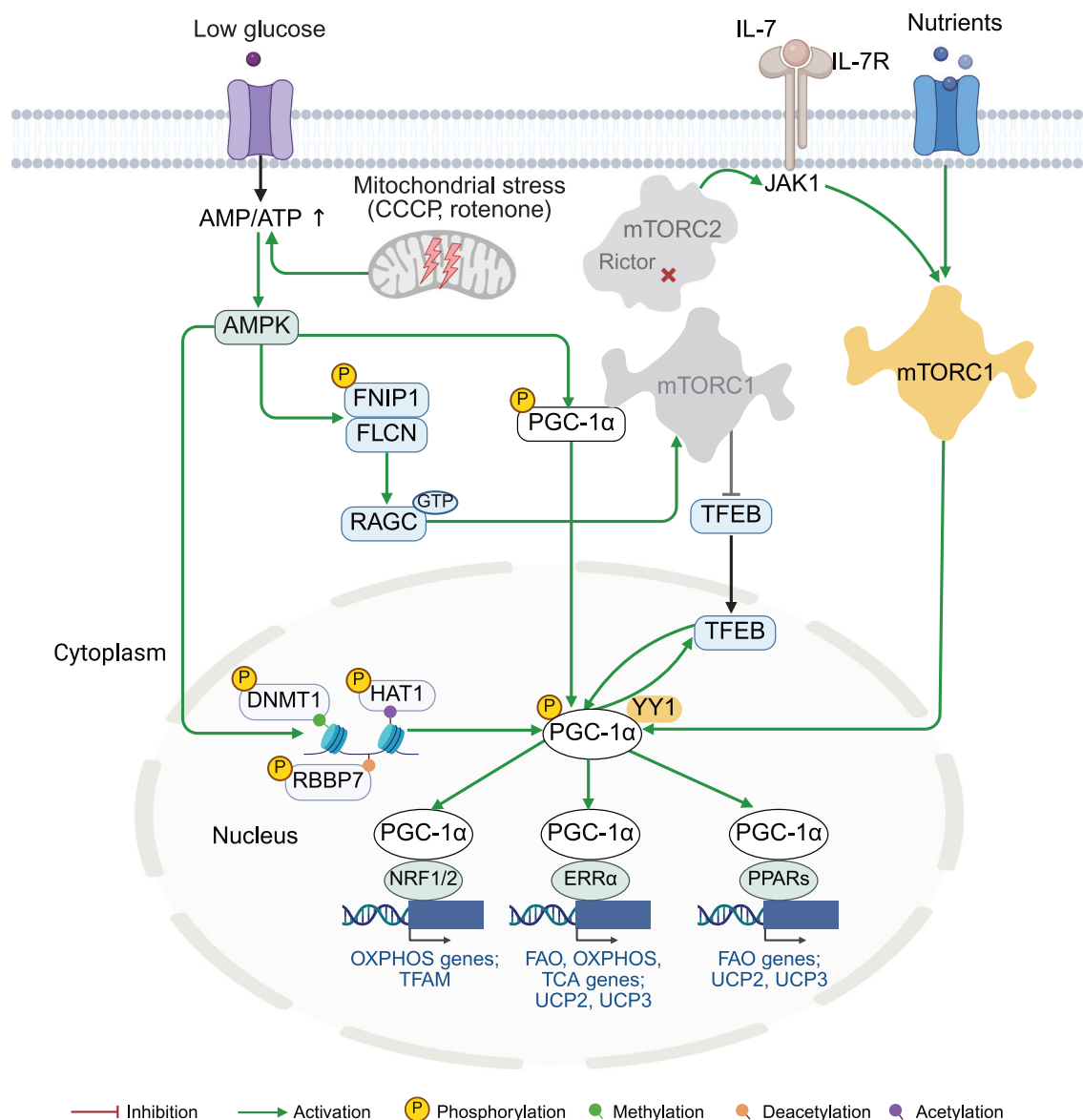


Fig. 3 Regulation of mitochondrial biogenesis by mTOR at the transcriptional level. mTORC1 regulates mitochondrial gene transcription by activating PGC-1 α through multiple mechanisms: (i) via interaction with the transcription factor YY1; (ii) via TFEB dephosphorylation and nuclear translocation; and (iii) through AMPK-mediated phosphorylation of PGC-1 α . Mitochondrial damage (for example, caused by CCCP or rotenone treatment) activates AMPK, which in turn phosphorylates FNIP1 and suppresses FLCN-FNIP1 GAP activity. This maintains RAGC in its GTP-bound form and promotes TFEB nuclear translocation. Nuclear TFEB induces PGC-1 α , which in turn enhances TFEB expression through a positive feedback loop. AMPK also phosphorylates three epigenetic factors: DNA methyltransferase 1 (DNMT1), retinoblastoma binding protein 7 (RBBP7) and histone acetyltransferase 1 (HAT1). This leads to nucleosome remodeling and activation of the promoters of PGC-1 α , TFAM, NRF1 and NRF2, as well as uncoupling of proteins 2 and 3 (UCP2 and UCP3). PGC-1 α couples with distinct transcription factors to regulate mitochondrial metabolism: with NRF1/2 to induce OXPHOS genes and TFAM; with estrogen-related receptor alpha (ERR α) to activate fatty acid oxidation (FAO), the TCA cycle, OXPHOS genes, and the UCP2 and UCP3 genes; and with peroxisome proliferator-activated receptors (PPARs) to drive FAO, UCP2 and UCP3 genes. mTORC2 does not directly control mitochondrial transcription; it modulates upstream IL-7R/JAK1 signaling to influence mTORC1 activity

1-alpha (PGC-1 α)²⁰⁸ (Fig. 3). PGC-1 α is a transcriptional coactivator that interacts with several transcription factors to control the transcription of more than 150 genes encoding mitochondrial proteins involved in fatty acid oxidation, the tricarboxylic acid (TCA) cycle, oxidative phosphorylation (OXPHOS), the mitochondrial ribosomal machinery and membrane transport.

The regulation of PGC-1 α by mTORC1 occurs through multiple mechanisms: by interacting with the transcription factor Yin-Yang 1 (YY1), through the upstream energy sensor AMPK, or via the mTORC1 downstream effector TFEB.

To interact with PGC-1 α via YY1, both the mTOR and the RAPTOR components of mTORC1 bind directly to different sites of YY1.²⁰⁸ This represents one of the rare examples of mTORC1's nuclear activity. Treatment with rapamycin disrupts the interaction between YY1 and PGC-1 α , resulting in decreased expression of mitochondrial genes.

In contrast to mTORC1, AMPK directly interacts with and phosphorylates PGC-1 α , leading to the autoactivation of the PGC-1 α promoter and the induction of several mitochondrial genes²⁰⁹ and epigenetic factors²¹⁰ (Fig. 3). AMPK-mediated

phosphorylation of epigenetic factors not only enhances mitochondrial biogenesis but also increases the membrane potential.

AMPK is also important for the activation of TFEB, which plays an essential role in mitochondrial biogenesis by upregulating PGC-1 α expression (Fig. 3). Mitochondrial stress, induced by the application of electron transport chain inhibitors, triggers the activation of AMPK, which in turn phosphorylates FNIP1. This phosphorylation inhibits FLCN-FNIP1 GAP activity, leading to the accumulation of the inactive RAGC-GTP-bound form, pushing TFEB off the lysosome, and facilitating its nuclear translocation. Once in the nucleus, TFEB enhances the transcription of PGC-1 α .²¹¹ PGC-1 α , in turn, interacts with the promoter region of the TFEB-encoding gene, creating a feedback loop that increases TFEB expression and activity. This loop supports mitochondrial biogenesis, enabling adaptation to genotoxic and metabolic stressors.²¹² Additionally, TFEB can drive mitochondrial biogenesis independently of PGC-1 α by upregulating NRF1/2 and transcription factor A (TFAM), leading to increased mtDNA synthesis.²¹³

mTORC1 stimulates the translation of nuclear-encoded mitochondrial proteins and promotes the transcription of mRNAs related to mitochondrial functions through the phosphorylation of 4E-BP1 and S6K1.^{214,215} On the other hand, mitochondrial activity and its tethering with the endoplasmic reticulum (ER) are linked to the compartmentalization of translation initiation factor eIF4E to polysomes attached to the rough ER (rER).²¹⁶ In mammalian cells with functional mitochondria and active mTORC1, 4E-BP1 is localized on polysomes attached to the rER, leading to the dissociation of eIF4E and its recruitment to the active translation initiation complex eIF4F, enabling protein synthesis. Impaired mitochondrial fusion disrupts the tethering of mitochondria to the rER, reducing eIF4E targeting to the rER. mTORC1 inhibition, which results in decreased 4E-BP1 phosphorylation, reduces the intracellular pool of free eIF4E, promoting its defective compartmentalization to polysomes attached to the rER membrane, which causes retarded translation.

mTORC2 does not directly regulate the transcription of mitochondrial genes but can influence this process indirectly through mTORC1 (Fig. 3). For example, the deletion of *Rictor* leads to an increase in the mitochondrial DNA content and overall mitochondrial mass in immature myeloid dendritic cells.²¹⁷ This effect is attributed to compensatory mTORC1 activity, characterized by increased expression of several upstream regulators of mTORC1 (e.g., IL-7R and JAK1) and activation of its downstream targets S6K1 and 4E-BP1.

Overall, transcriptional and translational regulation act as a feedforward mechanism, where the translation of nuclear-encoded mitochondrial mRNAs is controlled by the mTORC1 pathway to induce ATP production and ensure adequate energy availability for protein synthesis.

mTORC1 and mTORC2 in the regulation of mitochondrial dynamics

Mitochondria are highly dynamic and interconnected in a complex network.²¹⁸ Fusion and fission allow mitochondria to share membranes, metabolites, solutes, and proteins and maintain an electrochemical gradient.²⁰⁴ Nutrient availability drives mitochondrial fragmentation, whereas acute starvation results in the formation of an elongated, tubular-like mitochondrial network.^{219,220}

The fission–fusion cycle helps repair, regenerate, and reintroduce healthy daughter mitochondria into the mitochondrial pool. This process is essential for maintaining a healthy population of mitochondria. Moreover, mitochondrial fission isolates damaged or dysfunctional mitochondria from the healthy network, followed by their selective elimination through mitophagy.

Fission occurs near the smooth ER, which wraps around mitochondria. Fission is regulated by the cytosolic GTPase

dynamins-related protein 1 (DRP1). Upon nutrient sufficiency, DRP1 is recruited to the outer mitochondrial membrane (OMM), where it assembles into a multimeric ring complex that compresses the OMM to trigger division (Fig. 4). DRP1 interacts with OMM-residing proteins, fission 1 protein (FIS1) and mitochondrial fission factor (MFF), which function as DRP1 receptors.^{221,222} Fission is also regulated by posttranslational modifications of DRP1, especially its phosphorylation by cyclic AMP-dependent protein kinase (PKA) at Ser637 and cyclin-dependent kinase (CDK1) at Ser616.²¹⁹

Mitochondrial fusion is controlled by three GTPases from the dynamin superfamily: homologous mitofusin 1 and 2 (MFN1 and MFN2), which reside at the OMM, and inner membrane (IMM)-specific optic atrophy 1 protein (OPA1) (Fig. 4).²¹⁹

Together with its upstream regulators and downstream effectors, mTORC1 plays a significant role in regulating mitochondrial fission and fusion (Fig. 4). In vitro studies have shown that active mTORC1 promotes mitochondrial fission, whereas mTORC1 inhibition during amino acid starvation shifts the balance toward mitochondrial fusion.²¹⁹ Depletion of serum or glucose has the opposite effect, inducing mitochondrial fragmentation. Conversely, rapamycin treatment reduces the expression of FIS1 and DRP1,²²³ as well as DRP1 phosphorylation.²²⁴ This treatment can also increase ER–mitochondria coupling, facilitating mitochondrial fusion.²²⁵

In contrast to in vitro findings, studies in animals (mice^{226,227} and *C. elegans*²²⁸) have shown that fasting leads to mitochondrial fragmentation. This discrepancy may be explained not only by variations between cultured cells and living organisms but also by the time during which starvation is conducted in cell culture (1–2 h) and in animals (24–48 h). During acute starvation, mitochondria might elongate to cope with stress and promote cell survival after nutrient depletion. Long-term fasting induces fission to facilitate the clearance of damaged mitochondria via mitophagy and increases the intercellular supply of nutrients necessary to sustain cellular functions. Importantly, mTORC1 activity also varies depending on the duration of starvation: it is repressed during acute starvation, whereas prolonged nutrient deprivation leads to robust reactivation of mTORC1.^{229,230}

Deletion or overexpression of upstream mTORC1 regulators influences mitochondrial dynamics by promoting fission or fusion, depending on whether mTORC1 is activated or repressed. For example, RHEB knockout inhibits mTORC1, resulting in mitochondrial elongation and reduced DRP1 levels.²³¹ Upstream mTORC1 regulators can also work directly to affect proteins involved in mitochondrial dynamics. For example, AMPK phosphorylates MFF to increase DRP1 levels, thereby promoting mitochondrial fission.¹¹⁹

Among the mTORC1 downstream effectors involved in mitochondrial dynamics are 4E-BP1, S6K1 and TFEB. Phosphorylation of 4E-BP1 by mTORC1 regulates mitochondrial fission by modulating the translation of mitochondrial fission process protein 1 (MTFP1)²³² (Fig. 4). Loss of MTFP1 is associated with increased mitochondrial fusion, whereas its overexpression leads to mitochondrial fragmentation.^{232,233} Treatment of cells with down-regulated MTFP1 with rapamycin induces mitochondrial hyperfusion and branching, resulting in cell death.²³² The deletion of S6K1 in mouse embryonic fibroblasts enhances mitochondrial fission by increasing both total DRP1 and its S616-phosphorylated form (not a target of S6K1 but a substrate of PINK1, CDK1 and other kinases), which in turn stimulates glycolysis.²³⁴ The overexpression of TFEB in the hearts of mice subjected to oxidative stress reduces the expression of the Drp1 and Fis1 mRNAs, diminishing mitochondrial fission.²³⁵

On the other hand, changes in mitochondrial dynamics can also influence mTORC1 activity. For example, the mitochondrial phosphatase phosphoglycerate mutase 5 (PGAM5) promotes

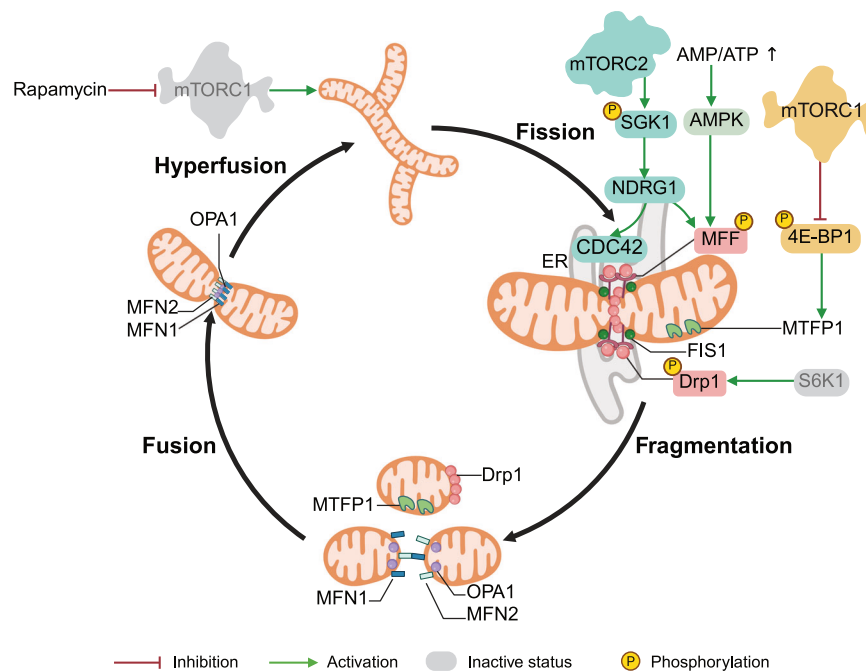


Fig. 4 Role of mTORC1 and mTORC2 in controlling mitochondrial dynamics. mTORC1 phosphorylates 4E-BP1, inactivating its repressor function and relieving cap-dependent blockade of MTFP1 mRNA translation, facilitating mitochondrial fission. Deletion of S6K1 in mouse embryonic fibroblasts enhances mitochondrial fission by increasing total DRP1 and its Ser616-phosphorylated form (a modification mediated by other kinases, not by S6K1). During extended nutrient deprivation, mTORC2 phosphorylates and activates SGK1, which in turn phosphorylates NDRG1 to promote mitochondrial fission. Fusion begins with the docking of two MFN1 molecules in trans, followed by conformational changes and GTP hydrolysis of MFN1, leading to OMM fusion. OPA1, in coordination with cardiolipins, is responsible for IMM fusion. mTORC1 inhibition in vitro (by rapamycin) or during acute starvation promotes mitochondrial fusion

mitochondrial fission by dephosphorylating DRP1.²³⁶ The depletion of PGAM5 accelerates senescence via mitochondrial hyperfusion, reduces mitochondrial turnover, and increases ATP levels, ROS production, and mTOR activity via the downregulation of AMPK and TSC2.²³⁷

mTORC2 also regulates mitochondrial dynamics (Fig. 4). Like mTORC1, prolonged fasting activates mTORC2, promoting mitochondrial fission.²²⁶ During extended nutrient deprivation, mTORC2 phosphorylates and activates SGK1, which subsequently phosphorylates N-myc downstream regulated gene 1 (NDRG1) to promote mitochondrial fission, which requires MFF and not DRP1.²²⁶ NDRG1 recruits the small GTPase CDC42 to mitochondria, where it interacts with the ER at mitochondria-associated membranes. Multiple effectors and regulators of CDC42 also participate in fission.

In *Drosophila*, the knockdown of *Rictor* blocks high-fat diet (HFD)-induced mitochondrial fragmentation and Drp1 recruitment, leading to the development of cardiomyopathy. Interestingly, knockdown of *Akt* did not affect HFD-induced mitochondrial fission. Like mTORC2 inhibition, *Drp1* knockdown prevents HFD-induced mitochondrial fragmentation in *Rictor*-knockdown flies, whereas the overexpression of *Drp1* restores mitochondrial fission.²³⁸

Impaired mitochondrial dynamics can, in turn, influence mTORC2 function. Depletion of *Drp1* in skeletal muscles activates mTORC1 via AKT/mTORC2. Moreover, AKT activation, resulting from *Drp1* deletion, is caused by the mitochondrial localization of mTORC2, which translocates RICTOR to deformed mitochondria-associated membranes, producing mito-bulbs structures. RICTOR is then activated by acetylation at these structures, presumably because of its proximity to mitochondrial acetyl-coenzyme A.²³⁹

Taken together, both mTORC1 and mTORC2 play distinct yet interconnected roles in regulating mitochondrial dynamics, which are critical for maintaining cellular homeostasis and responding to metabolic demands.

mTORC1 and mTORC2 in modulating communication between mitochondria and other organelles in cells

To obtain the necessary building blocks for biogenesis and maintain optimal function, mitochondria must communicate with other organelles. Unlike the ER and Golgi apparatus, which use vesicular transport to interact with different organelles, mitochondria primarily connect with other cellular compartments through contact sites, which facilitate the exchange of metabolites, lipid trafficking, and calcium signaling. The mTORC1 pathway plays a significant role in regulating mitochondrial communication with other organelles via these contact sites.

Mitochondria-lysosome (M-L) contact sites facilitate the direct transfer of lipids, calcium, and other small molecules between mitochondria and lysosomes and modulate mitochondrial and lysosomal network dynamics²⁴⁰ (Fig. 5). In the yeast *S. cerevisiae*, the SEACIT/SEACAT complexes (GATOR1/GATOR2 homologs), which localize to the vacuole membrane, are involved in the maintenance of vacuole-mitochondria contact sites (vCLAMPs).²⁴¹ Deletion of SEACIT components decreases the number of vCLAMPs.²⁴¹ The role of the GATOR1 complex in mammals in the formation of mitochondria-lysosomal contacts has not yet been addressed. Nevertheless, mTORC1 promotes the formation of M-L contact sites by tethering and mediating cholesterol transport from lysosomes to mitochondria. This occurs by regulating the interaction between the lysosomal protein NPC1 and the mitochondrial translocation protein TSPO.²⁴² Another example involves the mitochondrial outer membrane AAA+ ATPase Thorase, which directly interacts with mTOR at the lysosomal surface without binding to the lysosome, leading to mTORC1 disassembly.²⁴³ The number of mitochondria-lysosome contacts is reduced by half in the absence of Thorase. Additionally, mTOR dissociates from lysosomes upon contact with mitochondria-bound Thorase, and Thorase ATPase activity is important for this dissociation (Fig. 5).

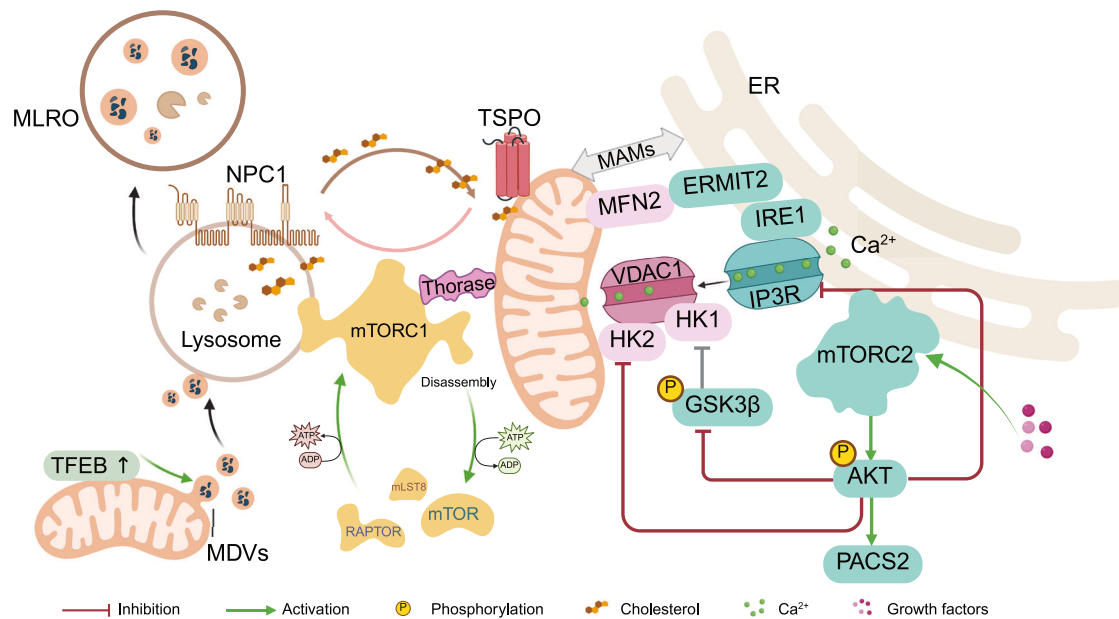


Fig. 5 mTOR mediates mitochondria-lysosome and mitochondria-endoplasmic reticulum crosstalk. mTORC1 promotes mitochondria-lysosome contact site formation and facilitates cholesterol transfer by regulating the interaction between the lysosomal cholesterol exporter NPC1 and the mitochondrial translocator protein TSPO. Damaged mitochondrial proteins are transferred to lysosomes via mitochondria-lysosome-related organelles (MLROs), which are formed through the fusion of mitochondria-derived vesicles (MDVs) with lysosomes. MLROs act as an alternative mitochondrial quality control pathway, selectively degrading mitochondrial proteins via lysosomal fusion, independent of autophagy. The overexpression of TFEB enhances the clearance of MLROs. Mitochondria-ER crosstalk is mediated by mTORC2, which promotes calcium transfer. The mitochondrial mitofusin MFN2 interacts with its splice variant ERMIT2 at the ER to tether mitochondria to the ER. The ER stress sensor inositol-requiring enzyme 1 (IRE1) is highly enriched at mitochondria-associated membranes (MAMs) and regulates calcium exchange between the ER and mitochondria. AKT-mTOR signaling suppresses IRE1 activity by promoting ER-mitochondria contacts. Hexokinase 1 (HK1) binding to the mitochondrial VDAC promotes the entry of metabolites into the mitochondria, increasing mitochondrial respiration. In addition, mTORC2 activation of AKT promotes the phosphorylation and inhibition of MAM-related proteins, such as the 1,4,5-trisphosphate receptor (IP3R) and hexokinase 2 (HK2), to modulate calcium release. Growth factor-stimulated mTORC2 further stabilizes MERCS through AKT-mediated phosphorylation of the MERCS-associated proteins IP3R3 and phosphofurin acidic cluster sorting protein 2 (PACS2)

A novel hybrid cellular structure termed mitochondria-lysosome-related organelles (MLROs) is formed through the fusion of mitochondria-derived vesicles (MDVs) with lysosomes, independent of DRP1. The overexpression of TFEB enhances the clearance of MLROs²⁴⁴ (Fig. 5).

Mitochondria and ER contact sites regulate calcium signaling, lipid transfer, and glucose metabolism.²⁴⁵ Protein folding in the ER is a highly energy-demanding process that requires large amounts of ATP.²⁴⁶ In turn, mitochondrial ATP synthesis depends on calcium transfer from the ER.²⁴⁷

The ER communicates with mitochondria via mitochondria-associated membranes (MAMs), which are also called mitochondria-ER contact sites (MERCs or ERMCSs) (Fig. 5). MERCs describes the structural and architectural organization of the contact sites between mitochondria and the endoplasmic reticulum. MAMs refer to the biochemical composition, including proteins and lipids, that characterize the MERCs (Fig. 5).

MAMs enable the bidirectional trafficking of lipids and calcium²⁴⁸ and play a role in glucose homeostasis.²⁴⁵ They also integrate signals from insulin, nutrients, and reactive oxygen species, allowing cells to adapt to autophagy and ER stress.²⁴⁹ In HeLa cells, treatment with rapamycin increases ER-mitochondria contacts and enhances mitochondrial Ca^{2+} uptake, whereas amino acid depletion does not alter ER-mitochondria colocalization (Fig. 5). This finding suggests that other signaling pathways, in addition to mTORC1, are probably required for the increase in organelle communication in this case.²²⁵

mTORC2 likely plays a more significant role than mTORC1 in the function of MAMs. mTORC2 localizes to MAMs and is essential for

their integrity in a growth factor-dependent manner²⁵⁰ (Fig. 5). Growth factors activate mTORC2, which in turn phosphorylates and activates AKT, which phosphorylates and inhibits GSK3 β at ER-mitochondria contact sites,¹⁹³ facilitating the recruitment of hexokinase 1 (HK1), which catalyzes the first step of glycolysis. The entry of metabolites into the mitochondria is facilitated by HK1 binding to the mitochondrial VDAC, which promotes mitochondrial respiration.²⁵¹

Deletion of *Rictor* results in fewer swollen and ruptured mitochondria, impairing ER-mitochondrial communication.^{250,252} On the other hand, MAM abrogation leads to reduced AKT-mTORC2 activity and insulin signaling.²⁵³

Finally, recent data show that mTORC1 is involved in mitochondrial transfer, the process by which mitochondria are transported from one cell to another, often via tunneling nanotubes (TNTs). TNTs transfer healthy mitochondria from donor cells to damaged recipient cells, thereby rescuing impaired mitochondrial function in recipient cells.²⁵⁴ ROS promote the formation of TNTs by activating PI3K/AKT/mTOR signaling, thereby facilitating the transfer of mitochondria between glial cells. This effectively increases the mitochondrial membrane potential, enhances ATP production, and reduces oxidative damage. Pharmacological inhibition of PI3K, AKT, or mTORC1 effectively blocks TNT formation.²⁵⁵

Taken together, the mTOR pathway has emerged as a key regulator of organelle contacts, which are essential not only for mitochondrial bioenergetics and biogenesis but also for broader cellular processes such as autophagy, protein folding, and insulin signaling. Furthermore, emerging evidence links mTORC1 to mitochondrial transfer between cells, underscoring its role in

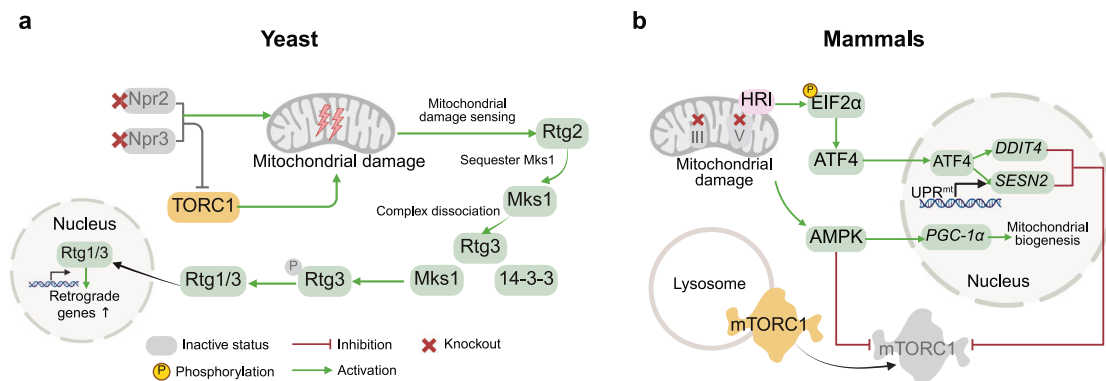


Fig. 6 TORC1/mTORC1 sensing of mitochondrial damage. **a** In yeast, retrograde signaling is dysregulated in Npr2- and Npr3-deleted cells. Mitochondrial damage is sensed by Rtg2, which sequesters Mks1 and drives dissociation of the Mks1-Rtg3-14-3-3 complex. Dephosphorylated Rtg3 dimerizes with Rtg1, translocates into the nucleus, and promotes the transcription of RTG response genes. **b** In mammals, the inhibition of complex III or V induces mitochondrial stress that activates heme-regulated inhibitor (HRI), which signals mitochondrial damage to mTORC1 in an ATF4-dependent manner. AMPK also responds to mitochondrial damage (see Fig. 3)

coordinating intercellular mitochondrial rescue mechanisms under stress. These findings highlight the central role of mTOR in orchestrating mitochondrial communication within and between cells, reinforcing its importance in maintaining mitochondrial and cellular homeostasis.

Role of mTORC1 in the sensing of defective mitochondria
mTORC1 contributes to the detection of defective mitochondria not only by regulating organelle dynamics and isolating damaged organelles through fission but also by modulating communication with the nucleus via retrograde signaling, particularly when the mitochondrial genome needs to be repaired (Fig. 6).

The mitochondrial genome, which comprises numerous mtDNA copies, is particularly vulnerable to mutations due to the absence of histones and has a mutation rate over 10 times greater than that of nuclear DNA. Excessive ROS production, often due to inefficient electron transport chain (ETC) function, can damage mtDNA²⁵⁶ and disrupt the proper folding of mitochondrial proteins, thereby initiating the unfolded protein response (UPRmt) in mitochondria. mTORC1 signaling is closely related to the response to DNA damage not only in the nucleus but also in the mitochondria.¹²⁸

To facilitate mitochondrial DNA repair, all the proteins necessary for this process must be imported from the cytoplasm because mtDNA does not encode them. Prior to this export, the required mRNAs must be transcribed from genes encoded in the nucleus. Consequently, effective communication between the mitochondria and nucleus is essential to ensure detection of mtDNA damage signals and activation of appropriate repair mechanisms. This communication occurs via the retrograde signaling process (RTG), which was initially described in yeast.²⁰⁷ Retrograde signaling enables mitochondria under stress to relay signals to the nucleus, thereby modulating gene expression programs to adjust nitrogen and carbohydrate metabolism. In yeast, RTG was found to be deregulated in deletion mutants of NPR2 and NPR3, components of the SEACIT complex, homologs of the mammalian GATOR1 complex's NPRL2 and NPRL3²⁵⁷ (Fig. 6). Since both SEACIT and TORC1 are localized to the vacuole membrane, information about dysfunctional mitochondria detected by vCLAMPs can be rapidly transferred to the adjacent TORC1 network to initiate degradation.²⁴¹

To reduce the misfolded protein load in the mitochondria, the nucleus initiates the upregulation of several antioxidant enzymes, mitochondrial import proteins, chaperones, and proteases.^{258,259} One of the mTORC1 targets, the ATF4 transcription factor, was identified as an essential player in the UPRmt.²⁶⁰ Mitochondrial stress induced by treatment with antimycin A (an

ETC inhibitor) or oligomycin (a mitochondrial ATP synthetase inhibitor) promotes lysosomal localization and activation of mTORC1. This leads to increased mTORC1-mediated phosphorylation of ATF4 at multiple sites and enhanced binding of ATF4 to the promoters of UPRmt genes (Fig. 6). This process is specific to mitochondrial dysfunction because disruption of mTORC1-dependent ATF phosphorylation blocks only the UPRmt but not the UPR of the ER.

Taken together, these findings indicate that mTORC1 plays a critical role as a sensor and integrator of mitochondrial dysfunction, enabling the cell to detect and respond to various forms of mitochondrial stress. It monitors mitochondrial health through multiple inputs, including changes in energy status, redox imbalance, impaired oxidative phosphorylation, and mtDNA damage. These dysfunctions activate retrograde signaling pathways that converge on mTORC1 to modulate its activity on the basis of the severity and duration of mitochondrial stress. However, the most critical outcome of the mTORC1 response to mitochondrial damage is the initiation of mitophagy.

mTORC1 in the regulation of mitophagy

The elimination of defective mitochondria or organelles in excess is one of the essential functions of mTORC1, which controls both general autophagy and mitophagy²¹ (Fig. 7), whereas mTORC2 is not directly involved in these processes. Overall, the mechanisms by which mTORC1 and AMPK induce autophagy are similar to the mechanisms involved in initiating mitophagy. Both mTORC1 and AMPK are implicated in removing mitochondria at the basal level and through mitophagy when damage to the organelle is excessive.

Mitophagy can be classified into two types on the basis of how mitochondria prime for degradation: ubiquitin-dependent (PINK-PARKIN mitophagy) and ubiquitin-independent pathways. In PINK-PARKIN mitophagy, the protein kinase PTEN-induced kinase 1 (PINK1) accumulates on the outer membrane of defective mitochondria, leading to ubiquitin phosphorylation and the recruitment of the E3 ubiquitin ligase PARKIN. In contrast, PINK1-PARKIN independent mitophagy relies on mitophagy receptors, mitochondrial membrane proteins (e.g., BNIP3, BNIP3L/NIX, FKBP8, FUNDC1), which can interact with LC3 or GABARAP proteins to recruit dysfunctional mitochondria to autophagosomes.²⁶¹

PINK-PARKIN mitophagy can be induced upon rapamycin treatment in cells harboring either large-scale partial deletions of mtDNA or complete depletion of mtDNA.²⁶² Importantly, the mitophagic response in such cells requires both mTORC1 inhibition and loss of mitochondrial membrane potential, which

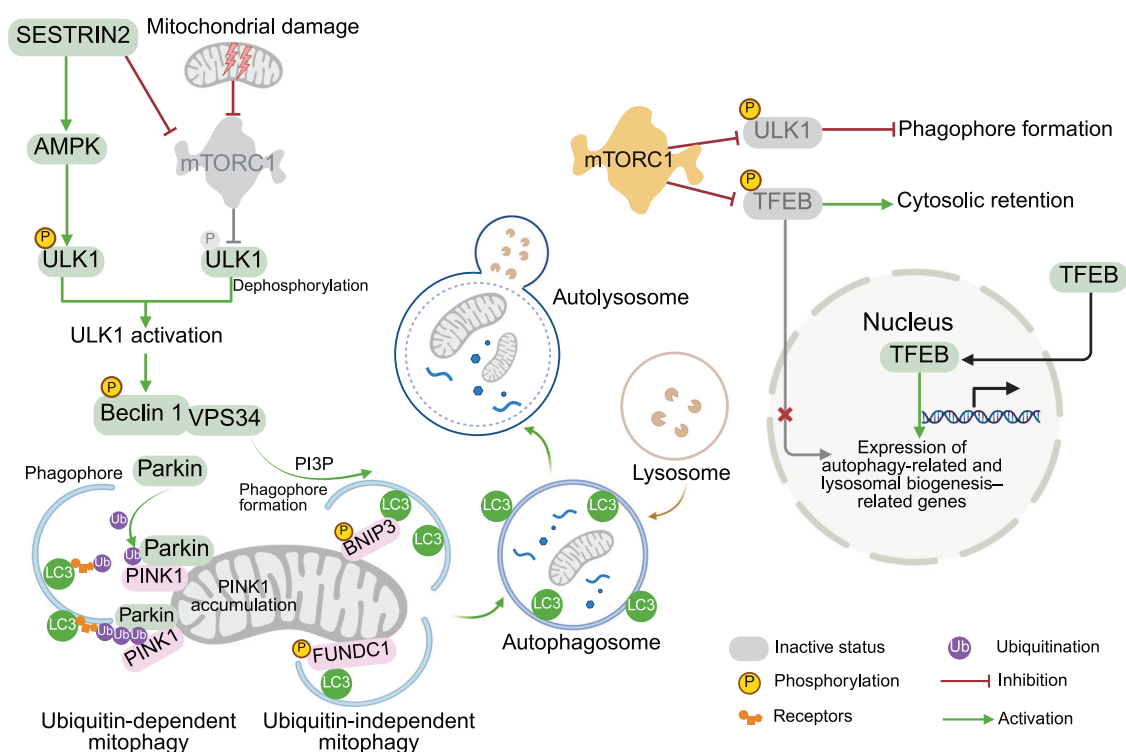


Fig. 7 mTORC1 regulation of ubiquitin-dependent and ubiquitin-independent mitophagy. mTORC1 regulates autophagy and mitophagy to eliminate damaged mitochondria. mTORC1 phosphorylates ULK1 and TFEB to inhibit phagophore formation and lysosomal biogenesis. Upon mitochondrial damage or the inhibitory effect of SESTRIN2, mTORC1 is inactivated and dissociates from lysosomes, allowing ULK1 dephosphorylation and AMPK-mediated phosphorylation. Active ULK1 then phosphorylates BECLIN 1 and recruits VPS34 to nucleate the phagophore, triggering PINK1/PARKIN (ubiquitin-dependent) mitophagy and BNIP3- or FUNDC1-mediated (ubiquitin-independent) mitophagy

activate PARKIN recruitment to mitochondria. Interestingly, mtDNA mutations that impair other mitochondrial functions, such as reduced ATP synthesis or respiratory chain function, are not important for PARKIN recruitment. As a result, mitophagy in these cells is not effective.

Recently, a new mechanism for regulating PINK-PARKIN mitophagy involving mTORC1 was described. Clustered mitochondrial homolog (CLUH) is a cytosolic protein that interacts with cytoplasmic mRNAs encoding mitochondrial proteins, facilitating their translation and distribution.²⁶³ Dysfunctional mitochondria that accumulate due to impaired protein distribution are likely candidates for mitophagy. CLUH RNP particles act as spatial compartments, preserving mRNAs involved in mitochondrial catabolic pathways and promoting their translation. Additionally, they function as signaling hubs by recruiting mTORC1. Upon starvation, CLUH inhibits mTORC1 activation and mitochondrial anabolic pathways, promoting mitochondrial turnover and allowing efficient metabolic reprogramming. In the absence of CLUH, mitophagy impairment causes mitochondrial clustering to be rescued by rapamycin treatment.²⁶⁴ Interestingly, a constitutive lack of CLUH accelerates autophagy but inhibits mitophagy.

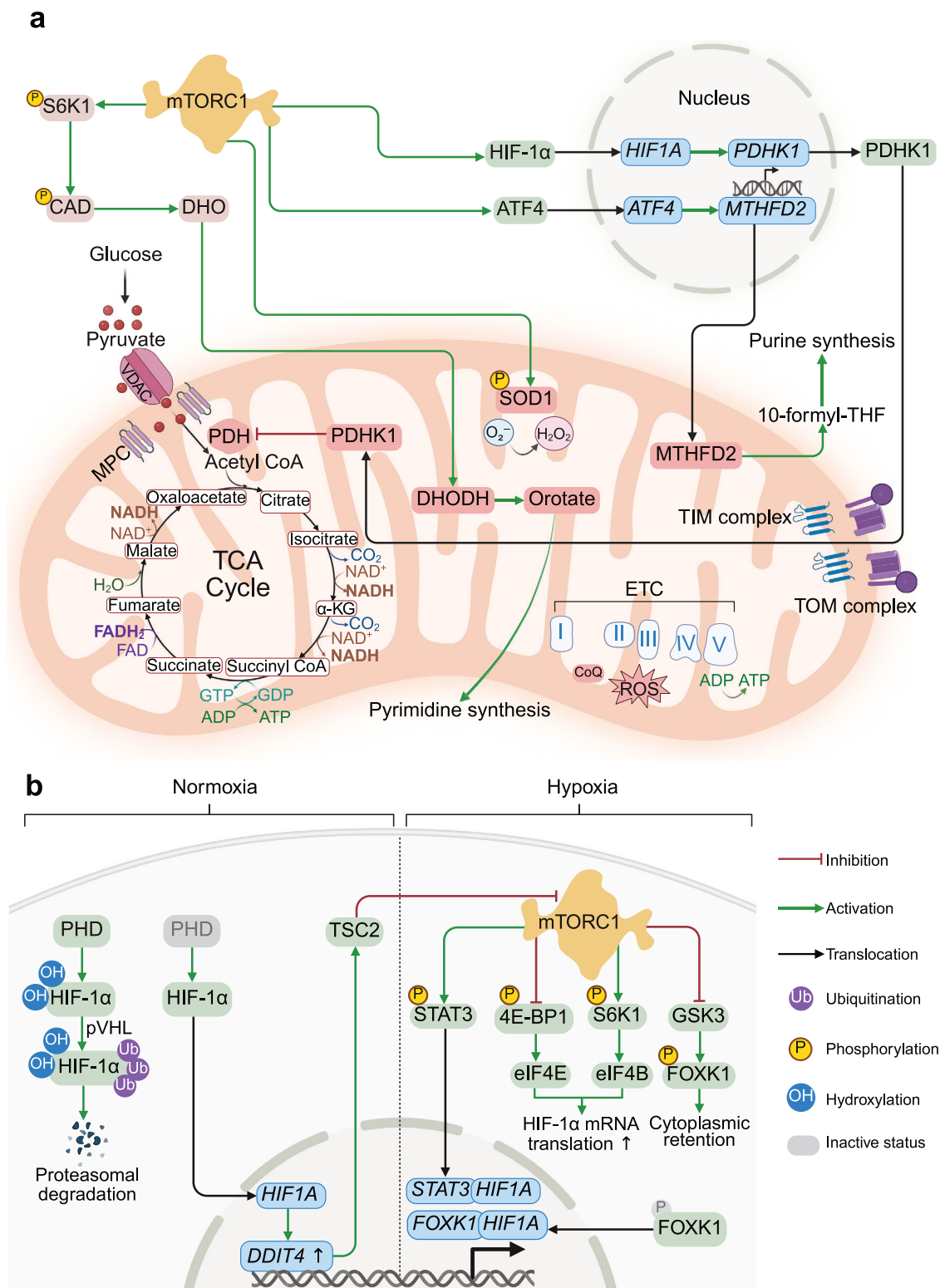
Another reason for mTORC1-related mitophagy defects is linked to mTORC1 hyperactivation due to the loss of function of its upstream negative regulators, such as mammalian TSC2,^{265,266} SESTRIN2²⁶⁷ and the SEACIT complex in yeast.^{241,268} For example, *Tsc2*-deficient neurons progressively accumulate mitochondria in their soma while becoming depleted of functional mitochondria in axons.²⁶⁵ Suppressing mTORC1 hyperactivity with rapamycin or inducing mTOR-independent autophagy with carbamazepine can restore the clearance of damaged mitochondria in *Tsc2*-deficient neurons. A similar effect was observed in pancreatic β cells, where *Tsc2* knockout in cells treated with the mitochondrial uncoupler

CCCP resulted in a reduced $\Delta\psi_m$. mTORC1 hyperactivation and the accumulation of damaged mitochondria with collapsed mitochondrial membrane potential are accompanied by a reduction in PINK1 expression and impaired translocation of PARKIN to uncoupled mitochondria.²⁶⁶ The mitophagy defects in *Tsc2*-deleted cells were rescued by inhibition of mTORC1 with rapamycin.

In addition to its function as a leucine sensor, SESTRIN2 is a stress-inducible antioxidant protein and one of the upstream AMPK activators²⁶⁹ (Fig. 7). It can negatively regulate mTORC1 through two parallel mechanisms: via AMPK during energy sensing and via GATOR2 during amino acid sensing. In mammalian cells, SESTRIN2 interacts with ULK1 and facilitates ULK1-mediated phosphorylation of BECLIN1, which is required for BECLIN1 binding with PARKIN prior to mitochondrial translocation. SESTRIN2 downregulation inhibits the interaction between BECLIN1 and PARKIN, preventing optimal mitochondrial accumulation of PARKIN.²⁶⁷

Finally, during PARKIN-dependent mitophagy, p62, which is phosphorylated by mTORC1, is recruited to ubiquitinated cargo and accumulates on damaged mitochondria.²⁷⁰ This phosphorylation enhances the binding of p62 to ubiquitinated substrates and LC3, thereby promoting autophagosome engulfment and subsequent lysosomal degradation of both p62 and damaged mitochondria.

In BNIP3-mediated mitophagy in mammals, ULK1 phosphorylates BNIP3, enhancing its interaction with LC3 and reducing BNIP3 proteasomal degradation of^{271,272} (Fig. 7). Phosphorylated BNIP3 more effectively facilitates the formation of autophagosomes.²⁷³ The mitochondrial localization of BNIP3 and the translocation of endonuclease G (ENDOG) from mitochondria to the nucleus are crucial steps in BNIP3-related autophagy.²⁷⁴ ENDOG suppresses the mTORC1 pathway by interacting with 14-



3-3y, which releases TSC2.²⁷⁵ Cytoplasmic ENDOG can also bind to 14-3-3y to release RICTOR, activating mTORC2 and promoting acetyl-CoA production.²⁷⁶

Interestingly, accumulation of the BNIP3L/NIX protein not only triggers mitophagy but also activates the mTORC1 pathway. This phenomenon can be observed during lipotoxicity in muscles, where lipid accumulation leads to mitochondrial dysfunction and insulin resistance. BNIP3L/NIX

expression is elevated in the muscle tissue of rodents fed a high-fat diet.²⁷⁷ This results in mitochondrial depolarization and mitophagy.^{278,279} In parallel, BNIP3L/NIX accumulation modulates RHEB-dependent phosphorylation of S6K1 by mTORC1. mTOR signaling is involved in a negative feedback pathway that limits glucose uptake through S6K1-mediated phosphorylation and suppression of IRS1.²⁷⁸ Impaired glucose uptake, in turn, stimulates BNIP3L-dependent mitophagy.

Fig. 8 mTOR regulates the TCA cycle, OXPHOS nucleotide biosynthesis, and hypoxia. **a** Before initiating the TCA cycle in mitochondria, glucose is first metabolized through glycolysis in the cytosol, generating pyruvate. Pyruvate then diffuses through VDAC in the outer mitochondrial membrane and is imported into the matrix by the mitochondrial pyruvate carrier (MPC). In the matrix, pyruvate dehydrogenase (PDH) converts pyruvate into acetyl-CoA, linking glycolysis to the TCA cycle and fueling OXPHOS. Inhibition of PI3K/mTOR signaling enhances phosphorylation of the PDH E1 α subunit, thereby suppressing PDH activity. mTORC1 promotes pyrimidine and purine synthesis through phosphorylating S6K1. mTORC1 promotes purine synthesis through ATF4. ATF4 induces the expression of the mitochondrial enzyme MTHFD2, which generates the 10-formyl-THF required for purine biosynthesis. mTORC1 promotes de novo pyrimidine synthesis via S6K1-mediated phosphorylation and activation of CAD, which catalyzes the early steps of the pathway, leading to dihydroorotate (DHO), which is subsequently converted by dihydroorotate dehydrogenase (DHOH) toward pyrimidine synthesis. mTORC1 promotes the phosphorylation of the superoxide dismutase SOD1 to decrease DHOH activity. **b** Under normoxia (left panel), prolyl hydroxylase (PHD) hydroxylates HIF-1 α for pVHL-mediated proteasomal degradation. In the absence of PHD, HIF-1 α is stabilized and translocates to the nucleus. HIF-1 α induces DNA damage-inducible transcript 4 (DDIT4), which activates TSC2 to inhibit mTORC1. Under hypoxia (right panel), mTORC1 promotes the phosphorylation of 4E-BP1 and S6K1 to increase HIF-1 α mRNA translation, whereas GSK3 inhibition-mediated FOXK1 translocation and STAT3 translocation further facilitates HIF-1 α and glycolytic gene expression. TCA cycle, tricarboxylic acid cycle; ETC, electron transport chain; O $_2^{\cdot -}$, superoxide; H $_2$ O $_2$, hydrogen peroxide; CoQ, coenzyme Q (ubiquinone); pVHL, von Hippel-Lindau protein

Another form of mitophagy that is considered ubiquitin independent is FUNDC1-mediated mitophagy. Under hypoxic conditions, ULK1 phosphorylates the mitochondrial outer membrane protein FUNDC1, enhancing its binding to LC3 and thereby initiating receptor-mediated mitophagy to remove damaged mitochondria.²⁸⁰ In doxorubicin-injured cardiomyocytes, AMPK phosphorylation activates ULK1, which subsequently elevates FUNDC1 and LC3-II/I levels, resulting in increased mitophagic flux.²⁸¹ Conversely, pharmacological inhibition of either AMPK or ULK1 abolishes FUNDC1-mediated mitophagy. Notably, ULK1 can also be locally activated independently of both AMPK and mTOR signaling.²⁸²

Yeast lacks direct homologs of mammalian PARKIN, PINK1, BNIP3, and BNIP3L but compensates with a unique mitophagy pathway centered on proteins such as Atg32.²⁸³ These differences reflect evolutionary divergence between unicellular and multicellular organisms in handling mitochondrial quality control. Nevertheless, the mTORC1 pathway is also involved in mitophagy regulation in yeast. Thus, in *S. cerevisiae*, cells lacking the SEACIT complex exhibited significant suppression of mitophagy during prolonged respiratory growth, whereas other types of selective autophagy were less affected.^{241,268,284} Treatment with rapamycin or deletion of the mTORC1-stimulating RAGA homolog Gtr1 downstream of SEACIT rescued mitophagy defects.²⁶⁸ Whether GATOR1 is involved in mitophagy in mammals remains to be determined.

In conclusion, mTORC1 plays a crucial role in mitochondrial degradation, ensuring proper cellular homeostasis. Alterations in mitochondrial mass due to impaired biogenesis, dynamics, and degradation ultimately impact mitochondrial functionality. As mTOR complexes play crucial roles in regulating these processes, they are indispensable for maintaining optimal mitochondrial performance.

mTORC1 in the regulation of mitochondrial respiration: the TCA cycle, ETC and OXPHOS

The regulation of mitochondrial respiration is one of the key functions of mTORC1.²⁸⁵ Mitochondrial respiration involves glycolysis in the cytoplasm, followed by the TCA (Krebs) cycle in the mitochondrial matrix (Fig. 8a). The electrons generated from these metabolic processes are transferred to the electron transport chain located in the inner mitochondrial membrane, where oxidative phosphorylation takes place, producing the majority of the cellular ATP.

Glucose is crucial for supplying the necessary substrates for mitochondrial respiration. When glucose uptake is impaired, mitochondrial respiration is negatively affected, leading to decreased ATP levels (Fig. 3). The mTORC1 pathway is involved in the expression of glucose transporters through the upregulation of PGC-1 α and in the maintenance of GLUT1 transporters at the plasma membrane.²⁸⁶ Under high-glucose conditions, active mTORC1 promotes the expression of YY1, which then translocates to the nucleus and inhibits PGC-1 α transcription. This inhibition results in a reduced mitochondrial membrane potential and decreased ATP production.²⁸⁷

During exercise, glucose uptake is independent of mTORC1 and PGC-1 α but instead is mediated by TFEB. The dephosphorylation of TFEB, which is necessary for its activation and translocation to the nucleus, is mediated by the calcium-dependent phosphatase calcineurin.²⁸⁸ Exercise-induced calcium influx activates calcineurin. During physical activity, TFEB translocates to the nucleus of skeletal muscle cells and regulates glucose uptake and glycogen content by controlling the expression of glucose transporters (e.g., GLUT4), glycolytic enzymes (e.g., hexokinase), and pathways related to glucose homeostasis (e.g., nitric oxide synthetase).²⁸⁹ In addition, AMPK and mTORC1 regulate mitochondrial respiration by controlling the interaction between the RNA-binding protein SMAUG1 and nuclear transcripts encoding mitochondrial enzymes.²⁹⁰

When glucose is abundant, it can be converted to glycogen for storage. Glycogen synthesis is modulated in an mTORC1-dependent manner.²⁹¹ In the absence of TSC2, hyperactivated mTORC1 phosphorylates S6K1 and inactivates GSK3 β , leading to the dephosphorylation and activation of glycogen synthase, which promotes glycogen synthesis.²⁹¹ Furthermore, because mTORC1 inhibits ULK1, autophagy is impaired, affecting glycogen clearance.²⁹¹

When a cell requires a sustained energy supply, glucose is converted into pyruvate through glycolysis. mTORC1 regulates pyruvate oxidation through the TCA cycle to promote OXPHOS²⁹⁰ (Fig. 8a). Pyruvate dehydrogenase (PDH) is the enzyme that catalyzes the transformation of pyruvate to acetyl-CoA, linking glycolysis and the TCA cycle to drive ATP production through OXPHOS. Inhibition of PI3K/mTOR signaling leads to increased phosphorylation of the E1 α subunit of the PDH, inhibiting its activity. This results in decreased mitochondrial respiration capacity due to impaired pyruvate entry into the TCA cycle.²⁹²

Conditional knockout of the GATOR1 subunit NPRL2 in skeletal muscle leads to chronic mTORC1 activation, which reduces glucose entry into the TCA cycle and decreases the levels of citrate and fumarate.²⁹³ In *S. cerevisiae* strains lacking the NPRL2 homolog Npr2, the levels of TCA cycle metabolites such as citrate, succinate and α -KG are also significantly reduced.²⁹⁴

The reversible hydration of fumarate to malate is catalyzed by fumarase. Fumarase deficiency halts the TCA cycle, leading to fumarate accumulation, which in turn contributes to the upregulation of hypoxia-inducible factor-1 α (HIF-1 α), a transcription factor that facilitates cellular adaptation to hypoxia by promoting aerobic glycolysis.^{295,296} mTORC1 regulates both glycolysis and mitochondrial oxygen consumption by controlling the transcription and translation of HIF-1 α .^{297,298} (Fig. 8b). Specifically, mTORC1 stimulates the expression of glycolytic enzymes by upregulating HIF-1 α through the transcription factor Forkhead box K1, thereby promoting glycolysis rather than OXPHOS.²⁹⁹ Conversely, OXPHOS contributes to lipid metabolism by maintaining phosphatidic acid levels and supporting mTORC1 activity through mtDNA replication. This helps suppress excessive HIF-1 α accumulation and maintains metabolic balance.³⁰⁰

During hypoxia, mTORC1 promotes HIF-1 α expression by directly phosphorylating signal transducer and activating transcription factor 3 (STAT3) to stimulate HIF-1 α mRNA transcription³⁰¹ (Fig. 8b). mTORC1 stabilizes and controls the translation of HIF-1 α through the phosphorylation of 4E-BP1 and S6K1.³⁰¹ Under normoxia, HIF-1 α is hydroxylated by prolyl-hydroxylases (PHDs) and then recognized and degraded by the ubiquitin–proteasome system, maintaining its low intracellular levels. Inhibition of PHD leads to suppressed phosphorylation of mTORC1, S6K1 and 4E-BP1, promoting HIF-1 α expression.³⁰²

mTORC1 inhibition through *Raptor* suppression decreases mitochondrial basal respiration and diminishes ATP production.³⁰³ In addition, mTORC1 inhibition reduces the expression levels of proteins from ETC complexes I–V, whereas mTORC2 inhibition does not affect the expression of ETC proteins. Nevertheless, mTORC2 is also involved in regulating mitochondrial function. The absence of mTORC2 leads to a decrease in the levels of TCA cycle intermediates, especially α -KG.¹⁵¹ In *S. cerevisiae*, TORC2 and Ypk1 (S6K1 homolog) control calcineurin activity, modulating mitochondrial respiratory activity.³⁰⁴

Importantly, mitochondrial dysfunction also alters mTORC1 activity, primarily through reduced oxidative phosphorylation, which lowers ATP production and increases the AMP/ATP ratio, activating AMPK, which phosphorylates TSC2 and Raptor, leading to mTORC1 inhibition and a shift toward energy conservation and stress adaptation.^{115,116} Simultaneously, AMPK activation promotes mitochondrial recovery by stimulating PGC-1 α .²⁰⁹ Thus, AMPK coordinates a dual response to mitochondrial stress: temporarily suppressing energy-intensive growth to conserve resources and enhancing the regenerative capacity of organelles to recover their function and energy supply.

A recent genome-wide screening design to identify mTORC1 regulators further highlighted the importance of mitochondrial health in mTORC1 function.³⁰⁵ This screen revealed that not only AMPK but also another kinase, HRI, signals mitochondrial distress to mTORC1 (Fig. 6). Through its action on ATF4, HRI upregulates SESTRIN2 and REDD1 (stress-induced protein, generally upregulated by DNA damage and ROS). AMPK inhibits mTORC1 shortly after mitochondrial dysfunction begins, whereas HRI do so at later stages. Loss of AMPK and HRI renders mTORC1 signaling resistant to mitochondrial dysfunction induced by oligomycin and antimycin treatments. Another example is the suppression of mTORC1 due to chronic energy stress and elevated AMPK activity in cells with mitochondrial DNA damage. Finally, high levels of ROS produced by defective mitochondria inactivate mTORC1 and initiate mitophagy.

Role of mTOR complexes in mitochondrial ROS production
Mitochondria generate approximately 90% of cellular ROS,³⁰⁶ which are primarily produced during OXPHOS in the ETC. The main sites of mitochondrial ROS production are complex I (NADH dehydrogenase) and complex III (cytochrome bcl complex). At these sites, electrons can leak and interact with molecular oxygen, leading to the formation of superoxide, the primary ROS produced in mitochondria. Superoxide is then converted to hydrogen peroxide by the antioxidant enzymes superoxide dismutases (SOD1 and SOD2). mTORC1 promotes the phosphorylation of SOD1 to decrease its activity.³⁰⁷

High levels of ROS lead to the inactivation of mTORC1, which functions as a feedback mechanism. This inactivation reduces the number of mitochondria through mitophagy, thereby preventing further ROS production.³⁰⁸ Interestingly, low ROS levels can activate mTORC1 through the oxidation of cysteine residues in the mTORC1 complex itself³⁰⁹ or in RHEB.³¹⁰ Rather than causing damage solely, mitochondrial ROS can initiate stress responses and trigger transcriptional changes in the nucleus through retrograde signaling. The RTG pathway can activate stress-defense mechanisms and even help eliminate

signaling molecules, including ROS. In addition, ROS can promote autophagy, which in turn reduces oxidative stress.^{311,312} These “beneficial” ROS can protect cells from additional stress-related damage.³¹³

Several upstream regulators of mTORC1 are crucial in shaping cellular responses to ROS and influencing mTORC1/mitochondria interactions. For example, overexpression of NPRL2, an mTORC1 inhibitor from the GATOR1 complex, promotes ROS production by activating the NOX2 enzyme and exacerbating mitochondrial dysfunction.³¹⁴

Another key player in the mitochondria-ROS-mTORC1 axis is SESTRIN2, which regulates mitochondrial respiration³¹⁵ and exhibits antioxidant properties that mitigate ROS levels.^{311,316} Structural studies on SESTRIN2 suggest that it may act as a direct ROS scavenger.³¹⁷ Moreover, SESTRIN2 expression is upregulated by UVA exposure (315–400 nm) via mitochondrial ROS, and its absence inhibits UVA-induced ROS production.³¹⁸ Therefore, mitochondrial damage caused by UVA contributes to SESTRIN2 induction.

Recently, mTORC2 has emerged as a crucial mediator of the cellular response to intracellular and mitochondrial ROS, particularly under pathological conditions. For example, in mutant KRAS-driven colorectal cancer cells, drug-induced activation of mutant KRAS promoted mitophagy and mitochondrial fission via phosphorylation of AKT and ROS generation, leading to cell death.³¹⁹ During stress conditions in the brain, high levels of ROS contribute to astrocytes dysfunction, which consequently impairs tissue regeneration or aggravates neurotoxicity. Mitochondrial NADPH oxidase (NOX4) is a major source of ROS production. Increased NOX4-dependent ROS production causes the oxidation of RICTOR, impaired mTORC2 activation and AKT phosphorylation during astrocyte differentiation.³²⁰

In summary, mitochondrial ROS are not only byproducts of cellular metabolism but also serve as dynamic signaling molecules that influence a range of cellular processes. Their dual role is exemplified by their ability to inactivate mTORC1 at high concentrations, triggering protective processes such as mitophagy, whereas at lower concentrations, they can activate mTORC1 and promote cell survival pathways. Thus, ROS orchestrate a feedback system that not only maintains mitochondrial health and energy balance but also determines cell fate.

mTORC1 functions in nucleotide biosynthesis

The interplay between mTORC1 and mitochondria plays a central role in coordinating nucleotide biosynthesis with cellular energy and nutrient availability (Fig. 8a). Together, they regulate the production of purines and pyrimidines by integrating metabolic signals to ensure efficient growth and proliferation.³²¹ Mitochondria provide the energy, precursors and cofactors required for nucleotide biosynthetic pathways. ATP generated by mitochondrial oxidative phosphorylation is required for energy-intensive steps in purine and pyrimidine synthesis. The TCA cycle provides precursors for nucleotide synthesis, such as aspartate and fumarate. Aspartate is synthesized from oxaloacetate in the mitochondria, whereas fumarate, released during purine biosynthesis, is fed back into the TCA cycle. In parallel, mTORC1 senses the AMP/ATP ratio via AMPK and promotes mitochondrial oxidative phosphorylation to ensure sufficient ATP for nucleotide synthesis. mTORC1-driven mitochondrial activity maintains high levels of oxaloacetate, which is transaminated to produce aspartate.

Carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD) catalyze the first three steps of de novo pyrimidine synthesis. mTORC1 promotes pyrimidine synthesis through S6K1-dependent phosphorylation of CAD.¹⁷⁸ In addition, RHEB³²² and mLST8³²³ interact with CAD and upregulate its activity. Loss of the mitochondrial NAD⁺-dependent deacetylase sirtuin 3 results in mTORC1 activation, induction of mTORC1-dependent CAD phosphorylation and increased pyrimidine synthesis.³²⁴

mTORC1 also promotes purine synthesis via the transcription factors ATF4, MYC and FOXK1,^{177,299} which control a variety of metabolic enzymes that contribute to purine biosynthesis. The expression of mitochondrial methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), which is necessary for purine synthesis, depends on ATF4.¹⁷⁷ In B cell germinal centers, however, MTHFD2 expression is driven by YY1 rather than by ATF4.³²⁵ Depletion or inhibition of MTHFD2 leads to a reduction in mTORC1 activity in B cells, whereas supplementation with purines and the antioxidant glutathione effectively restores mTORC1 activity in MTHFD2-deficient cells.

mTORC1 also stimulates the formation of “purinosomes”, enzyme complexes on the mitochondrial surface dedicated to de novo purine synthesis.³²⁶ mTOR inhibition reduces purinosome-mitochondrion colocalization and suppresses the purinosome formation stimulated by mitochondrial dysregulation.

In summary, mTORC1 and mitochondria synchronize cellular growth with metabolic capacity, ensuring that nucleotides are produced when energy and nutrients are sufficient. mTORC1 not only enhances mitochondrial ATP production and supplies biosynthetic precursors but also directly regulates key enzymes and transcription factors involved in purine and pyrimidine synthesis. This integration ensures that cells maintain a balanced supply of nucleotides to support proliferation and stress adaptation. Disruption of this axis impairs nucleotide metabolism and compromises mTORC1 activity itself, highlighting a reciprocal relationship that is critical for cellular growth and homeostasis.

mTORC1 and mitochondria in the regulation of cell death

mTORC1 and mitochondria are involved in modulating different forms of programmed cell death, including apoptosis, ferroptosis, cuproptosis, and pyroptosis. mTORC1 does not directly “select” a specific cell death pathway but rather modulates cellular conditions, such as energy status, redox balance, and autophagy activity, that favor or suppress distinct forms of cell death. For example, mTORC1 promotes survival by inhibiting apoptosis and autophagy, while its inhibition can sensitize cells to autophagy-dependent death, ferroptosis, or, in certain contexts, necroptosis and pyroptosis. This regulatory flexibility allows mTORC1 to influence cell fate decisions in a context-dependent manner.

The communication between mTORC1 and mitochondria during apoptosis is influenced by the metabolic state, ROS production, mitochondrial dynamics, and autophagy.³²⁷ BCL-associated X (BAX) and BCL2 homologous antagonist/killer (BAK) are key effectors that initiate the apoptotic pathway. They form pores on the OMM, increasing membrane permeability and allowing the release of mitochondrial apoptogenic factors such as cytochrome *c* into the cytosol.³²⁸ Consequently, cytochrome *c* binds to apoptotic protease-activating factor 1 (APAF-1), recruiting and activating caspase-9. This initiates the formation of apoptosomes and activates a cascade of downstream caspases, leading to cell death.^{329,330}

mTORC1 can also inhibit apoptosis through autophagy; conversely, inactivation of mTORC1 can induce apoptosis by promoting autophagy.³³¹ Necroptosis, pyroptosis and ferroptosis are also regulated by mTORC1 through autophagy.³²⁷

Ferroptosis is a type of programmed cell death dependent on the accumulation of lipid peroxides. One of the reasons for this accumulation is an increased mitochondrial membrane potential that occurs upon cysteine deprivation.³³² Cysteine is an essential amino acid that is important for the synthesis of the antioxidant glutathione, which protects cells from oxidative stress. Cysteine activates mTORC1 and facilitates the synthesis of the GPX4 enzyme, which suppresses ferroptosis by using the antioxidant glutathione to remove lipid peroxides.³³³ The inhibition of mTORC1 by Torin1 prevents ferroptosis, restoring mitochondrial ETC dysfunction and promoting the reformation of the tubular mitochondrial network.³³⁴ Thus, mTORC1 regulates ferroptosis both by autophagy and by modulating GPX4 activity.

Cuproptosis is triggered by the accumulation of copper ions, which target components of the TCA cycle. This binding leads to aberrant protein aggregation and destabilization of iron-sulfur cluster proteins, causing mitochondrial stress, oxidative damage, and eventual cell death. Ferredoxin 1 (FDX1), a reductase that reduces Cu²⁺ to its more toxic Cu¹⁺ form, and a number of factors involved in protein lipoylation (e.g., dihydrolipoamide S-acetyltransferase, DLAT) are key regulators of cuproptosis.³³⁵ The upregulation of DLAT is associated with mitochondrial dysfunction, activation of the PI3K/mTOR pathway, autophagy suppression, and eventual cell death.³³⁶

Pyroptosis is a pro-inflammatory form of programmed cell death characterized by gasdermin-mediated membrane pore formation and the release of IL-1 β and IL-18.³³⁷ Pyroptosis is typically initiated in response to lipopolysaccharide, extracellular ATP, mitochondrial DNA leakage, and certain chemical agents, such as cisplatin. These stimuli activate cytosolic pattern recognition receptors (PRRs), leading to the assembly of inflammasomes and culminating in membrane rupture and inflammatory cytokine release. mTORC1 inhibition via rapamycin can attenuate pyroptosis by reducing mitochondrial ROS and activating mitophagy, thereby limiting inflammasome activation. Adrenomedullin, a cytoprotective peptide hormone, further enhances this protective effect by activating the ROS-AMPK-mTOR axis, increasing mitochondrial clearance and suppressing pyroptotic signaling.³³⁸ mTOR also influences pyroptosis through the STAT3, PPAR γ , NRF2, and metabolic signaling pathways, underscoring its multifaceted role in controlling inflammatory cell death.^{339,340}

MTOR IN PHYSIOLOGY

mTOR signaling in metabolic tissues

While mTOR is active across all tissues, its regulatory effects are especially critical in metabolic organs such as the liver, skeletal muscle, and adipose tissue, which maintain glucose and lipid homeostasis. These tissues respond dynamically to nutrient levels, hormonal cues, especially insulin, and the cellular energy status, converging on mTOR complexes to determine whether cells undergo anabolic growth or catabolic breakdown (Fig. 9).

In the liver, mTOR signaling modulates energy metabolism in response to feeding and fasting. During fasting, hepatic glucose production occurs through glycogenolysis, which prevents hypoglycemia. Following a meal, glycolysis, glycogenesis, and lipogenesis become dominant.³⁴¹ During feeding, elevated insulin coactivates mTORC1 and mTORC2, facilitating nutrient storage and tissue growth. In contrast, fasting lowers insulin, shifting the metabolic program toward autophagy.³⁴² This transition is regulated by mTORC1 inhibition, enabling the degradation of cellular components to fuel gluconeogenesis. Fasting-induced liver mass loss (~25%) is abolished in mice lacking TSC1, RAPTOR,³⁴³ or ATG7,³⁴⁴ confirming the role of mTORC1 in switching from anabolism to catabolism. Mice with liver-specific knockouts of *Raptor* or *Rictor* exhibit hyperglycemia, glucose intolerance, and insulin resistance, mirroring aspects of type 2 diabetes.^{345,346}

The role of mTORC1 in liver fat synthesis is context dependent. Liver-specific mTORC1-deficient mice show reduced triglycerides and cholesterol only under high-fat diets, suggesting that mTORC1 is essential for hepatic steatosis under pathological conditions.¹⁸² This is relevant for understanding selective hepatic insulin resistance, where insulin fails to suppress gluconeogenesis but continues to activate lipogenesis, paradoxically causing both hyperglycemia and hyperlipidemia.³⁴⁷ In contrast to this model, mice with deletions of lymphatic *Tsc1*, which demonstrate persistent mTORC1 activation, are protected from hepatic steatosis.³⁴⁸ This is due to impaired AKT signaling via mTORC1-mediated negative feedback loop, as both AKT and mTORC1 are independently essential for full lipogenesis.^{343,349} AKT promotes

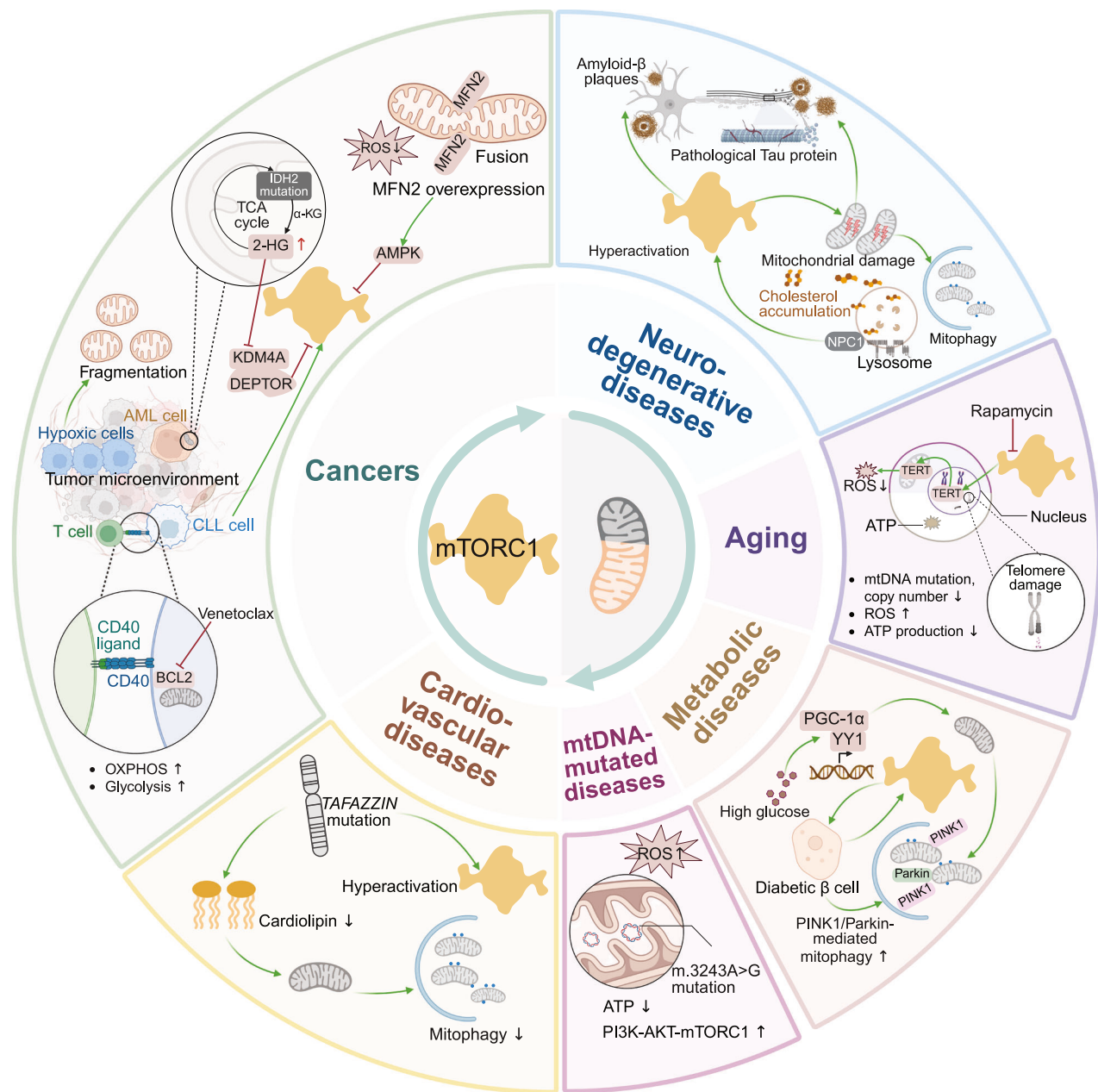


Fig. 9 Deregulation of mTORC1 signaling and mitochondrial dysfunction in human diseases. Schematic illustration showing crosstalk between mTORC1 signaling and mitochondrial homeostasis under different pathological conditions. Representative disease contexts are indicated: cancer (AML, CLL), neurodegenerative disorders (Alzheimer's disease with amyloid-β plaques and tau pathology), aging, metabolic disorders (diabetes, obesity), mtDNA mutation syndromes and cardiovascular diseases. Key mechanisms include altered fusion–fission balance (MFN2), impaired mitophagy (PINK1/Parkin), dysregulated bioenergetics (ATP, ROS), and transcriptional regulation (YY1/PGC-1α). Perturbations in the tumor microenvironment, hypoxia (KDM4A, IDH2 mutation, 2-HG), and therapeutic interventions (rapamycin, venetoclax, DEPTOR) are also illustrated

lipogenesis by suppressing INSIG2a, an SREBP inhibitor, whereas mTORC1 facilitates SREBP activation.

The role of mTORC2 plays an equally significant role in the liver is, acting as a key integrator of hepatic glucose and lipid metabolism. Liver-specific *Rictor* knockout mice exhibit total hepatic insulin resistance, as evidenced by hyperglycemia and defective lipogenesis.^{345,346,350} This results from impaired AKT phosphorylation, which reduces SREBP-1c activity. Rescue of AKT signaling restores lipogenesis, demonstrating that mTORC2 controls SREBP-1c via AKT but not through INSIG2a suppression.³⁴⁶ Additionally, mTORC2 enhances glycolysis through the activation of glucokinase and the transcription factor ChREBP and

suppresses gluconeogenesis by limiting nuclear FOXO1, a major gluconeogenic regulator.

Mice are unable to suppress mTORC1 during fasting because of constitutively active RAGA and suffer lethal hypoglycemia due to failure of autophagy and energy conservation.³⁵¹ Similar fatal outcomes occur in mice lacking Sestrins or autophagy-related genes.^{352,353}

mTOR complexes are vital for maintaining muscle mass. In skeletal muscle, mTORC2–AKT signaling enhances glucose uptake and glycogen synthesis, whereas mTORC1 supports protein synthesis from circulating amino acids. Muscle-specific deletions of mTOR³⁵⁴ or Raptor³⁵⁵ lead to progressive muscle

dystrophy, diminished oxidative capacity, and glycogen accumulation, despite hyperactivation of AKT caused by disruption of mTORC1 feedback inhibition, which is mediated through S6K1–IRS1 signaling. Cardiac-specific deletions of mTOR³⁵⁶ or Raptor³⁵⁷ result in dilated cardiomyopathy, which is attributed to loss of 4E-BP1 repression, leading to diminished protein synthesis and heart failure. Disruption of GATOR1 function in skeletal muscle leads to sustained mTORC1 activation, both in fed and fasted states.²⁹³ Muscle-specific NPRL2 knockout mice exhibit hypertrophic fibers and a shift in fiber-type distribution, characterized by an increase in fast-twitch glycolytic fibers and a reduction in slow-twitch oxidative fibers. These animals also display modified running performance and improved glucose tolerance.

White adipose tissue is the body's principal energy reservoir, and mTOR signaling regulates adipogenesis, lipid storage, and energy consumption. mTORC1 is essential for the differentiation of preadipocytes via the activation of PPAR γ and SREBP transcription factors, which play essential roles in adipogenesis.^{358–360} Mice lacking S6K1 have a lean phenotype and are protected from obesity,³⁶¹ whereas the deletion of 4E-BPs leads to increased obesity and decreased energy expenditure.³⁶² mTORC1 inhibition via rapamycin blocks adipocyte differentiation, whereas hyperactivation through TSC2 deletion enhances fat cell formation.³⁶³ Adipose-specific Raptor knockout mice are lean, resistant to diet-induced obesity, and show enhanced energy expenditure.³⁵⁸ These results underscore the mTORC1–S6K1 axis as the major effector controlling adipose physiology.

mTORC2 in adipose tissue affects systemic growth and metabolism through endocrine mechanisms. Adipose-specific *Rictor* knockout mice exhibit increased lean mass, likely due to elevated hepatic IGF-1 and pancreatic insulin secretion, as mTORC2 in white adipose tissue negatively regulates these hormones via feedback loops.³⁶⁴

Together, these findings highlight the tissue-specific complexity of mTOR signaling in metabolic regulation. The precise tuning of mTOR activity across these organs is critical for whole-body metabolic homeostasis, and its dysregulation contributes to the pathogenesis of insulin resistance, obesity, and type 2 diabetes.

mTOR in immunity

mTORC1 is essential for regulating immune cell metabolism, differentiation and effector functions.³⁶⁵ In the resting state, T lymphocytes rely on catabolic pathways and autophagy to generate essential biomolecules for maintaining protein synthesis and energy production. Upon activation, these cells undergo a metabolic shift toward anabolism, increasing glycolysis to meet the demands of rapid proliferation. This transition is tightly regulated by mTORC1, which activates key metabolic programs required for T-cell activation. CD4⁺ T cells differentiate into various effector subtypes, including Th1, Th2, and Th17, in response to specific cytokine signals. mTORC1 supports this differentiation by enhancing glycolysis and lipid biosynthesis.^{366,367} mTORC1-deficient T cells have an impaired ability to differentiate.³⁶⁶ Similarly, T cells lacking the mTORC1 activator RHEB also fail to differentiate into Th1 and Th17 lineages.³⁶⁸ Conversely, deletion of the mTORC1 inhibitor TSC1 leads to hyperactivation of the pathway, resulting in increased Th1 and Th17 differentiation and multiorgan inflammation in mouse models.³⁶⁹ Furthermore, deficiencies in amino acid transporters for leucine LAT1³⁷⁰ and ASCT2 for glutamine³⁷¹ disrupt Th1 and Th17 differentiation in an mTORC1-dependent manner. In CD8⁺ T cells, mTORC1 activity supports the generation of cytotoxic effector cells that eliminate infected or malignant cells. However, excessive mTORC1 signaling can impair the development of long-lived memory CD8⁺ T cells, ultimately weakening secondary immune responses.^{372,373}

Tregs are immune cells that negatively control both cytotoxic effector CD8⁺ T cells and effector CD4⁺ T cells. mTORC1 activity

has negative effects on Treg generation,^{374,375} while it is positively correlated with the inhibitory effects of Tregs on other T cells.³⁷⁶ Active mTORC1 promotes the conversion of Tregs to effector-like T cells and further impairs Treg stability and function.^{369,377,378} Signals from toll-like receptors, which stimulate Treg proliferation, also increase mTORC1 activity, glycolysis, and the expression of the glucose transporter Glut1.³⁷⁷

B cells undergo multiple steps of maturation before becoming plasma cells and acquiring antibody-secreting capacity, which is critical for defense against recurrent infections. B-cell development similarly depends on mTORC1, which is most active in early developmental stages and is gradually downregulated as cells mature.^{379,380} Deletion of RAPTOR prior to B-cell lineage specification results in a complete block in B-cell maturation.^{379,380}

mTORC1 and mitochondria are tightly interconnected hubs that orchestrate immune cell metabolism and function and participate in the metabolic reprogramming of immune cells.^{381,382} Like in many other cell types, mTORC1 promotes mitochondrial biogenesis and function via PGC-1 α , which enhances mitochondrial oxidative capacity in T cells and macrophages. mTORC1 also drives the metabolic switch to glycolysis in activated CD4⁺ and CD8⁺ T cells, supporting their proliferation and effector functions. Mitochondria play a crucial role in maintaining this metabolic switch by providing substrates for biosynthesis. Both mTORC1 and mTORC2 regulate the balance between effector and memory T-cell formation. Memory T cells rely more on mitochondrial metabolism, including fatty acid oxidation, to sustain their long-term survival and function. TORC1 promotes M1 macrophages (proinflammatory), which depend on glycolysis, while inhibiting M2 macrophages (anti-inflammatory), which rely on OXPHOS and mitochondrial metabolism. Additionally, mTORC1 regulates dendritic cell activation and antigen presentation by influencing mitochondrial dynamics and energy demands during the immune response.

Interestingly, B cells seem to have an mTORC1-independent mechanism for coordinating nutrient sensing and mitochondrial metabolism, which still depends on RAG-GTPases. Specifically, the RAGA/B complex constrains TFEB/TFE3 activity to prevent mitophagy dysregulation and maintain mitochondrial fitness, independent of canonical mTORC1 activation. RAGA/B deficiency results in reduced mitochondrial membrane potential and mitochondrial ROS, a phenotype distinct from that observed in *Raptor*-deficient B cells. This deficiency also leads to TFEB/TFE3 overactivation and abnormal mitophagy. TFEB/TFE3 deletion restores B-cell development and germinal center formation, whereas RAGA/B-deficient B cells fail to form germinal centers, which are crucial for B-cell proliferation and maturation, thus impacting humoral immunity.³⁸³

An emerging area of interest in immune regulation involves the cGAS–STING pathway, which connects mitochondrial stress to inflammatory signaling. Mitochondria, which retain ancestral bacterial motifs, are released into the cytosol or extracellular space as DAMPs upon cellular damage and are sensed by innate immune receptors to initiate inflammatory and antimicrobial defense responses.^{384,385} Upon stress, mitochondrial DNA leaks into the cytosol to activate the cGAS–STING pathway, while mitophagy is initiated to remove damaged mitochondria and suppress the inflammation triggered by mtDNA release.^{386,387} The mitophagy activator urolithin A, which indirectly inhibits mTOR through the PI3K/AKT/mTOR pathway, has been shown to reduce cytosolic mtDNA-dependent activation of the cGAS/STING pathway and subsequent inflammation.^{387,388} The cGAS–STING pathway also drives lysosome biogenesis via TFEB.³⁸⁹ Upon activation of cGAS–STING by cytoplasmic DNA, STING promotes lipidation of the autophagy-related protein GABARAP, which sequesters the FLCN–FNIP complex and blocks its GAP activity toward the RAGC/D GTPase. This selectively prevents mTORC1 from phosphorylating TFEB without affecting mTORC1's catalytic activity toward its other

substrates. Dephosphorylated TFEB then translocates to the nucleus to activate the transcription of lysosomal biogenesis genes, thereby enhancing lysosome formation and autophagic flux.

cGAS-STING activation drives the production of type I interferons (IFNs) as part of the innate immune response.³⁹⁰ Type I IFN- α signaling triggers mitochondrial oxidative stress and lysosomal alkalization via mTOR activation, impairing autophagic flux in monocytes. This leads to cytosolic mtDNA accumulation, which activates the cGAS-STING pathway, driving proinflammatory cytokine production. Inhibition of mTOR restores lysosomal acidification, rescues autophagy, and suppresses STING-dependent inflammation.³⁹¹

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that modulates macrophage glycolytic reprogramming by suppressing mTOR. Upon LPS stimulation, IL-10 prevents the metabolic shift from OXPHOS to glycolysis in macrophages. By inducing DNA damage inducible transcript 4, an mTOR inhibitor, IL-10 preserves the mitochondrial membrane potential, reduces ROS accumulation and facilitates mitophagy.³⁹²

These findings suggest that both the proinflammatory cGAS-STING-IFN axis and the anti-inflammatory cytokine IL-10 play crucial roles in mTOR-mediated mitochondrial inflammation and highlight mTOR inhibition as a potential therapeutic strategy for treating inflammatory diseases.

In summary, mTORC1 integrates both internal and external signals, including cues from the immune microenvironment, to orchestrate the development, differentiation, and functional specialization of immune cells and inflammatory responses.

mTORC1 modulation of aging and lifespan

mTORC1 is recognized as a central driver of aging (Fig. 9). Inhibition of mTORC1 via genetic deletion of mTOR, RAPTOR, or S6K1 extends lifespan in yeast,³⁹³ worms,³⁹⁴ flies,³⁹⁵ and mice.³⁹⁶ Pharmacological inhibition with rapamycin similarly enhances both lifespan and health span in middle-aged mice, delaying age-related functional decline.³⁹⁷

mTORC1 inhibition promotes longevity through multiple pathways. A major mode of action is the restoration of autophagy, which clears damaged proteins and organelles and decreases with age. Autophagy activation increases lifespan in mice,³⁹⁸ but mTORC1 inhibition does not extend lifespan in autophagy-deficient worms,³⁹⁹ suggesting that without functional autophagy, the longevity benefit of mTORC1 inhibition is limited in this organism.

Another key mechanism involves the suppression of protein synthesis. Inhibition of mTORC1 reduces translation-related stress, limits the production of misfolded proteins, and alleviates oxidative damage. Dietary restriction mimics this effect by reducing mTORC1 activity and protein synthesis. In support of this, loss of S6K1 extends the lifespan of *C. elegans*⁴⁰⁰ and mice,⁴⁰¹ likely by reducing the translation burden and improving proteostasis.

mTORC1 can regulate life span by modulating telomerase function. In telomerase-deficient mouse models, the mTORC1 pathway is activated, as manifested by increased phosphorylation of S6K1. Inhibition of the mTORC1 pathway in wild-type mice extends the median lifespan by 39%. However, in mice with telomerase deficiency, rapamycin reduces the median lifespan, indicating that mTORC1 inhibition may be detrimental to survival when telomeres are already shortened. In mice with dual deficiency of S6K1 and telomerase, rapamycin treatment also reduces lifespan. This finding suggests that inhibiting mTOR may be unfavorable for survival when telomeres are short, but mTORC1 inhibition can help delay aging when telomere length is normal.⁴⁰² In addition, inhibition of mTORC1 by rapamycin facilitates the relocation of telomerase reverse transcriptase (TERT) to mitochondria. This translocation decreases the levels of

mitochondrial superoxide and intracellular peroxides, thereby enhancing mitochondrial function and reducing ROS release.⁴⁰³

The accumulation of damaged mitochondria due to inefficient degradation and mtDNA instability is a significant hallmark of aging and age-associated diseases^{404,405} (Fig. 9). Because maintaining low mTOR activity contributes to longevity, low-dose rapamycin administration significantly extends the lifespan of mice with mtDNA depletion.⁴⁰⁶

Prolonged rapamycin administration facilitates the clearance of impaired mitochondria by increasing mitophagy, reduces the incidence of mtDNA deletion mutations, and has dose-dependent effects, with higher doses resulting in more significant outcomes.⁴⁰⁷

Chronic mTORC1 activation contributes to stem cell exhaustion and the accumulation of senescent cells.⁴⁰⁸ Senescent cells exhibit mTORC1 hyperactivity and secrete proinflammatory factors through translation programs controlled by mTORC1, thereby promoting systemic aging.^{409,410} Rapamycin mitigates this secretory phenotype, although whether it can reverse senescence or promote cell clearance remains unclear.

To date, the application of mTOR inhibitors to slow aging is limited by side effects such as insulin resistance and immunosuppression. However, these effects are typically associated with high-dose regimens used in cancer or transplant patients. Emerging evidence suggests that low or intermittent dosing can extend the lifespan of mice without adverse metabolic effects⁴¹¹ and may even enhance immune function in elderly humans.⁴¹² Finally, a recent study surveyed 333 adults who had used rapamycin off-label, primarily for health span and longevity purposes.⁴¹³ Self-reported outcomes indicated enhanced health, cognitive function, and emotional well-being. Importantly, no significant adverse effects were reported, suggesting that low-dose, intermittent rapamycin use may be safe in healthy adults. While the findings are promising, further research, including controlled clinical trials, is necessary to establish the efficacy and safety of rapamycin for healthspan extension.

mTOR in development

mTOR coordinates environmental cues with intrinsic developmental programs, ensuring that growth and differentiation occur at the right time and place. For example, mutations in *Drosophila* GATOR2 components suppress oocyte growth and differentiation⁴¹⁴ and are required for ovary growth and female fertility.⁴¹⁵ In *C. elegans*, NPRL2 and NPRL3 are required for postembryonic development⁴¹⁶ and NPRL3 deficiency in worm intestines triggers abnormal behavior and impaired growth.⁴¹⁷

Lowering the activity of the mTOR signaling pathway causes human stem cells and lab-grown embryo-like structures (blastoids) to enter a temporary "resting" or dormant state. Human blastoids treated with mTOR inhibitors have limited proliferation, developmental progression, and capacity to attach to hormonally stimulated endometrial cells. They can also give rise to stem cell derivatives of the blastocyst lineages and can continue to differentiate under post-implantation culture conditions.⁴¹⁸

Recently, the mTORC1 pathway was shown to play a role in facial phenotypic plasticity in mice.⁴¹⁹ When pregnant mice are fed diets with low or high protein levels, the activity of mTORC1 in their developing fetuses changes. This affects how facial bones form by influencing the size of the groups of cells that make up the skeleton. As a result, the diet during pregnancy plays a role in determining the shape and structure of the offspring's face.

Recent work in the axolotl (*Ambystoma mexicanum*), a highly regenerative urodele amphibian, has revealed a unique adaptation of the mTORC1 signaling pathway that underlies its exceptional regenerative capabilities by regulating translational control.⁴²⁰ Following injury, axolotls rapidly upregulate protein synthesis from preexisting mRNAs, particularly those encoding

ribosomal components and antioxidants, via a hypersensitive mTOR kinase variant. This response is absent in nonregenerative injury mouse models. Notably, the axolotl mTOR (axmTOR) protein has undergone unique evolutionary sequence expansions that are not found in mammals. These alterations confer heightened sensitivity to extracellular signals, enabling mTORC1 activation even in response to low amino acid levels at wound sites. Furthermore, a portion of axmTOR remains constitutively localized to lysosomes, priming the pathway for rapid activation upon injury. This hypersensitive mTOR system allows axolotls to repurpose a classical stress response pathway into a robust pro-regenerative signal, suggesting that species-specific mTOR tuning may be a critical determinant of regenerative capacity in vertebrates.

Together, these studies highlight the remarkable versatility of the mTORC1 signaling pathway across species and developmental stages. Understanding how mTORC1 is fine-tuned in diverse biological contexts may open new avenues for regenerative medicine, reproductive health, and developmental biology.

MTOR SIGNALING IN HUMAN DISEASES

Aberrant mTOR signaling is commonly associated with a wide range of diseases, including cancer, diabetes, obesity, various neurological disorders and other pathologies. This dysregulation can result from several mechanisms: (1) activating mutations within mTOR itself, mTOR complexes or their upstream regulatory proteins; (2) elevated expression levels of mTOR complex components; (3) inactivation or loss of function of negative regulators within the mTOR signaling pathway; and (4) changes in mTOR activity due to metabolic stress and mitochondrial dysfunction.

Although mTOR activation is often observed in the majority of disorders, it is not universally upregulated in all diseases. For example, in cancer-associated cachexia, mTORC1 signaling is downregulated in skeletal muscles. In advanced stages of nonalcoholic fatty liver disease, including cirrhosis, mTORC1 activity may also decline due to mitochondrial deregulation.⁴²¹

In an attempt to classify different mTOR-related diseases, several suggestions for the collective names of various disorders have recently been proposed. The term mTORopathy is used in the context of neurological diseases, especially epileptic encephalopathies and cortical malformations such as tuberous sclerosis due to mutations in *TSC1* or *TSC2*; focal cortical dysplasia, often caused by somatic mutations in *MTOR*, *PIK3CA*, or *AKT3*; hemimegalencephaly; brain malformation associated with mutations that hyperactivate mTOR signaling; and DEPDC5-related epilepsy.⁴²²

GATORopathies refer more specifically to disorders, other than cancer, caused by mutations in the genes in the GATOR1 complex.⁴²³ GATORopathies are mostly associated with familial focal epilepsy with variable foci, nonlesional focal epilepsies, and malformations of cortical development. Recently, mutations in DEPDC5 were also observed in autism spectrum disorders⁴²⁴ as were mutations in NPRL3 in Parkinson's disease,⁴²⁵ suggesting a broader neurological impact of GATOR1 dysfunction.

RAGopathies are a broader set of disorders resulting from mutations in the RAG GTPases,⁴²⁶ such as mutations of RAGA in cataracts,⁴²⁷ RAGC⁴²⁸ in dilated cardiomyopathy, RAGD in kidney tubulopathy and cardiomyopathy^{429,430} and RAGC in follicular lymphoma.⁴³¹

The upregulation of mTOR in diseases has profound effects on mitochondria (Fig. 9). Conversely, mitochondrial dysfunction can also influence mTOR signaling, creating a bidirectional relationship that plays a critical role in many pathological conditions. Thus, overactive mTORC1 inhibits mitophagy, leading to the accumulation of dysfunctional mitochondria, which contributes to increased ROS production and reduced cellular energy efficiency. On the

other hand, mitochondrial dysfunction in cancers forces cells to rely on glycolysis (the Warburg effect) for energy production, which is often accompanied by hyperactivation of mTORC1 to support anabolic needs.

mTOR signaling in cancer

mTOR signaling is altered in nearly 30% of cancers⁴³² (Fig. 9). The activating mutations within the kinase domain of mTOR can directly increase mTOR signaling. For example, approximately 33 mutations localized in six distinct regions of the mTOR gene C-terminal region have been identified in human tumors.⁴³³ These mutations promote mTOR kinase activity via the inhibition of DEPTOR binding in lung carcinoma, stomach cancer, endometroid carcinoma, colorectal cancer, melanoma and renal cell carcinoma. Owing to these mTOR mutations, various cell lines have become sensitive to mTOR inhibitors both in vitro and in murine models.⁴³³

RICTOR amplification leads to a poor prognosis and short survival in patients with squamous cell lung carcinoma.⁴³⁴ RICTOR is also robustly expressed in HER2-amplified breast carcinoma.⁴³⁵ In addition, its upregulation is associated with metastasis and drug resistance in triple-negative breast cancer and promotes the motility and proliferation of glioma cells.⁴³⁶

Hyperactivation of mTOR signaling can also be the result of mutations in upstream oncogenes and tumor suppressor genes. Mutations, amplifications, and overexpression of PIK3CA, KRAS, AKT, IGF1R and EGFR upstream of mTORC1 are very common in human malignancies.⁴³⁷ Additionally, the loss of function of p53, PTEN, and TSC1/2 contributes to mTOR signaling activation in a variety of tumors. For example, p53 depletion can enhance cell proliferation and survival, disrupt apoptosis and increase genomic instability by altering the expression of REDD1, an mTOR suppressor.⁴³⁸ In breast and endometrial cancers, alterations in PTEN expression increase the sensitivity of cells to mTOR inhibitors.⁴³⁹ Mutations in TSC1/2 identified in renal, liver, bladder, pancreatic and urothelial cancers are associated with tumorigenesis.^{440,441}

Several components of the nutrient-sensing input to mTORC1 have also been implicated in cancer progression. For example, RHEB1 upregulation, which increases mTORC1 activity, leads to worse median survival in acute myeloid leukemia patients.⁴⁴² RAGC⁴³¹ is mutated at a high frequency in follicular lymphoma. Causative damage caused by Birt-Hogg-Dube hereditary cancer syndrome is related to folliculin mutations.⁴⁴³ Mutations in NPRL3 and DEPDC5 were found in glioblastoma,⁴⁷ and DEPDC5 alterations were detected in colorectal cancer.⁴⁴⁴ NPRL2 has the most recurrent cancer-associated mutations in lung cancer, hepatocellular carcinoma, glioblastoma, and cancers of the ovary, breast, kidney, and colon.⁷⁶ Additionally, NPRL2 loss has been associated with decreased sensitivity to the chemotherapeutic agent cisplatin.^{188,445}

Healthy cells primarily carry out OXPHOS in mitochondria to generate ATP. Cancer cells tend to produce energy through glycolysis rather than OXPHOS even when oxygen is sufficient.⁴⁴⁶ Under hypoxic conditions, cells adjust their metabolism through the mTORC1 signaling pathway, promoting a shift from OXPHOS to glycolysis to adapt to the low-oxygen environment and sustain the energy supply during cancer cell proliferation.^{21,28} Dysregulated mitochondrial dynamics, abnormal mitophagy, mtDNA and TCA cycle enzyme mutations are implicated in cancer invasiveness, metastasis, progression and anticancer drug resistance⁴⁴⁷ (Fig. 9).

Mitochondrial fragmentation is common in various cancer types. Targeting mitochondrial fragmentation via the mTOR-MFN2 signaling axis may be a promising strategy to limit tumor growth. Indeed, MFN2 overexpression enhances mitochondrial fusion, reduces ROS generation, activates AMPK, inhibits mTOR, and promotes autophagy, ultimately suppressing ovarian cancer progression.⁴⁴⁸ Mitochondrial fragmentation is also induced by

the hypoxic tumor microenvironment, which leads to mTOR signaling activation. One consequence of this is that natural killer (NK) cells, which are crucial for tumor immunosurveillance, are unable to perform their normal antitumor functions, leading to evasion of immune surveillance and accelerating tumorigenesis.⁴⁴⁹ Restoring the antitumor capacity of NK cells can be achieved by inhibiting mitochondrial fragmentation.⁴⁴⁹

Mutations in metabolic enzymes, changes in cell signaling, and a hypoxic environment in cancer cells lead to altered metabolic pathways, driving mitochondria to produce oncometabolites such as lactate, fumarate, succinate and 2-hydroxyglutarate (2-HG).⁴⁵⁰ Although these compounds are normal intermediates in metabolism, their pathological accumulation disrupts cellular homeostasis.

mTOR promotes the accumulation of oncometabolites through mitochondrial biogenesis, glutamine metabolism, and HIF-1 α signaling. For example, inactivation of *Tsc1* leads to mTORC1 upregulation, fumarate accumulation and mTOR-dependent downregulation of the TCA enzyme fumarate hydratase (FH). Treatment with rapamycin reduces fumarate levels, affecting renal cancer progression in mice.⁴⁵¹ mTORC1 upregulation is correlated with FH downregulation and fumarate accumulation in human renal cell carcinoma.^{451,452}

Activation of mTORC1 by tumor-derived lactate leads to TFEB retention in the cytoplasm, which in turn decreases the expression of the macrophage-specific vacuolar ATPase subunit ATP6V0d2, which is required for lysosomal-mediated degradation of hypoxia-induced factor 2 α (HIF-2 α). In turn, HIF-2 α promotes expression of protumoral genes in macrophages.⁴⁵³

In acute myeloid leukemia and gliomas, cancer-associated mutations in the TCA enzymes IDH1 and IDH2 lead to the production of 2HG from α -KG.⁴⁵⁴ This abnormal generation of 2HG results in mTOR activation through the inhibition of KDM4A, an α -KG-dependent lysine demethylase that stabilizes the mTOR suppressor DEPTOR. KDM4A inhibition decreases DEPTOR stability, promoting mTOR activation.

Another important axis of tumor metabolism is the mTOR-related and mitochondria-driven synthesis of purines, pyrimidines, and amino acids. The excess production of these molecules fuels the uncontrolled proliferation of cancer cells.

Aspartate, especially under conditions of impaired mitochondrial metabolism, is an important factor for tumor growth. The conversion of aspartate to asparagine is crucial for the proliferation of cancer cells.^{452,455} Inhibition of the ETC leads to aspartate depletion, triggering ATF4 upregulation and increasing the transcription of genes related to amino acid transport and synthesis. Asparagine deficiency reduces mTORC1 activity,⁴⁵⁵ whereas aspartate or adenylate supplementation restores mTORC1 activity.⁴⁵²

The byproducts of tyrosine catabolism serve as intermediates in the TCA cycle, and dysregulation of this process contributes to the progression of hepatocellular carcinoma.⁴⁵⁶ Deficiencies in the tyrosine metabolic enzyme 4-hydroxyphenylpyruvate dioxygenase (HPD) lead to mTOR activation, promoting the accumulation of TCA cycle intermediates, such as citrate, α -KG, succinate, fumarate, and malate. Inhibition of mTOR or p70S6 kinase helps control the progression of liver cancer in HPD-deficient patients.⁴⁵⁶

Chronic lymphocytic leukemia (CLL) patients with high proliferation features have upregulated OXPHOS and the mTOR pathway.⁴⁵⁷ Venetoclax, a BCL-2 inhibitor, is used in CLL treatment. In the tumor microenvironment, CLL cells are stimulated by various signals, including the activation of CD40 signaling, which enhances OXPHOS and glycolysis, increases the expression of antiapoptotic proteins, and is associated with venetoclax resistance.^{458,459} AZD8055, which suppresses both mTORC1 and mTORC2, effectively reduces mitochondrial mass and respiratory activity and downregulates the expression of antiapoptotic proteins induced by CD40 stimulation to alleviate venetoclax resistance.

The biguanides metformin and phenformin inhibit mitochondrial electron transport chain complex I. Inhibition of mTORC1 restored autophagy in cells treated with these drugs, helping cancer cells bypass the blockage of phosphorylation of the E1 subunit α of PDH and making it a potential therapeutic target in tumor cells that are resistant to mTOR-mediated therapies.⁴⁶⁰

mTOR signaling in neurological disorders

mTOR signaling is involved in regulating neurological processes⁴⁶¹ and psychiatric and autism spectrum disorders.^{462,463} Deletions of RAPTOR or RICTOR decrease neuron size, impair oligodendrocyte differentiation and myelination in the central nervous system and are associated with early death, thus affecting the underlying functions of mTORC1 and mTORC2 in the development of the brain.⁴⁶⁴

Hyperactivation of mTORC1, which leads to impaired mitochondrial function, is observed in many neurodegenerative diseases (Fig. 9). For example, one of the main characteristics of Parkinson's disease is defective PINK/PARKIN-mediated mitophagy.⁴⁶⁵ Treatment with rapamycin enhances mitophagy, prevents mitochondrial dysfunction, and protects against neuronal apoptosis, safeguarding vascular dementia mice from brain injury and cognitive impairment.⁴⁶⁶

In Alzheimer's disease (AD), mTORC1 overactivation and mitochondrial dysfunction contribute to neurodegeneration by impairing autophagy and mitophagy, increasing ROS production, reducing glucose utilization, disrupting energy metabolism, and promoting the accumulation of amyloid-beta oligomers as well as hyperphosphorylated and aggregated forms of pathological tau protein⁴⁶⁷ (Fig. 9). The induction of synaptic activity has been shown to protect against AD by promoting autophagic flux and the lysosomal degradation of tau.⁴⁶⁸

Hyperactivation of mTORC1 reduces the autophagic clearance of amyloid-beta, promoting its accumulation in the brain. Amyloid-beta directly interacts with mitochondrial membranes, impairing ETC function, increasing ROS production, and blocking communication between lysosomes and mitochondria in vivo.⁴⁶⁹ mTORC1 dysregulation exacerbates mitochondrial vulnerability to amyloid-beta, creating a feedback loop that amplifies neurodegeneration. Amyloid-beta oligomers, which are precursors to amyloid plaques in the Alzheimer's disease brain, stimulate mTORC1 kinase activity at the plasma membrane but not at lysosomes and block nutrient-induced mitochondrial activity (NiMA) through a mechanism dependent on tau.⁴⁷⁰

Niemann-Pick type C disease is a lethal neurodegenerative disorder caused by mutations in the cholesterol exporter NPC1, leading to aberrant cholesterol accumulation in lysosomes, hyperactivation of mTORC1 signaling, and compromised mitochondrial function (Fig. 9). Treatment of NPC1-null cells with the mTOR inhibitor Torin1 or downregulation of mTORC1 activity via knockdown of LAMTOR5 restores impaired lysosomal and mitochondrial functions in these cells.⁴⁷¹

In patients with TSC1/2 loss, mTORC1 hyperactivation leads to severe epileptic seizures, which are reduced after rapamycin treatment.⁴⁷² In addition, epileptic disorders can also be caused by mutations within the KICSTOR and GATOR1 complexes.⁷⁶

In recent years, the GATOR1 complex has been shown to play an important role in the pathogenesis of focal epilepsies, with DEPDC5 exhibiting a higher mutation frequency (approximately 85% of reported cases) than NPRL2 (5%) and NPRL3 (10%). Among these GATOR1 variants, most are loss-of-function mutations, suggesting that GATOR1 genes, especially DEPDC5, should be prioritized in clinical genetic screening for focal epilepsy patients.⁴⁷³ Clinical studies have also identified loss-of-function mutations in GATOR1 components, especially DEPDC5 and NPRL3, in FCD type IIa. These mutations are associated with impaired autophagic flux, as indicated by the accumulation of p62 in autophagosomes in approximately half of the biopsy samples.⁴⁷⁴ A retrospective study of 50 children with GATOR1 variant-related

epilepsy revealed that the majority of patients were resistant to antiseizure medications and presented altered brain structures.⁴⁷⁵ Epilepsy surgery significantly improved seizure outcomes, indicating that surgery might be a more effective therapeutic approach for patients with GATOR1 variants.

Dietary restriction, specifically acute fasting, has been shown to decrease seizure susceptibility, which is linked to a reduction in mTORC1 activity. Interestingly, in neuron-specific DEPDC5 knockout mouse models, increased susceptibility to seizures and seizure-induced death was observed, suggesting the critical role of DEPDC5 in modulating seizure susceptibility following acute fasting.⁴⁷⁶

A recent study identified three novel NPRL3 loss-of-function mutations in families with focal epilepsy.⁴⁷⁷ These mutations disrupt NPRL3 protein stability, impairing the ability of the GATOR1 complex to inhibit mTORC1, leading to increased phosphorylation of S6K1.

mTOR signaling in cardiovascular diseases

Dysregulation of mTORC1, which leads to mitochondrial dysfunction, contributes to atherosclerosis, cardiomyopathy, ischemia-reperfusion injury, hypertension, and hypertrophy. For example, in high-protein diet-induced atherosclerotic models, amino acid starvation-induced mTORC1 inhibition promotes mitophagy, whereas leucine supplementation activates mTORC1 and suppresses mitophagy, causing macrophage apoptosis. The macrophage mTORC1-mitophagy axis should be considered an important target in high-protein diet-related cardiovascular risk.⁴⁷⁸

PTEN, an upstream negative regulator of mTOR, has been shown to be regulated by YAP, the downstream effector of the Hippo pathway.⁴⁷⁹ Activation of this pathway suppresses the expression of mitochondrial genes, contributing to mitochondrial damage and the progression of cardiomyopathy.⁴⁸⁰

Barth syndrome (BTHS), characterized by dilated cardiomyopathy, is an X-linked genetic disease caused by *TAFAZZIN* gene mutation. The transacylase encoded by *TAFAZZIN* is crucial for the remodeling of cardiolipin phospholipids, which are essential for maintaining mitochondrial structure and function. *TAFAZZIN* deficiency leads to mTORC1 hyperactivation, reduced mature cardiolipin levels, mitochondrial dysfunction, and defective mitophagy (Fig. 9). Treatment with rapamycin in *TAFAZZIN*-knockdown mice restores mitophagy, improves impaired mitochondrial morphology and respiratory function, reduces ROS, and ameliorates BTHS-related cardiomyopathy.⁴⁸¹ Therefore, targeting the mTORC1 pathway could serve as a novel therapeutic approach for BTHS, a disease that currently lacks effective treatment.

On the other hand, mTORC1 activation has a protective effect in cardiac reperfusion injury. Reduced phosphorylation of TSC2 enhances mTORC1 activity, followed by an increased mitochondrial oxygen consumption rate and a metabolic shift from fatty acid oxidation to glycolysis, which helps attenuate mitochondrial damage during ischemia and reperfusion.⁴⁸²

mTOR signaling in diabetes

The abnormal activation of mTORC1 and impairment of the TCA cycle jointly contribute to the dysfunction of diabetic β -cells, including reduced insulin secretion and increased apoptosis (Fig. 9). Upregulated mTORC1 contributes to the inhibition of pyruvate dehydrogenase, further reducing the entry of pyruvate into the TCA cycle. By restricting glycolysis during hyperglycemia, these metabolic changes may be prevented, potentially slowing the progression of diabetes.⁴⁸³

In diabetic nephropathy, high glucose conditions induce the upregulation of YY1, promoting the assembly of the mTOR-YY1 heterodimer. This complex then binds to the PGC-1 α promoter and suppresses PGC-1 α activity. This inhibition leads to mitochondrial dysfunction, which in turn promotes renal fibrosis in early diabetic nephropathy. Treatment with rapamycin reverses this process by downregulating YY1 expression.²⁸⁷

During the progression of diabetic retinopathy, the expression of the mitophagy proteins PARKIN and PINK1 is upregulated, and mTOR signaling is activated. Overexpression of Wnt inhibitory factor 1 (WIF1) suppresses the Wnt/ β -catenin signaling pathway, reduces the activity of the AMPK/mTOR signaling pathway, and improves the mitochondrial function caused by diabetic retinopathy.⁴⁸⁴

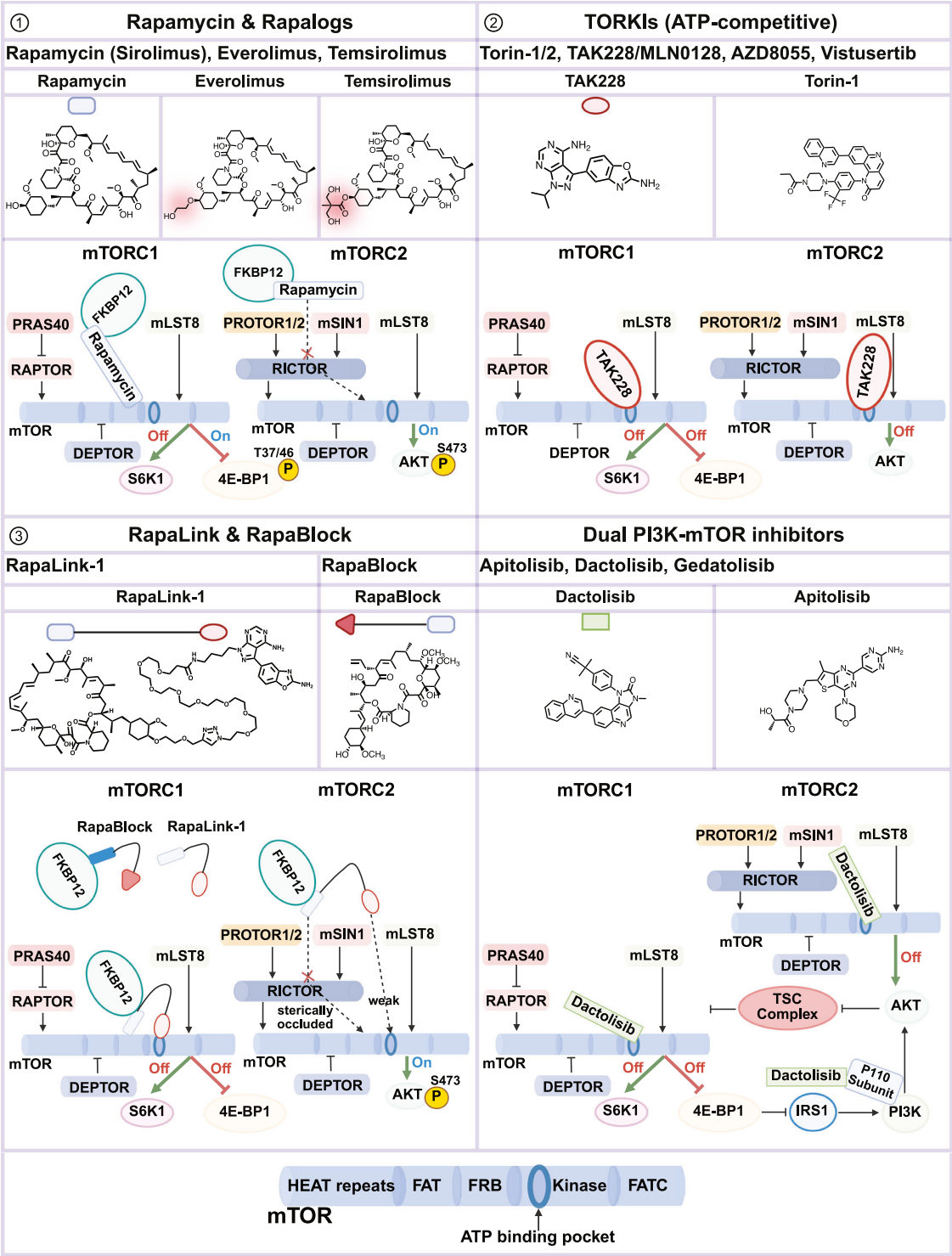
mTOR signaling in other diseases

Lymphangioleiomyomatosis (LAM) is a rare multisystem disorder that predominantly affects women and is characterized by progressive cystic lung destruction, renal angiomyolipomas (AMLs), and lymphangioleiomyomas.⁴⁸⁵ Its hallmark is the proliferation of abnormal smooth muscle-like perivascular epithelioid cells (LAM cells) within the lungs and lymphatics. LAM occurs in two forms: TSC-associated LAM, caused by germline mutations in TSC1 or TSC2, and sporadic LAM, which most often results from somatic loss-of-function mutations in TSC2. TSC1 or TSC2 germline mutations and sporadic LAM are often caused by somatic loss-of-function mutations in TSC2. Both forms share dysregulation of the mTORC1 pathway due to loss of TSC gene function, leading to uncontrolled cell growth and infiltration. In recent years, clinical studies have demonstrated that sirolimus and everolimus can stabilize lung function, shrink AMLs, reduce lymphatic involvement, improve quality of life, and establish mTORC1 inhibition as the current standard of care for LAM patients.

The m.3243 A > G mutation is the most common pathogenic mtDNA mutation in humans, which causes severe conditions, known as MELAS (myopathy, lactic acidosis and stroke episodes) and less severe MIDD (maternally inherited diabetes and deafness). This mutation results in mitochondrial dysfunction and elevated ROS and is associated with constitutive activation of the PI3K-AKT-mTORC1 pathway⁴⁸⁶ and suppressed autophagy and mitophagy (Fig. 9). Inhibition of mTORC1 with rapamycin in patient fibroblasts reduced the mutation load, rescued mitochondrial bioenergetic function, and reduced glucose dependence. Effective mitophagy triggered by mTORC1 inhibition is necessary to eliminate the m.3243 A > G mtDNA mutation.⁴⁸⁷ mTOR hyperactivation and suppressed autophagy are also observed in Down syndrome disease, where restoration of mitophagy and autophagic flux by blocking mTOR activity with the dual mTORC1/mTORC2 inhibitor AZD8055 has been proposed as a promising therapeutic strategy.⁴⁸⁸

Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are metabolic disorders characterized by significant lipid metabolism abnormalities. Mitochondrial dysfunction is implicated in the progression of NAFLD. Neddylation, a druggable and reversible posttranslational modification, is increased in different liver diseases, including NAFLD.⁴⁸⁹ Neddylation inhibition reduces oxidative stress, lipid peroxidation, fatty acid oxidation, and OXPHOS in preclinical NAFLD models. These effects are linked to mTOR suppression via DEPTOR accumulation.⁴⁸⁹ Another role of mTOR has been identified through its connection with endonuclease G, which promotes NAFLD via mTORC2 upregulation and ER stress.²⁷⁶

Taken together, these findings indicate that mTOR signaling has a profound impact across a diverse spectrum of human diseases. While hyperactivation of mTORC1 is a hallmark of many malignancies, metabolic disorders, and neurological pathologies, its role is not universally pro-growth or pro-survival. In fact, under certain pathological conditions, such as cancer-associated cachexia, cirrhosis, and advanced mitochondrial dysfunction, mTOR activity may decline, revealing the context-dependent complexity. As such, a growing body of evidence suggests that mTOR and mitochondria function as interdependent hubs, coordinating metabolic responses, cell fate decisions, and inflammatory signaling. This intricate crosstalk becomes especially relevant in the context of chronic diseases, where metabolic stress and immune dysfunction converge.



Understanding how mTOR and mitochondria coregulate cellular fate across tissues and disease states will be essential for designing targeted interventions that restore homeostasis without disrupting essential physiological functions.

MODULATING MTOR ACTIVITIES WITH MTOR INHIBITORS

Given the central role of mTOR in numerous pathological conditions, it is important to design therapeutic strategies that can support appropriate pathway activity in health and disease. A

key aspect of this effort lies in the development of various mTOR inhibitors, along with strategies to increase their efficacy through drug combinations (Fig. 10). This section outlines the core mechanisms of these inhibitors and their interactions, while more specific data, such as clinical trial results and IC₅₀ values in models of cancer and other disorders, are covered in detail in several dedicated reviews.^{490–495}

Rapamycin, which is used clinically under the generic name sirolimus or marketed under the brand name rapamune by Pfizer and Novartis, was the first drug developed to inhibit mTOR,

Fig. 10 Classification and mechanisms of action of mTOR inhibitors. The names of different generations of mTOR inhibitors are indicated at the top layer and marked by a corresponding number. The second layer lists representative drugs commonly utilized within each inhibitor category. The third layer shows the chemical structures of selected representative compounds. The final layer illustrates the distinct molecular mechanisms of inhibitor action, depicting interactions with mTORC1 and mTORC2 complexes and highlighting specific binding sites, inhibition targets, and downstream effects. The domain composition of mTOR is shown at the bottom. The following classes of mTOR inhibitors are represented: (1) Rapamycin/sirolimus (blue rectangle) and rapalogs (first-generation inhibitors). Red shading indicates key structural variations of everolimus and temsirolimus relative to rapamycin. Sirolimus is primarily used in organ transplantation to prevent rejection, whereas everolimus is more commonly employed in oncology (advanced renal cell carcinoma, hormone receptor-positive breast cancer and pancreatic neuroendocrine tumors). Temsirolimus, a water-soluble prodrug of rapamycin that is administered intravenously, is used for the treatment of advanced renal cell carcinoma. (2) ATP-competitive TOR kinase inhibitors (TORKIs, second-generation inhibitors). The chemical structures of TAK228/MLN0128 (pink oval) and Torin-1 are shown. (3) RapaLink-1 (third-generation) and RapaBlock. In RapaLink-1, rapamycin (blue rectangle) is connected to TORKI TAK228/MLN0128 (pink oval) by a linker composed of eight ethylene glycol units ($-(CH_2CH_2O)_8-$). Dual PI3K–mTOR inhibitors are illustrated in the lower right box. Phosphorylation by mTORC1 activates S6K1 (green arrow) but inhibits 4E-BP1 (red inhibition sign), whereas phosphorylation by mTORC2 activates AKT (green arrow). First-generation inhibitors block the phosphorylation of S6K1 by mTORC1 but not T37/46 of 4E-BP1 or S473 of AKT by mTORC2. Second-generation inhibitors block all three substrates, whereas RapaLink-1 selectively blocks mTORC1 targets. FKBP12 binding is necessary for the activity of rapalogs and RapaLink-1 but not for TORKIs. Compared with RapaLink-1, RapaBlock binds FKBP12 with higher affinity, preventing FKBP12 from interacting with the mTORC1–RapaLink-1 complex

followed by several analogs that constitute first-generation mTOR inhibitors (Fig. 10). These rapalogs differ in their chemical modifications, which determine their pharmacokinetics and routes of administration.

Although rapalogs have achieved great success in the field of organ transplantation, where their immunosuppressive properties have proven highly effective in preventing graft rejection, their application in oncology has been far less successful. Clinical trials in cancer patients with rapalogs, particularly as monotherapies, revealed only modest efficacy, with limited and inconsistent therapeutic responses across tumor types.

This suboptimal effectiveness reflects not only the complexity of mTOR signaling in cancer but also specific mechanistic limitations inherent to the rapalog mechanism of action. First, the 4E-BP signaling branch downstream of mTORC1 remains largely unresponsive to rapamycin. Second, selective inhibition of mTORC1, without concurrent suppression of mTORC2, triggers compensatory activation of the PI3K–AKT pathway via an S6K1–IRS1 feedback loop, promoting cell survival rather than suppression. Additionally, rapamycin has been shown to promote proliferation within hypoxic, poorly vascularized tumor regions by stimulating lysosomal degradation of extracellular proteins.^{496,497}

In response to these limitations, a second generation of mTOR inhibitors, also referred to as pan-mTORC1/2 inhibitors or TORKIs, was developed to more comprehensively inhibit mTOR signaling (Fig. 10). These compounds act as ATP-competitive inhibitors, effectively blocking both mTORC1 and mTORC2 by directly targeting the mTOR kinase domain.^{498,499} Examples include Torin1,⁴⁹⁹ AZD2014 (vistusertib),⁵⁰⁰ and AZD8055.⁵⁰¹ These inhibitors exhibit greater selectivity for mTOR than for PI3K and other PIKK family kinases. A more advanced generation of TORKIs, such as Torin2, also inhibits the DNA damage-sensing kinases ATM, ATR, and DNA-PK while retaining limited activity against PI3K.⁵⁰² TORKIs have shown superior ability to sensitize cancer cells to DNA-damaging agents by disrupting DNA damage response pathways and interfering with cell cycle checkpoint control, a capability that rapalogs notably lacking.⁵⁰³

Despite these advances, the use of first- and second-generation mTOR inhibitors has been associated with a range of side effects such as insulin resistance, hyperglycemia, impaired wound healing, hypertension, and various metabolic, renal, and pulmonary complications. A major contributor to this is the nonselective inhibition of both mTORC1 and mTORC2 during extended treatment. Even if intermittent dosing of rapamycin extends the lifespan of mice and has a milder impact on glucose homeostasis compared to continuous administration, without triggering the glucose intolerance,⁴¹¹ a long-term administration of rapamycin eventually inhibits not only mTORC1 but also mTORC2 and may lead to hepatic insulin resistance.^{345,504} Acute mTOR inhibition by

AZD8055 also leads to insulin resistance and glucose intolerance.⁵⁰⁵

Building on the quest for selectivity and improved efficacy, a major breakthrough came with the development of third-generation mTOR inhibitors (Fig. 10). These novel agents, exemplified by RapaLink-1, represent a significant leap forward in targeted therapy. RapaLink-1 is a bitopic chimeric molecule that simultaneously engages both the FRB domain and ATP-binding site of mTOR.⁵⁰⁶ RapaLink-1 demonstrates improved potency, durable intracellular retention, reduced toxicity and efficient suppression of downstream targets such as 4E-BP1. Designed by linking an FRB-binding moiety to a TORKI structure, RapaLink-1 is able to overcome resistance in cancer cells that harbor mutations in either the FRB or kinase domains of mTOR. It can also cross the blood–brain barrier, leading to potent antitumor activity in glioblastoma xenograft mouse models with minimal systemic toxicity.⁵⁰⁷

An important complementary tool in the RapaLink-1 system is RapaBlock (Fig. 10), which selectively binds to FKBP12 and prevents its association with rapamycin.⁵⁰⁸ RapaBlock is not considered a third-generation mTOR inhibitor, as it lacks the dual-site targeting and therapeutic activity characteristic of RapaLink-1. Due to the significantly greater stability of the FKBP12–rapamycin complex than the FRB–rapamycin interaction, RapaBlock effectively inhibits the assembly of the near-irreversible FKBP12–rapamycin–FRB tripartite complex.⁵⁰⁹ As a result, TORC1 remains predominantly active and unbound. Importantly, RapaBlock cannot cross the blood–brain barrier, enabling the selective activity of RapaLink-1 in the central nervous system. In the brain, RapaLink-1 forms a stable ternary complex with mTORC1 and FKBP12, effectively silencing mTORC1 activity. This tissue-selective targeting strategy, when RapaLink-1 is combined with RapaBlock, has been shown to prolong survival in mouse models of glioblastoma, highlighting its potential for treating brain tumors while minimizing systemic toxicity.

While third-generation mTOR inhibitors represent major advances in specificity and potency, alternative approaches have also been pursued to increase therapeutic efficacy and overcome pathway redundancies. In particular, dual PI3K–mTOR inhibitors such as NVP-BEZ235 (dactolisib),⁵¹⁰ GDC-0980 (apitolisib),⁵¹¹ and PKI-587 (gingatolisib)⁵¹² are ATP-competitive compounds designed to block both PI3K and mTOR signaling (Fig. 10). By simultaneously targeting these two nodes, they mitigate the compensatory PI3K–AKT activation often seen with selective mTORC1 inhibition. Dual inhibitors are especially effective in sensitizing cancer cells to genotoxic and replication stress, further expanding the arsenal of strategies for targeting the PI3K–mTOR axis in oncology.

mTOR activity can also be modulated indirectly through the actions of several metabolic drugs on mitochondria. For example,

metformin, a widely used antidiabetic drug, inhibits mitochondrial complex I, leading to AMPK activation and downstream inhibition of mTORC1.^{513–515} Similarly, resveratrol⁵¹⁶ and AICAR⁵¹⁷ activate AMPK and promote mitochondrial biogenesis while suppressing mTOR signaling. The novel mitochondrial inhibitor IACS-010759 (OPI) suppresses mTOR by enhancing the activity of the AMPK pathway and inhibiting OXPHOS.⁵¹⁸ Drugs such as bezafibrate support fatty acid oxidation and mitochondrial health while also downregulating mTOR signaling.⁵¹⁹ These mitochondria-targeting agents offer promising avenues for modulating mTOR activity in a context-dependent manner and may complement direct mTOR inhibition strategies, particularly in diseases where metabolic dysfunction and mitochondrial decline are key pathological features, such as neurodegeneration, aging, and certain cancers. On the other hand, pharmacological modulation of mTOR can substantially influence mitochondrial health by promoting mitophagy, enhancing mitochondrial biogenesis, and reducing oxidative stress.⁴⁸⁸ For example, treatment with rapamycin demonstrates mitochondrial protection in models of Parkinson's and Alzheimer's diseases.^{520,521} Compared to rapalogs, more potent mitophagy and metabolic reprogramming, as well as effects on mitochondrial dynamics and biogenesis, were observed upon treatment with second-generation mTOR inhibitors.⁵²²

Current therapeutic strategies increasingly favor combination treatments, where mTOR inhibitors are paired with genotoxic agents. For example, targeting mTORC1 with rapamycin and AKT with MK2206 has been shown to increase radiation sensitivity in breast and non-small cell lung cancer models.⁵²³ Similarly, the dual PI3K–mTOR inhibitor NVP-BE225 improved the response of glioblastoma cells to ionizing radiation.⁵²⁴ Finally, mTOR represents an attractive target for enhancing sensitivity to cisplatin, one of the mainstream chemotherapeutic agents.¹⁸⁸

An emerging strategy in cancer treatment involves combining mTOR inhibitors with hormonal therapy to stop the growth of hormone-sensitive cancers.⁵²⁵ These immunomodulatory effects provide a rationale for the synergistic efficacy when paired with immune checkpoint inhibitors. Combinations of everolimus with antibodies against the key immune checkpoint proteins programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) are under investigation to overcome the immunosuppressive tumor microenvironment and improve therapeutic outcomes in renal cell carcinoma.⁵²⁶

Recently, several combination therapies have been developed that, rather than targeting mTOR directly, focus on its upstream regulators in conjunction with immunotherapy. These strategies aim to modulate tumor growth and enhance immune responses by influencing signaling pathways that intersect with mTOR signaling. For example, combining PI3K inhibitors with antibodies against the key immune checkpoint proteins PD-1/PD-L1 has improved antitumor responses in preclinical models by augmenting T-cell-mediated tumor cell killing and reducing immunosuppressive cell populations within the tumor microenvironment.^{527,528} AKT inhibitors can also increase the effectiveness of immunotherapies by promoting apoptosis in tumor cells and improving T-cell responses.⁵²⁹ Finally, immediate upstream regulators of mTOR can also serve as effective targets in combination therapies. Recent studies have demonstrated that restoring NPRL2 expression via cationic lipid nanoparticle-mediated gene delivery in humanized mouse models represents a promising therapeutic strategy.⁵³⁰ Restoration of NPRL2 expression in KRAS/STK11-mutant anti-PD1-resistant NSCLC models induced DNA damage, cell cycle arrest, and apoptosis while also eliciting a robust antitumor immune response. NPRL2 gene therapy synergized with immune checkpoint blockade enhanced immune-mediated tumor suppression.

Together, the expanding arsenal of mTOR inhibitors, which are capable of targeting different branches of mTOR signaling, either alone or in combination with genotoxic agents or

immunotherapies, represents a powerful toolkit for the treatment of cancer and other diseases.

CONCLUSIONS AND PERSPECTIVES

Over the past five decades, the discovery of rapamycin and the subsequent characterization of the TOR pathway have revolutionized our understanding of cellular growth, metabolism, and aging. From the initial identification of rapamycin as an antifungal compound to the current recognition of mTOR as a central modulator of cellular physiology, the trajectory of mTOR research represents a compelling story of scientific progress. Today, the mTOR complexes serve as critical hubs that integrate many environmental cues (nutrients, energy status, oxygen levels, growth factors and DNA damage) to orchestrate cellular and organismal homeostasis.

Mechanistically, significant advances have been made in delineating the regulation and spatial dynamics of mTORC1 signaling, particularly its localization to lysosomes, and evidence of nonlysosomal activity at the Golgi, ER, and even within the nucleus has emerged. Nevertheless, how mTORC1 and mTORC2 interact with their substrates at different cellular locations and how compartment-specific signaling contributes to distinct physiological outcomes are still unclear. It is also poorly understood how mTORC1/2 complexes are targeted to specific subcellular locations and whether there are specific signatures (for example, posttranslational modifications or scaffolding interactions) that maintain them at these sites. The structure and function of the proposed mTORC3 complex also need further investigation.

From a clinical perspective, rapamycin and rapalogs have transitioned from laboratory-approved therapies to FDA-approved therapies for certain cancers and transplant rejection. However, their modest efficacy in solid tumors, coupled with metabolic side effects such as insulin resistance and immunosuppression, highlights the need for more selective and context-dependent modulators. The development of dual PI3K/mTOR inhibitors and third-generation compounds such as Rapalink-1 reflects this shift toward rational, structure-guided drug design aimed at overcoming resistance and achieving deeper therapeutic responses. However, challenges remain in designing therapies that preserve beneficial mTOR activity in healthy tissues.

Recent insights also underscore the systemic role of mTOR in interconnecting metabolic diseases, neurodegeneration, and cancer. The broad involvement of the pathway across tissues means that targeted inhibition can potentially improve multiple pathologies and age-associated disorders. However, this raises the risk of adverse effects, increasing the importance of tissue- and isoform-specific strategies. Moreover, the integration of mTOR-targeting agents with dietary programs, nanoformulations, and immune therapies is emerging as a promising frontier for personalized medicine. Future studies should address how systemic interventions (e.g., intermittent fasting and caloric restriction mimetics) interact with pharmacological mTOR inhibition and whether combinatorial approaches can be tailored to patient-specific metabolic and genetic backgrounds.

As our understanding of the role of mTOR in cellular metabolism, immune regulation, and disease progression deepens, it becomes increasingly clear that future therapies must strike a delicate balance, sufficiently inhibiting pathological mTOR activity while preserving its essential physiological functions. The integration of direct inhibitors with modulators of mitochondrial function and immune responses holds great promise for achieving this goal. Ultimately, rational design of targeted, context-specific, and combination-based interventions will be key to unlocking the full therapeutic potential of mTOR inhibition in both cancer and other diseases.

The next decades of mTOR research must aim for a systems-level understanding of the pathway across physiological contexts and

disease states. A key priority will be identifying novel regulatory nodes, particularly in understudied cellular compartments and tissues. At the translational level, there is a need to refine therapeutic regimens and optimize dosing, timing, and combination strategies to balance efficacy with safety. Finally, as mTOR research moves increasingly toward precision and integrative medicine, the challenge will be to apply its therapeutic potential in a manner that is both specific and comprehensive, transforming decades of fundamental research into clinical outcomes.

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