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Differential control of ageing and lifespan by isoforms and splice variants across the mTOR network

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Abstract

Ageing can be defined as the gradual deterioration of physiological functions, increasing the incidence of age-related disorders and the probability of death. Therefore, the term of ageing does not only reflect the lifespan of an organism but also refers to progressive functional impairment and disease.

The nutrient-sensing kinase mTOR (mammalian target of rapamycin) is a major determinant of ageing. mTOR promotes cell growth and controls central metabolic pathways including protein biosynthesis, autophagy, and glucose and lipid homeostasis. The concept that mTOR has a crucial role in ageing is supported by numerous reports on the lifespan prolonging effects of the mTOR inhibitor rapamycin in invertebrate and vertebrate model organisms. Interestingly, rapamycin not only increases lifespan but also delays the appearance of age-related metabolic phenotypes and diseases. Dietary restriction increases lifespan and delays ageing phenotypes as well, and mTOR has been assigned a major role in this process. This may suggest a causal relationship between the lifespan of an organism and its metabolic phenotype.

More than 25 years after mTOR's discovery, a wealth of metabolic and ageing-related effects have been reported. In this review we cover the current view on the contribution of the different elements of the mTOR pathway to lifespan and age-related metabolic impairment. We specifically focus on distinct roles of isoforms and splice variants across the mTOR network. The comprehensive analysis of mouse knockout studies targeting these variants does not support a tight correlation between lifespan prolongation and improved metabolic phenotypes and questions the strict causal relationship between them.

Summary

- mTOR inhibition prolongs lifespan and delays the appearance of age-related metabolic diseases, suggesting that mTOR is a major determinant of ageing and metabolic balance.
- The mTOR network in higher organisms harbours many different isoforms and splice variants, which exert complex effects on lifespan and metabolism.
- A comprehensive analysis of mouse knockout studies targeting these variants does not support a tight causal relationship between an improved metabolic phenotype and lifespan prolongation.

Abbreviation list

4E-BP1	eukaryotic translation initiation factor 4E-binding protein 1
4E-BP2	eukaryotic translation initiation factor 4E-binding protein 2
4E-BP3	eukaryotic translation initiation factor 4E-binding protein 3
aa	amino acids
AGC	protein kinase A/ protein kinase G/ protein kinase C
AMPK	AMP-activated protein kinase
ApoE	apolipoprotein E
Atg13	autophagy related 13
BAD	BCL2 associated agonist of cell death
BD	binding domain
C. elegans	*Caenorhabditis elegans*
CaMKKβ	Ca2+/calmodulin-dependent protein kinase kinase-beta
Cdc42	cell division cycle 42
CTD	C-terminal domain
CREM	cAMP responsive element modulator
D. melanogaster	*Drosophila melanogaster*
d4E-BP	eukaryotic translation initiation factor 4E-binding in *Drosophila melanogaster*
DEPTOR	DEP domain containing mTOR-interacting protein
dS6K	ribosomal S6 kinase in *Drosophila melanogaster*
dTOR	TOR in *Drosophila melanogaster*
eEF2K	eukaryotic elongation factor-2 kinase
eIF4B	eukaryotic translation initiation factor 4B
eIF4E	eukaryotic translation initiation factor 4E
EMT	endothelial-mesenchymal transition
Erα	estrogen receptor alpha
FAT	FRAP-ATM-TTRAP domain
FATC	FAT-carboxy terminal domain
FoxO	Forkhead box O
GAP	GTPase-activating protein

GLUT4	glucose transporter type 4
Grb2	growth factor receptor-bound protein 2
Grb10	growth factor receptor-bound protein 10
GSK-3	glycogen synthase kinase 3
GWAS	genome-wide association studies
HEAT	huntingtin-elongation factor 3-regulatory subunit A of PP2A-TOR1 repeats
HM	hydrophobic motif
IGF-1	insulin like growth factor 1
IGF1-R	insulin like growth factor 1 receptor
InR	insulin receptor
IRS1	insulin receptor substrate 1
IRS2	insulin receptor substrate 2
IRS3	insulin receptor substrate 3
IRS4	insulin receptor substrate 4
KD	kinase domain
KRLB	kinase regulatory loop binding
MAPK	mitogen-activated protein kinase
Mdm2	Mdm2 proto-oncogene
mLST8	mammalian lethal with SEC13 protein 8
mSin1	mammalian stress-activated protein kinase interacting protein 1
mTOR	mammalian target of rapamycin
mTORC1	mTOR complex 1
NLS	nuclear localization sequence
NTD	N-terminal domain
PDK1	3-phosphoinositide-dependent kinase-1
PH	pleckstrin homology
PHLPP1	PH domain and leucine-rich repeat protein phosphatase 1
PI3K	phosphatidylinositol 3-kinase
PIP2	phosphatidylinositol-3,4-biphosphate
PIP3	phosphatidylinositol-3,4,5-triphosphate
PKC	protein kinase C
PRAS40	proline-rich Akt substrate of 40 kDa
Protor-1	protein observed with rictor 1/ proline-rich protein 5
Protor-2	protein observed with rictor 1/ proline-rich protein 5-like
PTEN	phosphatase and tensin homolog
Rac-1	ras-related C3 botulinum toxin substrate 1
Rag	ras-related GTP-binding proteins
Raptor	regulatory associated protein of mTORC1
RBD	ras-binding domain
Rheb	ras-homologue-enriched-in-brain
Rictor	rapamycin-insensitive companion of mTOR
RNC	raptor N-terminal conserved
RORγ	RAR-related orphan receptor gamma
S6	ribosomal protein S6
S6K1	ribosomal protein S6 kinase 1
S6K2	ribosomal protein S6 kinase 2
S. cerevisiae	*Saccharomyces cerevisiae*

SGK	serum and glucocorticoid-regulated kinase
SHP2	src homology 2 domain-containing phosphatase
SIRT	sirtuins
TBC1D7	Tre2-Bub2-Cdc16 1 domain family member 7
Tel2	telomere maintenance 2
TFE3	transcription factor binding to immunoglobulin heavy constant mu enhancer 3
Tti1	TELO2 interacting protein 1
TOR	target of rapamycin
TOS	TOR signalling sequence
TSC	tuberous sclerosis complex
TTP	tristetraprolin
ULK1	unc-51 like autophagy activating kinase 1
ULK2	unc-51 like autophagy activating kinase 2
YY1	yin yang 1

1. The mTOR pathway in mammals

Target of rapamycin (TOR) was discovered in 1991 in the budding yeast *Saccharomyces cerevisiae* (*S. cerevisiae*) (1) and is structurally and functionally conserved in all eukaryotes, including mammals where it is called mammalian TOR (mTOR). mTOR is a serine/threonine kinase that functions as a master regulator of cellular growth and metabolism. mTOR forms two structurally and functionally distinct complexes, mTOR complex 1 (mTORC1) and mTORC2 (2-4). mTORC1's specific binding partners are the scaffold protein raptor (regulatory associated protein of mTORC1) (5, 6), and the inhibitory protein PRAS40 (proline-rich Akt substrate of 40 kDa) (7-11). mTORC2 is formed by rictor (rapamycin-insensitive companion of mTOR) (12, 13), mSin1 (mammalian stress-activated protein kinase interacting protein 1) (14, 15) and Protor (protein observed with rictor) (7, 16). In addition, mTORC1 and mTORC2 share the binding partners mLST8 (mammalian lethal with SEC13 protein 8) (13, 17), DEPTOR (DEP domain containing mTOR-interacting protein) (18) and the Tti1 (TELO2 interacting protein 1)/ Tel2 (telomere maintenance 2) complex (19). Both mTORC1 and mTORC2 are activated by growth factors (e.g., insulin) and amino acids, and mTORC1 is positively regulated by energy (ATP/AMP ratio) (2) (**Figure 1**).

Insulin activates mTORC1 via a well-described signalling cascade that is initiated either by the InR (insulin receptor) or the IGF1-R (insulin like growth factor 1 receptor) (**Figure 1**). Upon insulin/ IGF-1 (insulin like growth factor 1) binding, the receptors dimerize and trans-phosphorylate their cytoplasmic domain (20). This leads to the recruitment of IRS (insulin receptor substrate), which is phosphorylated at tyrosine residues by the InR and IGF-1R, and in response acts as a scaffold for many proteins (20). Class-I PI3Ks (phosphatidylinositol 3-kinases) bind to IRS, where they are activated, and thus, phosphorylate PIP2 (phosphatidylinositol-3,4-biphosphate) to form PIP3 (phosphatidylinositol-3,4,5-triphosphate). PIP3 is converted back to PIP2 by the phosphatase PTEN (phosphatase and tensin homolog) (21). Via binding to PIP3, proteins containing a PH (pleckstrin homology) domain are recruited to the membrane. The AGC (protein kinase A/protein kinase G/protein kinase C) kinases PDK1 (3-phosphoinositide-dependent kinase-1) and Akt both contain PH domains (21). Upon PIP3 formation they translocate to the plasma membrane, where PDK1 phosphorylates Akt within its kinase domain (22). Akt downstream of PDK1 activates mTORC1, by phosphorylating and inhibiting PRAS40 (23, 24), and the TSC (tuberous sclerosis complex) complex, formed by TSC1, TSC2 and TBC1D7 (Tre2-Bub2-Cdc16 (TBC) 1 domain family member 7) (25, 26). The TSC complex acts as a GAP (GTPase-activating protein) toward the small GTPase Rheb (ras-homologue-enriched-in-brain) (27). Therefore, TSC complex inhibition by Akt de-represses Rheb, which directly binds and activates mTORC1 (28). For Rheb to act on mTORC1, mTORC1 must be localized to lysosomes. Translocation of mTORC1 from the cytosol to the lysosomal surface occurs upon amino acid stimulation and is mediated by the Rag GTPases (Ras-related GTP-binding proteins) (29-32) (reviewed by (2, 4)). In addition, amino acids suppress lysosomal localisation of the TSC complex, leading to de-repression of Rheb (33-35). Amino acids also activate PI3K via an unknown mechanism (36, 37), and AMPK (AMP-activated protein kinase) in a CaMKKβ (Ca2+/calmodulin-dependent protein kinase kinase-beta) dependent manner (37) (**Figure 1**). Next to growth factors and amino acids, also the cellular energy status modulates mTORC1 activity (2). When the ATP/AMP ratio is low, AMPK (AMP-activated protein kinase) is allosterically activated by AMP, and inhibits mTORC1 by activating the TSC complex (38), and by directly

phosphorylating raptor (39) (**Figure 1**). mTORC1 affects virtually all metabolic processes to ultimately regulate cellular growth and survival (reviewed by Saxton and Sabatini (4)). We focus here on protein homeostasis which mTORC1 controls by activating translation and by inhibiting autophagy (4). mTORC1 positively regulates translation by activation of S6K (ribosomal protein S6 kinase) (40) and inhibition of 4E-BP (eukaryotic translation initiation factor 4E-binding protein) (41), leading to increased translation capacity (42) and enhanced translation initiation (43), respectively (**Figure 1**). mTORC1 inhibits autophagy by phosphorylating and inhibiting ULK1/2 (unc-51 like autophagy activating kinase 1/2) (44) (**Figure 1**), which phosphorylates FIP200 and thereby enhances autophagosome formation (45-48). AMPK activates ULK1, acting as an mTORC1 antagonist in autophagy (44, 49). In addition, mTORC1 and its substrate S6K reduce insulin sensitivity via negative feedback mechanisms. S6K phosphorylates IRS, leading to IRS degradation (50, 51), and mTORC1 phosphorylates and stabilizes Grb10 (growth factor receptor-bound protein 10), leading to InR inhibition (52, 53). Both events render cells refractory to insulin and consequently reduce PI3K and Akt activity.

mTORC2 also controls central metabolic pathways, including glucose metabolism (54), cell survival (54) and cytoskeletal organization (12, 13). The mechanisms leading to mTORC2 activation are relatively poorly explored. Previous research has established that insulin activates mTORC2 (13) in a PI3K dependent manner (55) at the ribosomes (56) (**Figure 1**). However, this PI3K differs from the PI3K upstream of mTORC1, in that mTORC2 is not regulated by mTORC1-driven negative feedback to PI3K or Akt (57). Different PI3K inputs to the two mTOR complexes could be mediated by distinct isoforms of the PI3K catalytic subunit p110, such as in hippocampal progenitor cells, where p110α activates both mTOR complexes while p110β activates mTORC2 only (58). Amino acids activate mTORC2 as well, but the exact mechanism remains so far unknown (36). The best described downstream effector of mTORC2 is Akt, which is phosphorylated by mTORC2 within the so-called hydrophobic motif (HM) (59). Akt downstream of mTORC2 inhibits the transcription factors FoxO1/3A (forkhead box proteins O1 and O3A) (60, 61) (**Figure 1**) which enhance apoptosis (60, 62, 63) and mediate stress responses (64-67). Therefore, mTORC2 promotes cellular survival. mTORC2 also activates the AGC kinase SGK (serum and glucocorticoid-regulated kinase) (55) that induces proliferation, migration and cell survival (68).

Isoforms and splice variants across the mTOR network

Next to the complex wiring of the mTOR network, most of its components occur as different variants, contributing to the network's complexity and versatility. These variants comprise similar proteins originating from different genes, referred to as "isoforms" (**Table 1**), and "splice variants" originating from alternative splicing or alternative translation initiation of the same gene (**Table 2**). Isoforms and splice variants can have overlapping, yet also different biological functions. In the following we focus on the differences between these variants regarding their protein structure, tissue expression pattern, and regulation and function within the mTOR network. Isoforms and splice variants of PI3K and FoxO1/3A have been reviewed extensively earlier (69, 70) and are therefore not covered here.

In mammals, the InR occurs in two different splice variants, named InR-A and InR-B (71) (**Table 2**). IR-A is expressed predominantly in the central nervous system and hematopoietic cells, while IR-B is found in adipose tissue, liver, and muscle (72). Both InR-A and InR-B have

alpha and beta domains, but only InR-B has an extra stretch of 12 amino acids in the alpha domain that changes its binding characteristics toward its ligands (72). IR-A and IR-B can form homo- or hetero-dimers and have similar affinities for insulin. However, InR-A binds IGF-I and IGF-II with higher affinity than InR-B (73, 74). This difference in affinity may allow them to activate the mTOR pathway differently in response to the same ligands.

Downstream of the InR, IRS has four different isoforms originating from different genes (IRS1-4), with IRS3 exclusively present in rodents (75). Therefore, we focus here on the differences between IRS1, IRS2, and IRS4 (**Table 1**). IRS1 and IRS2 are ubiquitously expressed (76) and, thus, the most widely studied isoforms. They are highly similar in their domain structure, but IRS2 contains an extra KRLB (kinase regulatory loop binding) domain that binds to the InR and the IGFI-R (77, 78). The KRLB domain limits the tyrosine phosphorylation on IRS2 by the InR and IGF1-R, and thereby inhibits IRS2 function (79). An *in vitro* study in skeletal muscle cells has shown that IRS1 and IRS2 tyrosines are dephosphorylated at different rates by phosphatases upon insulin or IGF-1 stimulation (80). While IRS2 becomes rapidly dephosphorylated after 3-10 min, IRS1 remains phosphorylated for up to one hour (80). It is unknown if this difference is mediated by distinct phosphatases, or by different dynamic behaviour of the same phosphatase toward IRS1 *versus* IRS2. Such differences in dynamic regulation might contribute to the distinct functional outcomes of IRS1 and IRS2. IRS1 enhances phosphorylation of both Akt1 and Akt2, whereas IRS2 signals mainly through Akt2 (81). IRS1 but not IRS2 enhances actin remodelling and GLUT4 (glucose transporter type 4) translocation (81). IRS1 and IRS2 induce the MAPK pathway with IRS2 having a stronger effect than IRS1 (81, 82). IRS1 and IRS2 seem to have opposite effects on metastasis, as IRS2 promotes metastasis in breast cancer cells (83, 84), whereas IRS1 suppresses metastatic spread in mice. Only little is known about IRS4, whose protein expression is limited to brain, kidney, thymus and liver (85). Recent evidence suggests a function of IRS4 during adenoviral infection, where IRS4 upregulation leads to constitutive Akt activation even in the absence of insulin (86).

The PI3K antagonist PTEN exists in three reported variants, termed PTEN, PTEN-Long (87) and PTENα (88), originating from alternative translation initiation (**Table 2**). PTEN-Long and PTENα both originate from the start codon CUG^{513} and have the same apparent molecular weight and predicted number of amino acids, suggesting that these two variants could be the same (87, 88). PTEN is ubiquitously expressed while PTENα is predominantly expressed in skeletal and cardiac muscle. All variants contain the functional PTEN domains, including the phosphatase domain, the C2 domain and the tail domain. PTENα and PTEN-Long contain an extra N-terminal domain which may lead to different subcellular localizations (87, 88). While PTEN localizes mainly to the cytosol, PTENα resides at mitochondria (88), and PTEN-Long is secreted to the microenvironment (87). This difference in localization results in distinct functions, as PTEN is the counterpart and antagonist of PI3K at the plasma membrane, whereas PTENα targets the mitochondrial complex IV (cytochrome c oxidase) to regulate the cellular energy status (88). PTEN-Long also decreases PI3K signalling in a phosphatase-dependent manner, however, as it is secreted to the microenvironment and able to enter neighbouring cells, it could have its main role in controlling signalling at the tissue level (87).

There are three Akt isoforms in mammals, Akt1 (PKBα), Akt2 (PKBβ) and Akt3 (PKBγ) (**Table 1**) which are similar in structure, as they all contain a PH domain, a kinase domain and the HM. Akt3 is also present as a shorter splice variant Akt3-1 (**Table 2**), which lacks the

mTORC2 phosphorylation site within the HM, and is less responsive to growth factor stimulation (89, 90). The three Akt isoforms display unique tissue expression patterns. While Akt1 is ubiquitously present (76), Akt2 resides primarily in brown fat, skeletal muscle and liver (91, 92), and Akt3 is mainly expressed in brain and testis (93). Akt1 and Akt2 also differ in their sub-cellular localization: in rat adipocytes, Akt1 is mainly distributed in the cytosol, whereas Akt2 localizes to GLUT4 containing vesicles (94). The phosphatases PHLPP1 (PH domain and leucine-rich repeat protein phosphatase1) and PHLPP2 (95) differently act on the mTORC2-substrate site within the HM of the three Akt isoforms (95): PHLPP1 specifically dephosphorylates Akt2 and Akt3, while PHLPP2 dephosphorylates Akt1 and Akt3 (95). The three Akt isoforms also differ regarding their substrate specificity, as only Akt1 and Akt2 phosphorylate TSC2 (95) to promote cell growth, whereas all three isoforms phosphorylate FoxO1/3A (95) to inhibit apoptosis. It is unknown if the substrate specificity of the different Akt isoforms also relates to PRAS40. It is likely as a number of further Akt-isoform specific substrates have been described: Akt1 phosphorylates palladin, an actin-binding protein, to inhibit cell migration (96) while Akt2 phosphorylates Ankrd2/ARPP, a muscle-specific protein that, when phosphorylated by Akt, prevents muscle differentiation (97). Their different substrate specificities grant the Akt isoforms distinct roles in tumour formation. Akt1 specifically promotes cell survival (98, 99) and cell growth (100, 101), whereas Akt2 is more important for glucose metabolism (102, 103), cell migration (100), tumour metastasis (100), and EMT (endothelial-mesenchymal transition) (101). In contrast, Akt3 inhibits migration (104), and has been linked to neuronal growth and development (105, 106). Interestingly, Akt3 overexpression confers resistance to the Akt inhibitor MK2206 (107) suggesting that Akt3 can be pro-tumourigenic in the context of targeted cancer therapies.

Downstream of Akt, TSC2 is present in healthy individuals in several variants originating from alternative splicing of exons 25 and 31 (108-110) (**Table 2**). TSC2 transcripts lacking exons 25 (Uniprot ID: P49815-2, P49815-3, P49815-5, P49815-6 and P49815-7) and/or 31 (Uniprot ID: P49815-4, P49815-5, P49815-6 and P49815-7) occur at higher levels as compared to the full length variant (Uniprot ID: P49815-1) (110). Exon 25 is detectable in lymph node, muscle and thyroid tissue whereas Exon 31 is found in adipose, adrenal, brain, breast, colon, kidney, lung, prostate, skeletal muscle, testes, and thyroid tissue (110). Deletion of exons 25 and 31 does not affect the TSC complex-dependent inhibition of mTORC1 (110). However, it may affect TSC2's properties as a substrate for Akt and AMPK. Akt phosphorylates TSC2 at residues S939, S981 and T1462 (26, 111, 112) and variants missing exon 25 do not contain the target site at S981.The TSC2 residues phosphorylated by Akt serve as a scaffold for 14-3-3 protein binding to and subsequent degradation of TSC2 (112). This suggests that TSC2 containing exon 25 has a higher 14-3-3 affinity and higher turnover than TSC2 variants without that exon (108). AMPK activates TSC2 by phosphorylation at S1387 and T1271 (38). The AMPK target site at T1271 is contained in exon 31 and, therefore, TSC2 variants that lack exon 31 may be less sensitive to AMPK activation. Further studies are required to delineate the biological consequences of the different structures of the TSC2 splice variants upon Akt and AMPK activation. TSC1 variants originating from alternative splicing have so far not been identified experimentally under healthy conditions. However, mutations in either TSC1 or TSC2 that lead to aberrant splicing and generation of multiple splice variants are commonly found in a wide range of pathologies such as cancer and tuberous sclerosis complex (TSC) (113). TSC is a rare genetic disorder caused by mutations in the TSC1 and TSC2 genes, leading to benign tumors in multiple organ systems (110). The TSC Leiden Open Variation Databases (TSC LOVD, www.lovd.nl) displays all variants reported for TSC1 and TSC2, being at the moment

(May 2017), 870 and 2473 respectively. For TBC1D7, the third member of the TSC protein complex, no isoforms or splice variants have been found experimentally. Also Rheb, the target of the TSC complex, does not present any isoform or splice variant.

Components of the mTOR complexes occur in different variants (**Table 2**). mTOR itself exists in two splice variants, mTORα and mTORβ, of which mTORβ is the shorter version (114). mTORα contains the protein-protein interaction domains HEAT (huntingtin-elongation factor 3-regulatory subunit A of PP2A-TOR1 repeats), FAT (FRAP–ATM–TTRAP) and FATC (FAT-carboxy terminal domain) and a kinase domain, while mTORβ lacks the HEAT and FAT domains (114). Studies in rats suggest that mTORα is ubiquitously expressed whereas mTORβ is mainly expressed in lung, heart, stomach, intestine and liver (114). mTORβ can still form the two mTORC1 and mTORC2 complexes, and phosphorylates the mTORC1 substrates S6K1 and 4E-BP1 as well as the mTORC2 substrate Akt. Cells overexpressing mTORβ have a shorter G1 phase, suggesting a role in cell cycle progression (114). This may be due to a stronger association of c-Myc, a transcription factor that controls cell cycle progression, with mTORβ than with mTORα. Also raptor exists in two splice variants, termed raptor and raptor-v2. Raptor-v2 (**Table 2**) is the shorter variant lacking the HEAT repeats, which are needed for binding to mTORC1 substrates (115). Raptor is ubiquitously expressed but levels are higher in brain, immune cells, the gastrointestinal tract and kidney (76). Raptor-V2 mainly localizes to the pituitary, nasal mucosa and muscle (115). Raptor-v2 binds mTORC1 but cannot bind S6K1, as it lacks the HEAT repeats (116). This suggests that raptor-v2 inhibits mTORC1 by sequestering mTOR away from the functional complex. However, further studies are required to characterize the biological function of raptor-v2.

Regarding the members of mTORC2, no isoform or splice variant for rictor has been so far described. However, five mSin1 splice variants have been experimentally identified, mSin1.1 - mSin1.5 (14) (**Table 2**). No information is available on their expression pattern. Only mSin1.1, mSin1.2 and mSin1.5 (also termed mSin1, mSin1α and mSin1β (117)) are found in mTORC2 and mediate Akt phosphorylation. mSin1.1 is the full-length protein, and contains a ras-binding domain (RBD) and a PH domain. mSin1.2 and mSin1.5 lack part of the RBD, and mSin1.5 also lacks the PH domain (14). mSin1.1 and mSin1.2 overexpression increases Akt phosphorylation in response to insulin, whereas mSin1.5 overexpression enhances Akt phosphorylation independently of insulin (14). This suggests that mTORC2 containing mSin1.1 or mSin1.2 is activated by insulin, whereas mTORC2 with mSin1.5 is constitutively active or responds to signals other than insulin. Protor has two isoforms, Protor-1 and Protor-2 (**Table 1**) (7, 16), that are ubiquitously expressed (16). They do not contain any known functional domain, but their amino-terminal sequence is highly conserved and they both bind mTORC2 via rictor (7, 16). Protor-1 also exists in 3 splice variants, Protor-1α, Protor-1β and Protor-1γ, of which only Protor-1α and Protor-1β can form part of mTORC2 (16). In kidney, Protor-1 in mTORC2 is required for the phosphorylation of SGK and activation of sodium transport (118). Protor-2 interacts with and activates the RNA binding protein TTP (tristetraprolin) to ensure mRNA turnover under stress conditions (119). Further research is required to better understand the differences in expression, function and regulation between these variants.

Protein variants downstream of mTORC1 have been extensively characterized, and we discuss them here in the context of cell growth, translation and autophagy. Firstly, S6K has two isoforms encoded by different genes, S6K1 and S6K2 (120-122) (**Table 1**). They both have an N-terminal domain (NTD), a C-terminal domain (CTD) and a kinase domain. In

addition, S6K2 has a nuclear localization sequence (NLS) next to its CTD (123). Both S6K1 and S6K2 are ubiquitously expressed (76) and, although they are both activated via phosphorylation by PDK1 (122, 124) and mTORC1 (40, 122), different isoform-specific signalling inputs contribute to their activation (123). S6K1 is inhibited by leucine starvation (125) and activated by the actin-binding protein neurabin (126), phospholipase D1 (127), the Rho family G proteins Rac1 and Cdc42 (cell division cycle 42) (128), and the deacetylases sirtuin 1 (SIRT1) and SIRT2 (129). S6K2 is activated in response to the cytokine IL-3 (interleukin 3) (130), by the MAPK pathway (131), and PKC (protein kinase C) (132). Both S6K1 and S6K2 phosphorylate S6 (ribosomal protein S6), a protein that forms part of the ribosomal 40S subunit (133) and, thus, the distinct activators of S6K1 and S6K2 may be a means to specifically control translation upon different cellular conditions, such as growth factor stimulation, different cell cycle phases or during the immune response. In addition to acting on S6, S6K1 also activates translation by phosphorylating eIF4B (eukaryotic translation initiation factor 4B) (134) and eEF2K (eukaryotic elongation factor-2 kinase) (135). Furthermore, S6K1 promotes cell survival by inhibiting BAD (BCL2 associated agonist of cell death) (136), Mdm2 (Mdm2 proto-oncogene) (137) and GSK-3 (glycogen synthase kinase 3) (138), and induces transcription by activating Erα (estrogen receptor alpha) (139) and CREM (cAMP responsive element modulator) (140). S6K2 activates transcription by binding to the transcription factors YY1 (yin yang 1) (141) and RORγ (RAR-related orphan receptor gamma) (142). Both S6K isoforms exist as several variants (**Table 2**). The S6K1 mRNA yields two differently translated variants originating from different translational start sites, p70-S6K1 and p85-S6K1 (143), the latter containing an N-terminal NLS. There is also a shorter S6K1 with a truncated kinase domain originating from alternative splicing, and termed p31-S6K1 in mice and hS6K1-h6A and hS6K1-h6C in humans (144). The tissue expression of the S6K1 variants is unknown but within the cell p70-S6K1 localizes to the cytoplasm (145), p85-S6K1 to both cytoplasm and nucleus (145, 146) and p31-S6K1 locates to the nucleus (147). Both p70-S6K1 and p85-S6K1 have been shown to be targeted by mTORC1, as rapamycin treatment decreases both phosphorylation of p70-S6K1 at pT389 and of p85-S6K1 at pT412 (147). However, in cells arrested in prometaphase, p85-S6K1 is phosphorylated at pT412 in an mTORC1 independent manner (148) and hence p85-S6K1 might not be regulated by mTORC1 under all circumstances. There is no conclusive data confirming if p31-S6K1 is targeted by mTORC1 (147). Further studies are needed to discriminate the biological functions of each of these variants. Alternative translation also occurs for S6K2, giving rise to two variants, p56-S6K2 and p54-S6K2, that differ by the presence of an NLS in p56-S6K2 in the NTD, but not in p54-S6K2 (122). Hence, they reside in different compartments, p56-S6K2 in the membranous fraction and p54-S6K2 in the soluble fraction (122). Further work is required to establish the exact sub-cellular localization and distinct functions of the S6K2 variants.

4E-BP, another direct mTORC1 substrate, exists in three isoforms (4E-BP1-3) (**Table 1**). All 4E-BP isoforms share an eIF4E (eukaryotic translation initiation factor 4E) binding domain and a TOS (TOR signalling sequence) motif. In addition, 4E-BP1 and 2 contain a RAIP motif, named after its amino acid sequence. Whereas 4EB-P2 is ubiquitously expressed (149), 4E-BP1 and 4E-BP3 are primarily found in the pancreas and skeletal muscle (149, 150). In addition, 4E-BP1 is expressed in adipose tissue (149), and 4E-BP3 in the kidney (150). mTORC1 inhibits 4E-BP1 and 2 by phosphorylation at their RAIP motifs. Phosphorylated 4E-BP1 and 2 are released from eIF4E, and consequently eIF4E can bind the 5' cap of mRNAs to initiate translation (43, 151). 4E-BP3 does not contain a RAIP motif and therefore is not inhibited by mTORC1, although mTORC1 phosphorylates 4E-BP3 at other sites (151).

Interestingly, prolonged mTORC1 inhibition enhances 4E-BP3 expression at the transcriptional level, and this is mediated by the transcription factor TFE3 (transcription factor binding to immunoglobulin heavy constant mu enhancer 3) (152). In addition to inhibiting eIF4E binding to the cap, 4E-BP3 regulates eIF4E at the nucleus to regulate nuclear mRNA export (153).

mTORC1 regulates autophagy by phosphorylating ULK. ULK has 4 isoforms, ULK1-4, but only ULK1 and ULK2 are inhibited by mTORC1 (47) (**Table 1**). ULK1 and ULK2 are ubiquitously expressed (76) and both induce autophagy (154, 155). ULK1 has a stronger affinity than ULK2 towards the other members of the autophagy-inducing complex, Atg13 (autophagy related 13) and FIP200 (48). This suggests different functions of the ULK1/Atg13/FIP200 and ULK2/Atg13/FIP200 complexes during autophagy, but further studies are needed to test this hypothesis.

2. mTOR and ageing

Ageing can be defined as the gradual deterioration of the physiological functions necessary for survival (156). This concept relates both to the lifespan of an individual and to the manifestation of age-related disorders, such as obesity, diabetes and myopathy. In other words, increased longevity can reflect either an increase in lifespan or a reduction of age-related diseases. The role of mTOR in the ageing process has been a topic of research over the last decades, and we give here an overview of the key findings in invertebrate and vertebrate model organisms and humans.

TOR and lifespan in invertebrates

The process of ageing has been widely studied in model organisms such as the budding yeast *Saccharomyces cerevisiae (S. cerevisiae)*, the nematode *Caenorhabditis elegans (C. elegans)*, and the fruit fly *Drosophila melanogaster (D. melanogaster)*. Much research on ageing has been performed in these organisms due to their short lifespan, their easy manipulation and the availability of powerful genetic tools. Studies in *S. cerevisiae* have shown that inhibition of TORC1 with rapamycin increases the chronological life span (duration of time that cells in stationary phase remain viable) (157). In addition, the longevity phenotype induced by dietary restriction was found to be TOR dependent (157). By generating yeast deletion collections, several long-lived mutants were identified. Among them were strains with mutations in genes of the TOR signalling axis (157) and the TOR substrate Sch9/S6K (158), as well as transcription factors that upregulate genes encoding amino acid biosynthetic enzymes and amino acid permeases (157).

Studies in *C. elegans* provide evidence that rapamycin (159) and dietary restriction (160) increase the lifespan of multicellular organisms as well. CeTORC1, TORC1 in *C. elegans*, inhibition by mutation or inhibition of let-363/CeTOR (161), DAF-15/raptor (162), raga-1/Rag GTPases (159, 163), or RHEB-1/Rheb (164) causes longevity phenotypes. Also mutations in CeTOR's upstream regulators DAF-2/InR (165) and AGE-1/PI3K (166) dramatically extend the lifespan of *C. elegans*. In contrast to the increased longevity of worms in which let-363/CeTOR or daf-15/raptor are targeted by RNAi or mutation, RICT-1/rictor deficiency causes short-lived worms (167), suggesting that the two complexes have opposite roles in the lifespan regulation of *C. elegans*.

Further evidence on the evolutionary conservation of TOR's role in lifespan and ageing arose from the confirmation of these phenotypes in *D. melanogaster*. Rapamycin (168) or dietary

restriction (169) increase lifespan also in flies. Inhibition of the insulin pathway by mutations in the InR gene (170) or in the InR substrate CHICO (171) results in increased longevity. Additionally, overexpression of Tsc1/TSC1 or Tsc2/TSC2, or dominant-negative forms of dTOR (*D. melanogaster* TOR) and its substrate dS6K/S6K, cause lifespan extension (172). Furthermore, the TORC1 substrate d4E-BP/4E-BP has a pivotal role delaying fly ageing (173). Hence, inhibition of the signalling axis converging on TOR and its substrates prolongs lifespan in non-vertebrate model organisms.

mTOR and ageing in mice

The before mentioned studies provide strong evidence that signalling through insulin and TOR restricts the lifespan of invertebrates. Evidence that this mechanism is also conserved in mammals came from mouse studies in which rapamycin or dietary restriction increased lifespan (174, 175). However, dissecting the mechanisms underlying mTOR's role in mammalian ageing proved to be even more challenging than in invertebrates. One reason is the higher complexity of the mammalian insulin-mTOR axis with several isoforms and often various splice variants at almost all levels of the pathway. As detailed earlier, tissue and subcellular distribution varies greatly for the different variants, leading to tissue-specific biological outcomes of mTOR. It is therefore not surprising that knockout of the same protein in different tissues leads to divergent phenotypes (176).

Several knockout studies in mice suggest functions of components of the insulin-mTOR pathway in ageing (**Table 3**). Lifespan extension has been observed in a female mouse model with heterozygous whole body double knockout of the mTOR and mLST8 genes, *Mtor* and *Mlst8* (177), or with a homozygous whole body knockout of Irs1 (178). Also a homozygous whole body knockout of *Rps6kb1*, the S6K1 gene, extends lifespan in mice (179). Lifespan extension has been also reported for heterozygous knockouts of *Irs2* in the whole body, or in brain only (180), but the reproducibility of this phenotype has been questioned (181). In agreement with the studies in *C. elegans (167)*, mTORC2 specific knockouts enhance ageing also in mice, as a whole body knockout of *rictor* decreases lifespan (182).

Lifespan studies in mice are relatively rare due to their comparatively long lifespan. In contrast, studies that focus on the role of the mTOR pathway in age-related, metabolic phenotypes and diseases are much more common (**Table 3**). Adipose tissue-specific knockouts of the InR gene *Insr* (183) or the raptor gene *Rptr* (184) result in mice with substantially less fat that are protected against obesity and hypercholesterolemia. In addition, knockout of either *Tsc1* or *Tsc2* enhances tumour formation (185, 186). This suggests that inhibition of the insulin-mTORC1 axis protects higher organisms against age-related metabolic and tumour disorders. However, this concept is challenged by the fact that most other knockout models of the insulin-mTOR pathway in mice develop phenotypes that positively link with age-related disease and could hence be considered as phenotypes of accelerated ageing (**Table 3**). Such phenotypes encompass for example impaired glucose tolerance, insulin resistance, obesity and myopathy, which correlate with increased age in mice and men (187-189). Impaired glucose tolerance has been observed in whole body knockouts of *Irs4* (190), and *Akt2* (102), in tissue specific knockouts of the *Insr* in muscle (191), beta cells (192) and brain (193), or of the PDK1 gene, *Pdpk1*, in the liver (194). Insulin resistance occurs in mice lacking *Irs2* (195) or *Akt2* (102), and in tissue specific knockouts of the *Insr* in liver (196) or brain (193). Obesity is also observed for whole body *Irs2* knockout mice (195), as well as for specific knockouts of the *Insr* in muscle (191) or brain (193). Myopathy is a characteristic phenotype of mice with muscle specific

knockouts of *Pdpk1* (197), *Mtor* (198), *Rptr* (199) and in a double knockout model of *Rps6kb1* and *Rps6kb2* (200).

The observation that so many mouse knockout models of the insulin-mTOR pathway develop metabolic phenotypes which can be linked with age-related metabolic impairment may seem at odds with the studies in invertebrates where a large majority of insulin-TOR pathway mutants display a prolonged lifespan. However, metabolic phenotypes in mouse studies must be interpreted with caution regarding their relationship with lifespan and ageing. The reason is that it is not possible to discriminate whether a detrimental metabolic phenotype in a mouse knockout model is due to an age-inhibitory role of the targeted gene, or due to a potentially essential role of this gene in metabolic processes. A well-known example of such a seemingly-contradictory phenotype has been observed for rapamycin in mice. Rapamycin does increase lifespan via mTORC1 inhibition but, when chronically administered, rapamycin causes secondary effects leading to mTORC2 inhibition and substantial impairment of glucose tolerance and insulin action (177). Hence, rapamycin extends lifespan and severely impairs metabolism at the same time, via distinct mechanisms. The fact that lifespan extension can be observed concomitantly with metabolic impairment suggests that metabolic alterations and lifespan are not always strictly causally linked.

The notion that lifespan and metabolic outcomes of genes can be separated is further strengthened when taking a closer look at isoform specific effects of genes in the insulin-mTOR axis on ageing and metabolic phenotypes in mice (**Table 3**). For example, different IRS isoforms govern metabolism and ageing in distinct, often opposite ways. A homozygous whole body knockout of *Irs1* prolongs lifespan of female mice (178), although they develop insulin resistance (201). In contrast a whole body knockout of *Irs2* shortens the lifespan of both male and female mice (178), and leads to a diabetic phenotype (195, 202). *Irs4* knock out mice have a milder phenotype than *Irs1* or *Irs2* knockouts, with regard to insulin sensitivity defects (190), and no effects on lifespan have been reported. Distinct phenotypes are also observed for knockouts of the different Akt isoforms. Mice with heterozygous *Akt1* knockout display a prolonged lifespan (203). In contrast, Akt2 deficient mice are insulin resistant with elevated plasma triglycerides and diabetes in males (102). An *Akt3* knockout does not seem to have an ageing-related metabolic phenotype (106). Downstream of mTORC1, a whole body knockout of *Rps6kb1* prolongs lifespan (179), whereas a *Rps6kb2* knockout shows no obvious phenotypic abnormalities (133). More extensive characterization of the *Rps6kb2* knockout model would allow for better understanding of S6K2's potential role in aging. Mice with a knockout of *Eif4ebp1,* 4E-BP1's gene, exhibit no difference in lifespan, although they present an increased metabolic rate and a reduction of adipose tissue (204), again questioning the strict causal relationship between beneficial metabolic features and lifespan extension. The role of 4E-BP2 has only been studied by double knockout of *Eif4ebp1* and *Eif4ebp2*, and these mice are obese and insulin resistant (205). However, no information about the lifespan of this model is available and no 4E-BP3 knockout has been so far reported.

mTOR and ageing in humans

Most research on mTOR in humans focuses on age-related diseases such as cancer and diabetes. Regarding ageing itself, studies in long-lived primates, specifically in Rhesus monkeys, have shown that calorie restriction delays disease onset and possibly mortality (206).The effect of rapamycin on primate lifespan has not yet been reported, but rapamycin improves immune function in elderly humans (207). Given that intervention studies on longevity and ageing in higher primates and humans are scarce, candidate gene and genome-

wide association studies (GWAS) are the main tools to understand the relationship between genetic makeup and human lifespan and disease susceptibility. Such studies use cohorts of advanced age and focus on genetic factors that correlate with exceptional longevity and healthy ageing.

GWAS studies in long-lived humans have so far yielded only very few gene associations that correlate with ageing. Indeed, only associations of ApoE (apolipoprotein E) and FoxO3a genes have been replicated in several studies (208). It is surprising that components of the insulin-mTOR pathway have not been identified in these studies. Possibly the GWAS study does not have enough power to detect such correlations, as this approach only allows the detection of common genetic variations. Development of new techniques such as whole-genome sequencing permit the detection of rare potentially functional genetic variants and raise much hope for the detection of further genetic correlations with lifespan and age-related processes. In a recent study, whole-genome sequencing was used to analyse human healthy ageing, defined as disease-free ageing without medical intervention (209). However, in this study no major genetic contributors to healthy ageing could be identified.

In contrast to these unbiased approaches, analyses of specific sets of candidates in the insulin-mTOR signalling pathway to unravel their potential role in ageing has yielded more success. A study that included 122 Japanese “semisupercentenarians” (older than 105 years) found polymorphic variations of the InR and IRS1 genes that are more frequent than in the control group (210). In addition, a polymorphism in Akt1 that significantly associates with lifespan has been found in three independent Caucasian cohorts (211). In the Leiden Longevity Study, gene expression analysis of nonagenarians shows that expression of 4E-BP1 and PRAS40, two negative effectors of the mTORC1 pathway, is higher in the aged group compared to the middle-aged control group (212). Moreover, raptor is expressed to a lower level in middle-aged members of the longevity families as compared to similarly aged controls (212). Finally, low insulin signalling has been associated with improved old-age survival in women (213). Of note, these targeted studies do not include isoforms or splice variants of the different members of the mTOR signalling pathway and, therefore, some relevant candidates may have been overlooked.

3. Discussion

mTOR signalling is widely recognized as a key element in ageing and age-related metabolic conditions in a wide range of organisms from yeast to rodents (157, 159, 168, 174). In invertebrates, inhibition of the insulin- TOR pathway by mutations or RNA interference extends lifespan, suggesting a positive link between TOR activity and ageing progression. When studying the same genes in higher organisms such as mammals, the relationship between mTOR and ageing becomes more complex. Although there are some clear examples of inhibition of the mTOR pathway that lead to lifespan extension, most knockouts result in the development of metabolic conditions that could be rather be considered as a sign of accelerated ageing. A limitation is that most of these mouse studies only analyse metabolic parameters and not lifespan, and conclusions cannot be drawn from metabolic phenotypes on shortened or prolonged lifespan. The concomitant occurrence of prolonged lifespan and detrimental metabolic phenotypes, or beneficial metabolic features with no lifespan effect in

some of these models challenge the idea of a strict and direct relationship between metabolic alterations in knockout models of genes of the insulin-mTOR axis and lifespan.

This review emphasizes the need to identify and characterize the isoforms and splice variants within the mTOR pathway to achieve a better understanding of the contribution of these different elements to metabolism and ageing, and the interrelationship of both. Although many of these isoforms have been identified long ago, they have been considered as proteins with overlapping function for a long period. This is reflected by the fact that isoforms with the number 1 in their name are often much better studied than their counterparts with higher numbers. Hence, the extent of knowledge on these variants often relates to their arbitrary numbering in databases.

For splice variants we lack even more knowledge as they are ignored by most experimental studies even though the majority of the genes that code for mTOR pathway components have been predicted to produce several splice variants. Furthermore, RNA splicing is required for longevity downstream of dietary restriction and the CeTORC1 pathway in *C. elegans* (214). The gap in our knowledge becomes even more apparent when considering that additional, abnormal splice variants occur in many genetic diseases and cancers (215). A recent global study on the interactomes of splice variants have shown that splice variants share only half of their interaction partners and have distinct tissue expression and, therefore, should be considered as distinct proteins (216). Thus, further work is required to experimentally identify and functionally characterize both natural-occurring and disease-causing variants in the mTOR pathway, and to better understand the relationship of these genes and their splice products with metabolic regulation, ageing and lifespan, and age-related diseases.

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Declarations of interest

The authors declare no competing interests

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Figure/Table legends

Figure 1. The mTOR pathway. Insulin binds and activates the insulin receptor (InR) which recruits phosphatidylinositol 3-kinase (PI3K) via the insulin receptor substrate (IRS). Once recruited to the membrane, PI3K phosphorylates phospholipid phosphatidylinositol-3,4-biphosphate (PIP2) to form phosphatidylinositol,3,4,5-triphosphate (PIP3). PIP3 is converted back to PIP2 by PTEN (phosphatase and tensin homolog). PIP3 serves as a membrane anchor for the 3-phosphatidylinositol-dependent kinase 1 (PDK1) and Akt. PDK1 activates Akt, which in turn phosphorylates and inhibits the tuberous sclerosis (TSC) complex and the proline-rich Akt substrate of 40 kDa (PRAS40). Once the TSC complex is inhibited, ras-homologue enriched in brain (Rheb) can exert its activating action on the mammalian target of rapamycin (mTOR) complex 1 (mTORC1). mTORC1 negatively regulates the eukaryotic translation initiation factor 4E-binding protein (4E-BP) and unc-51 like autophagy activating kinase 1/2 (ULK1/2) and positively regulates the ribosomal protein S6 kinase (S6K). Both mTORC1 and S6K contribute to negative feedback mechanisms at the level of the InR and IRS. Insulin also activates mTORC2 in a PI3K dependent manner. mTORC2 activates Akt, which inhibits the forkhead box O transcription factors FoxO1/3A. Amino acids have several activating inputs on the network, at the level of mTORC1 via the Rag GTPases (Ras-related GTP-binding proteins) and at the level of PI3K. A high AMP/ATP ratio leads to allosteric AMP-activated protein kinase (AMPK) activation that inhibits mTORC1 by activating the TSC complex and by directly inhibiting mTORC1. AMPK also phosphorylates and activates ULK1/2. Only the functional mTORC1 and mTORC2 outputs discussed in this review are shown. More extensive overviews of the processes downstream of the mTOR complexes are provided for example by Saxton and Sabatini (4) and Ben-Sahra and Manning (3).

Table 1. Isoforms across the mTOR network. This table highlights the differences among the various isoforms of proteins in the insulin-mTOR axis. Differences are categorized according to different structure, regulation, expression pattern and biological function**.** References are indicated in brackets. The colour code refers to the different signalling modules of the mTOR network as indicated in Figure 1. *Abbreviations: autophagy related 13 (ATG13), BCL2 associated agonist of cell death (BAD), cell division cycle 42 (Cdc42), epithelial to mesenchymal transition (EMT), estrogen receptor alpha (Erα), eukaryotic elongation factor-2 kinase (eEF2K), eukaryotic translation initiation factor 4B (eIF4B), eukaryotic translation initiation factor 4E-binding protein (4E-BP), forkhead box O (FoxO), glucose transporter type 4 (GLUT4), glycogen synthase kinase 3 (GSK-3), growth factor receptor-bound protein 2 (Grb2), interleukin 3 (IL-3), insulin receptor substrate (IRS), Mdm2 proto-oncogene (Mdm2), mitogen activated protein kinase (MAPK), PH domain and leucine-rich repeat protein phosphatase (PHLPP), protein kinase 3 (PKC), protein observed with rictor (Protor), RAR-related orphan receptor gamma (RORγ), ras-related C3 botulinum toxin substrate 1 (Rac1), ribosomal S6 kinase (S6K), serum and glucocorticoid-regulated kinase (SGK), sirtuins (SIRT), tuberous sclerosis complex 1 (TSC1), tuberous sclerosis complex 2 (TSC2), unc-51 like autophagy activating kinase (ULK), yin yang 2 (YY2).*

Table 2. Splice variants across the mTOR network This table indicates the predicted splice variants in human and mouse (source: uniprot.org) and the experimentally validated splice variants including functional differences. References are indicated in brackets. The colour code refers to the different signalling modules of the mTOR network as indicated in Figure 1.

Abbreviations: 3-phosphoinositide-dependent kinase-1 (PDK1), amino acids (aa), DEP domain containing mTOR-interacting protein (DEPTOR) , insulin-like growth factor 1 (IGF-1), insulin receptor (InR), mammalian stress-activated protein kinase interacting protein 1 (mSin1), mammalian target of rapamycin (mTOR), mTOR complex 2 (mTORC2), phosphatase and tensin homolog (PTEN), phosphatidylinositol 3-kinase (PI3K), phosphatidylinositol-3,4-biphosphate (PIP2), phosphatidylinositol-3,4,5-triphosphate (PIP3), proline-rich Akt substrate of 40 kDa (PRAS40), protein observed with rictor (Protor), rapamycin-insensitive companion of mTOR (rictor), regulatory associated protein of mTOR (raptor), ribosomal S6 kinase 1 (S6K1), ribosomal S6 kinase 2 (S6K2), TBC1 domain family member 7 (TBC1D7), tuberous sclerosis complex 1 (TSC1), tuberous sclerosis complex 2 (TSC2).

Table 3. Knockout phenotypes for the insulin-mTOR pathway in mice. This table highlights the phenotypes of knockout mouse models that display delayed or accelerated ageing phenotypes. Each group is subdivided depending on whether the phenotype relates to the lifespan of the mice or to their metabolic profile. For each mouse model, the target gene is indicated. Whole body knockout was performed if not indicated otherwise. Reference are indicated in brackets. The colour code refers to the different signalling modules of the mTOR network as indicated in Figure 1. As Mlst8 and Mtor are part of both mTOR complexes, the knockout models are indicated for mTORC1 and mTORC2. A more detailed comparison of the phenotypes with knockouts of the complex-specific components Rptor and Rictor is provided in the text. *Abbreviations: 3-phosphoinositide-dependent kinase-1 (Ppdk1),* eukaryotic translation initiation factor 4E-binding protein 1 *(Eif4ebp1), insulin receptor (Insr), insulin receptor substrate 1 (Irs1), insulin receptor substrate 2 (Irs2), insulin receptor substrate 3 (Irs3), mammalian lethal SEC13 protein 8 (Mlst8), mammalian target of rapamycin (Mtor), rapamycin-insensitive companion of mTOR (rictor), regulatory associated protein of mTORC1 (Rptr), ribosomal S6 kinase 1 (Rps6kb1), tuberous sclerosis complex protein 1 (Tsc1), tuberous sclerosis complex protein 1 (Tsc2).*

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Figure 1.

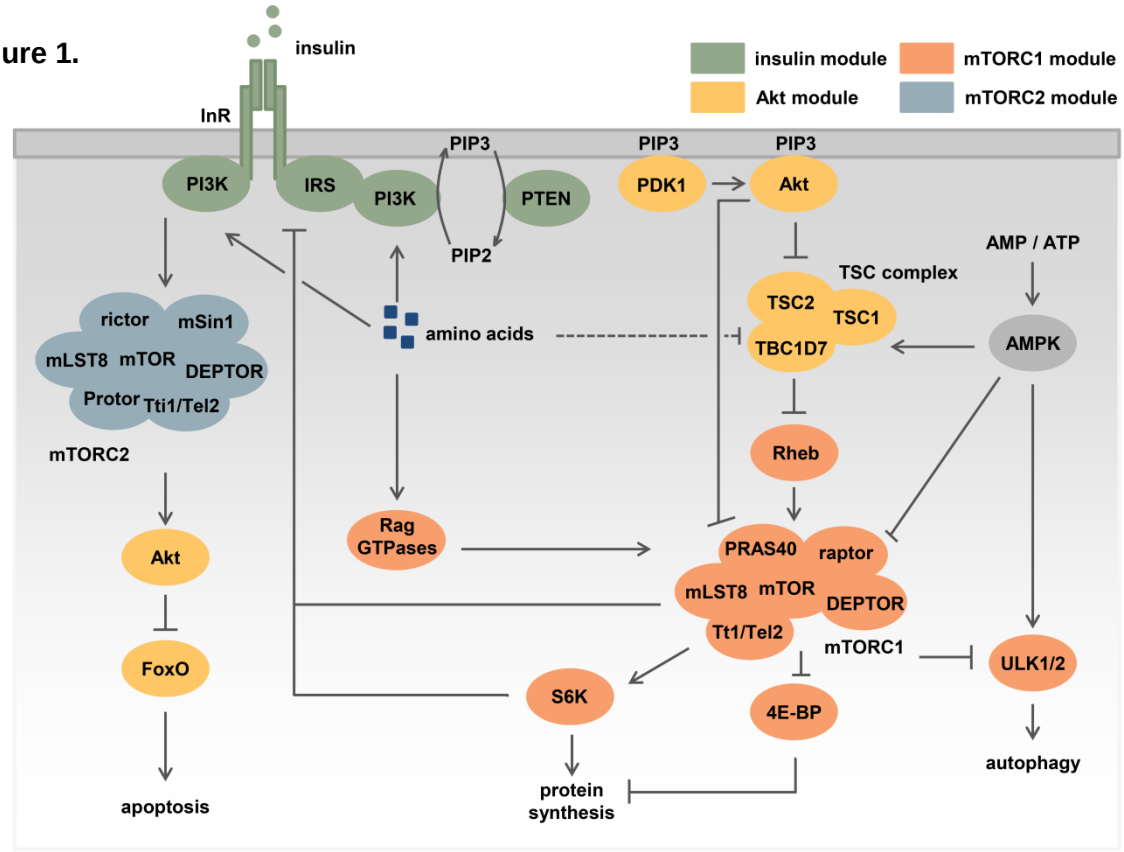

insulin
InR
insulin module
mTORC1 module
Akt module
mTORC2 module
PI3K
IRS
PI3K
PIP3
PIP2
PTEN
PIP3
PDK1
PIP3
Akt
AMP / ATP
TSC complex
TSC2
TSC1
TBC1D7
AMPK
rictor
mSin1
mLST8
mTOR
DEPTOR
Protor
Tti1/Tel2
mTORC2
amino acids
Rheb
Rag
GTPases
Akt
FoxO
apoptosis
PRAS40
raptor
mLST8
mTOR
DEPTOR
Tt1/Tel2
mTORC1
ULK1/2
autophagy
S6K
4E-BP
protein
synthesis

Table 1.

Isoforms across the mTOR network

		Name UniProt ID	Regulatory domains	Isoform regulation	Expression pattern	Isoform functions
Signalling modules	insulin	IRS	**IRS1** P35568	PH, PTB, PI3K-BD, Grb2-BD, SHP2-BD	Dephosphorylated 60 min after insulin stimulation (80) Strong Grb2 association (82)	Ubiquitous (76)	Signals to Akt1, Akt2 and MAPK pathway (81, 82) Actin remodelling(81) GLUT4 translocation(81)
			IRS2 Q9Y4H2	PH, PTB, KRLB, PI3K-BD, Grb2-BD, SHP2-BD	Dephosphorylated 10 min after insulin stimulation (80) Weak Grb2 association (82)	Ubiquitous (76)	Signals to Akt2 and MAPK pathway (81) Metastasis (83, 84) Maintenance of β-cell mass (217, 218)
			IRS4 O14654	PH, PTB, PI3K-BD, Grb2-BD	Upregulated upon viral infection(86)	Brain, kidney, thymus, liver (85)	Upon viral infection, leads to constitutive Akt activation (86)
	Akt	Akt	**Akt1** P31749	PH, KD, HM	Localizes at the cytosol (94) Dephosphorylated by PHLPP2 (95)	Ubiquitous (76)	Phosphorylates TSC2 (95), FoxOs (95) and palladin (96) Cell survival (98, 99) Cell growth (100, 101)
			Akt2 P31751	PH, KD, HM	Localizes in GLUT4 containing vesicles (94) Dephosphorylated by PHLPP1 (95)	Brown fat, skeletal muscle and liver (91, 92)	Phosphorylates TSC2 (95), FoxOs (95) and Ankrd2/ARPP (97) Glucose metabolism(102, 103) Cell migration/ metastasis(100, 101) EMT(100, 101)
			Akt3 Q9Y243	PH, KD, HM	Dephosphorylated by PHLPP1 and PLHPP2 (95)	Brain and testes (93, 219)	Phosphorylates FoxOs (95) Akt3 inhibition induces migration (104) Neuronal growth (105, 106)
	mTORC1	S6K	**S6K1** P23443	NTD, KD, CTD	Activated by neurabin, phospholipase D1, Rac1, Cdc42, SIRT1/SIRT2 (123) Inhibited by leucine starvation (125)	Ubiquitous (76)	Activates S6 (133) Targets BAD, Mdm2 GSK-3, eIF4B, eEF2K, Erα and CREM (123) Induces cell survival, translation initiation and elongation and transcription (123)
			S6K2 Q9UBS0	NLS, NTD, KD, CTD	Activated by IL-3, MAPK pathway and PKC (123) Not inhibited by leucine starvation (125)	Ubiquitous (76)	Activates S6 (133) Regulates YY2 and RORγ (123) Induces cell survival, translation and transcription (123)
		4E-BP	**4E-BP1** Q13541	RAIP, eIF4E-BD, TOS motif	Inhibited by mTORC1 (151)	Adipose tissue, pancreas, skeletal muscle (149)	Redundant function with 4E-BP2 (220, 221) Inhibits cap-dependent translation (43)
			4E-BP2 Q13542	RAIP, eIF4E-BD, TOS motif	Inhibited by mTORC1 (151)	Ubiquitous (149)	Redundant function with 4E-BP1 (220, 221) Inhibits cap-dependent translation (43)
			4E-BP3 O60516	eIF4E-BD, TOS motif	Not inhibited by mTORC1 (151) Transcription regulated by mTORC1 (152)	Skeletal muscle, heart, kidney, pancreas (150)	Inhibits cap-dependent translation (153) Regulation of eIF4E at the nucleus to regulate mRNA nuclear export (153)
		ULK	**ULK1** O75385	KD, Pro/Ser region, CTD	Inhibited by mTOR (48) Strong affinity to Atg13 and FIP200 (48)	Ubiquitous (76)	Induces autophagosome formation (154)
			ULK2 Q8IYT8	KD, Pro/Ser region, CTD	Inhibited by mTOR (48) Weak affinity to Atg13 and FIP200 (48)	Ubiquitous (76)	Induces autophagosome formation (155)
	mTORC2	Protor	**Protor-1** P85299	No functional domain known	Binds mTORC2 via rictor (16)	Ubiquitous (76)	Promotes SGK phosphorylation (118)
			Protor-2 Q6MZQ0	No functional domain known	Binds mTORC2 via rictor (16)	Spleen and intestine (76)	Regulates mRNA stability during stress (119)

Protein domains: pleckstrin homologue (PH), phosphotyrosine-binding (PTB), phosphatidylinositol 3-kinase binding domain (PI3K-BD), binding domain (BD), growth factor receptor-bound protein 2 bidning domain (Grb2-BD), src homology 2 domain-containing phosphatase binding domain (SHP2-BD), kinase regulatory loop binding (KRLB), kinase domain (KD), hydrophobic motif (HM), nuclear localization sequence (NLS), N-terminal domain (NTD), C-terminal domain (CTD), eukaryotic translation initiation factor 4E binding domain (eIF4E-BD), TOR signalling sequence (TOS),Proline/Serine (Pro/Ser).

Table 2.

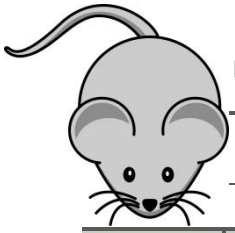
Variants across the mTOR network arising from alternative splicing or alternative translation initiation

		UniProt predicted variants		Name UniProt ID	Regulatory domains	Functional differences (citations)
Signalling modules	insulin	**InR**	Mouse: 1 Human: 2	**InR-A** P06213-2	α domain + β domain	High affinity for insulin, IGF-1 and IGF-2 (73, 74)
				InR-B P06213-1	α domain + β domain (+12 aa)	High affinity for insulin, low for IGF-1 (73, 74)
		PTEN	Mouse: 1 Human: 3	**PTEN** P60484-1	Phosphatase domain, C2 domain, tail	Counterpart of PI3K. Converts PIP3 into PIP2 (21)
				PTENα^{T} P60484-2	NTD, phosphatase domain, C2 domain, tail	Induction of cytochrome c oxidase activity (88)
				PTEN-Long^{T} P60484-2	NTD, phosphatase domain, C2 domain, tail	Counterpart of PI3K. Secreted in the microenvironment (87)
	Akt	**Akt1**	Mouse: 1 Human: 2	*	*	*
		Akt2	Mouse: 1 Human: 2	*	*	*
		Akt3	Mouse: 2 Human: 2	**Akt3** Q9Y243-1	PH, KD, HM	Activated by mTORC2 and PDK1 (90)
				Akt3-1 Q9Y243-2	Lacks mTORC2 target site	Activated by PDK1. Less responsive to growth factors (90)
		TSC1	Mouse: 4 Human: 2	*	*	*
		TSC2	Mouse: 7 Human: 8	**TSC2** P49815-1	Full protein	Akt target sites pS939, pS981 and T1462 (108) AMPK target sites pS1387 and pT1271 (38)
				P49815-2, P49815-3, P49815-5, P49815-6 P49815-7	Lacks exon 25	Akt target sites pS939 and pT1462 (108)
				P49815-4, P49815-5, P49815-6 P49815-7	Lacks exon 31	AMPK target site pS1387 (38)
		TBC1D7	Mouse: 2 Human: 4	*	*	*
	mTORC1	**mTOR**	Mouse: 2 Human: 1	**mTORα** P42345-1	HEAT, FAT, KD, FATC	Fully active mTOR. Weak binding to c-Myc (114)
				mTORβ^{L}	KD, FATC	Strong binding to c-Myc that allows control of cell cycle (114)
		DEPTOR	Mouse: 3 Human: 2	*	*	*
		raptor	Mouse: 5 Human: 3	**raptor** Q8N122-1	RNC, HEAT, WD40 repeats	Forms part of mTORC1. Mediates binding with mTORC1 substrates
				raptor-v2 Q8N122-3	RNC, WD40 repeats	Forms part of mTORC1. Cannot bind substrates (115)
		PRAS40	Mouse: 1 Human: 3	*	*	*
		S6K1	Mouse: 2 Human: 5	**p70-S6K1**^{T} P23443-2	NTD, KD, CTD	Targeted by mTORC1 (147)
				p85-S6K1^{T} P23443-1	1xNLS, NTD, KD, CTD	Contradiction if targeted by mTORC1 (147, 148)
				p35-S6K1^{L}	1xNLS, NTD, partial KD	Not known if targeted by mTORC1 (147)
		S6K2	Mouse: 1 Human: 2	**p54-S6K2**^{T} Q9UBS0-1	1xNLS, NTD, KD, CTD	Resides in soluble fraction of cells (122)
				p56-S6K2^{T,L}	2xNLS, NTD, KD, CTD	Resides in particulate fraction of cells (122)
	mTORC2	**rictor**	Mouse: 2 Human: 3	*	*	*
		mSin1	Mouse: 3 Human: 6	**mSin1.1** Q9BPZ7-1	RBD, PH	Forms part of mTORC2 (14)
				mSin1.2 Q9BPZ7-2	Partial RBD, PH	Forms part of mTORC2 (14)
				mSin1.3 Q9BPZ7-3	Partial RBD, PH	Does not form part of mTORC2 (14)
				mSin1.4	Partial RBD, PH	Does not form part of mTORC2 (14)

		Q9BPZ7-4		
		mSin1.5[L]	Partial RBD	Forms part of mTORC2 (14)
Protor-1	Mouse: 2 Human: 5	**Protor-1α** P85299-1	Full protein.	Forms part of mTORC2 (16)
		Protor-1β P85299-3	Shorter variant	Forms part of mTORC2 (16)
		Protor-1γ P85299-4	Shorter variant	Does not form part of mTORC2 (16)
Protor-2	Mouse: 1 Human: 4	*	*	*

*Protein domains: N-terminal domain (NTD), pleckstrin homologue (PH), kinase domain (KD), hydrophobic motif (HM), huntingtin-elongation factor 3-regulatory subunit A of PP2A-TOR1 repeats (HEAT repeats), FRAP-ATF-TTRAP (FAT), FRAP-ATM-TTRAP domain (FATC), raptor N-terminal conserved (RNC), C-terminal domain (CTD), nuclear localization signal (NLS), ras-binding domain (RBD). The variants arise from alternative splicing unless marked otherwise. Variants marked [T] originate from alternative translation initiation. *Asterisks designate splice variants that have been predicted, but have not yet been experimentally confirmed. [L] Refer to primary literature as this variant is not listed in UniProt.*

Table 3.



Phenotypes of knockout mouse models

	Delayed ageing phenotype		Accelerated ageing phenotype	
	Lifespan	**Metabolic profile**	**Lifespan**	**Metabolic profile**
Insulin	*Irs1*$^{(-/-)}$ (178) *Irs2*$^{(+/-)?}$ (180, 181) brain *Irs2*$^{(+/-)?}$ (180, 181)	adipose *Insr*$^{(flox/flox)\ L}$ (183)	*Insr*$^{(-/-)\ I}$ (222)	muscle *Insr*$^{(flox/flox)G,O}$ (191) pancreas *Insr*$^{(flox/flox)G}$ (192) brain *Insr*$^{(flox/flox)G,I}$ (193) liver Insr$^{(flox/flox)I}$ (196) *Irs2*$^{(-/-)\ I,O}$ (202) *Irs4*$^{(-/-)\ G}$ (190)
Akt	*Akt1*$^{(+/-)}$ (203)			liver *Pdpk1*$^{(-/-)\ G}$ (194) muscle *Pdpk1*$^{(-/-)\ M}$ (197) *Akt2*$^{(-/-)\ G}$ (102) *Tsc1*$^{(+/-)\ T}$ (185) *Tsc2*$^{(+/-)\ T}$ (186)
mTORC1	*Mtor*$^{(+/-)}$ *Mlst8*$^{(+/-)}$ (177) *Rps6kb1*$^{(-/-)}$ (179)	adipose *Rptor*$^{(-/-)\ L}$ (184) *Eif4ebp1*$^{(-/-)\ L}$ (204)		muscle *Mtor*$^{(flox/flox)\ M}$ (198) muscle *Rptor*$^{(flox/flox)\ M}$ (199)
mTORC2	*Mtor*$^{(+/-)}$ *Mlst8*$^{(+/-)}$ (177)		*Rictor*$^{(+/-)}$ (182) *Rictor*$^{(-/-)}$ (182)	muscle *Mtor*$^{(flox/flox)\ M}$ (198) liver *Rictor*$^{(flox/flox)\ G,I}$ (223)

^{L} Less adipose tissue, ^{G} Impaired glucose tolerance, ^{I} Insulin resistance, ^{O} Obesity, ^{M} Myopathy, ^{T} Appearance of tumours, $^{?}$Phenotype not reproduced