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Bidirectional crosstalk between endoplasmic reticulum stress and mTOR

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Abstract

Many cellular processes including apoptosis, autophagy, translation, energy metabolism, and inflammation are controlled by the mammalian target of rapamycin (mTOR) kinase and the endoplasmic reticulum (ER) stress pathway, also known as the unfolded protein response (UPR). While both of these signaling nodes have attracted wide attention in fundamental cell biology and drug discovery, crosstalk between the two pathways has emerged only very recently. mTOR complex 1 (mTORC1) operates both upstream and downstream of ER stress signals, which can either enhance or antagonize the anabolic output of mTORC1. Upon prolonged ER stress, mTORC1 contributes to apoptotic signaling by suppressing the survival kinase Akt through feedback inhibition. Likewise, chronic ER stress obstructs activation of Akt by mTOR complex 2. This review surveys existing knowledge on mTOR–ER stress intersections and highlights potential therapeutic implications.

Introduction

Cell growth, proliferation, survival, and energetic maintenance are intimately connected processes. External signals such as nutrient availability, growth factors, or inflammatory mediators are decoded by cellular sentinels, which – where appropriate – can remodel cell physiology. Thus, cells respond to positive growth signals by promoting anabolism (i.e. the buildup of macromolecules and the inhibition of degradative reactions) and to unfavorable growth conditions by eliciting stress pathways. The mammalian target of rapamycin (mTOR) signaling pathways and the endoplasmic reticulum (ER) stress response (the so-called "unfolded protein response", UPR) play increasingly recognized roles in this interplay [1, 2]. These two apposing signaling networks have traditionally been considered separate pathways and the identification of mTOR–UPR interconnections, which is reviewed herein, is a relatively new area of research.

The atypical serine/threonine kinase mTOR is a master regulator of cell growth and metabolism. It exists in two complexes, mTORC1 and mTORC2, which exhibit different subunit compositions and execute distinct cellular tasks [3, 4] (boxes 1 and 2). mTOR complexes reside in the cytoplasm, where they are often found in association with cellular membranes (see sections "Signal integration by mTORC1" and "mTORC2 and the ER"). Several pathways downstream of the mTOR complexes are known [4] and new ones are constantly being discovered. The UPR, on the other hand, is a conglomeration of signaling pathways originating from the ER (box 3). It is known to be triggered when the protein folding capacity in the ER is overwhelmed and "ER stress" ensues. The membrane-bound network of the ER, which extends from the nuclear envelope to the periphery of the cell and maintains vital contact

zones with many other cell organelles, is a highly metabolic organelle [1]. It mediates many anabolic processes such as lipid synthesis, gluconeogenesis, and the biogenesis of peroxisomes and lipid droplets, but also the catabolic turnover of proteins and organelles through autophagy or proteasomal hydrolysis.

Signaling through mTORC1 and mTORC2 is activated by extracellular and intracellular cues when conditions are favorable for growth. Both mTOR complexes in turn facilitate cell growth, survival, and proliferation (boxes 1 and 2). The metabolic classification of the UPR stress pathway is less straightforward, since it would be a gross oversimplification to state that it generally antagonizes cellular anabolism, which is orchestrated by mTOR. Indeed, the UPR does not only signal stress, catabolism, and cell death [5], but also, for instance, the anabolic expansion of ER membranes [6]. In the same vein, activation of the UPR is also achieved by stimuli which are not necessarily linked to unfavorable ("stressful") growth conditions (see section "Upstream of the UPR").

Given their central influence on cell viability, both mTOR and UPR have been subject to extensive biomedical and pharmacological research activity [7-9], for instance in the search for new cancer treatments. Thus, evaluating and understanding the intersections and synergisms (or antagonisms) between the outputs of mTOR and UPR is of importance; possible routes of crosstalk between these signaling networks are fundamental for cell health. Here, we do not intend to provide a comprehensive synopsis of the upstream and downstream signaling networks surrounding mTOR and UPR for which the reader is referred to other recent articles [3-5, 10]. Instead, this review focuses on the interdependence of these two pathways in health and disease. Recent pioneering studies on molecular links between mTORC1, mTORC2, and the

ER stress response will be summarized along with a tentative preview to where these new insights may guide future therapeutic strategies.

Signal integration by mTORC1

Our understanding of the molecular mechanisms leading to the activation of mTORC1 has tremendously increased over the past few years. Known pathways culminate in the association of mTORC1 with the active, GTP-bound forms of the small GTPases Rheb and Rag, which initiate mTORC1 signaling [11]. Thus, mTORC1 activation is regulated by at least two independent inputs. One is the regulation of the GTP-binding status of Rheb in response to growth factors. The other is the GTP loading of Rag and the recruitment of mTORC1 to the lysosomal membrane in response to amino acids [4, 11].

Growth factors and hormones, which operate upstream of Rheb–mTORC1, are recognized by cell surface receptors. These in turn initiate intracellular signaling cascades, the majority of which act on the heterodimeric TSC1–TSC2 (also called hamartin–tuberin) complex, a GTPase activating complex and a negative regulator of GTP-bound Rheb (Fig. 1). Examples of cell surface receptors which enhance mTORC1 signaling via inhibition of TSC1–TSC2 are the insulin receptor and Frizzled (the receptor of the Wnt signaling pathway). The insulin receptor signals via PI3K and PDK1 to the AGC kinase family member Akt. It also activates the MAP kinase cascade Raf–Mek–Erk. Activated Akt and Erk phosphorylate and inactivate TSC2 on multiple serine/threonine residues [12-14] (Fig. 1). In contrast, phosphorylation by GSK3, which is inactivated by the Wnt–Frizzled pathway, activates TSC2 [15]. Further regulatory inputs on TSC1–TSC2 by intracellular energy

levels, cytokines, and hypoxia are summarized in Figure 1. Thus, the TSC1–TSC2 complex operates as an integrator of complex signaling information upstream of mTORC1. Loss of either tumor suppressing subunit of TSC1–TSC2 leads to constitutive activation of mTORC1 by Rheb–GTP and to an autosomal dominant disease (tuberous sclerosis complex; TSC) characterized by the widespread accumulation of benign tumors [16].

A second form of regulatory input on mTORC1 is spatial organization. Activation of mTORC1 by Rheb–GTP occurs on the cytosolic surface of lysosomes. Recruitment of mTORC1 to these sites is mediated by binding to heterodimeric Rag GTPases and signaled by the availability of amino acids in lysosomes [17-19] (Fig. 1). In what way this pathway is integrated with the input of alternative amino acid-sensing machineries upstream of mTORC1 [20, 21] (Fig. 1) is currently unknown.

Collectively, activation of mTORC1 signaling is a multi-step process that depends on the inputs of cellular signaling cascades and the availability of nutrients, energy, and oxygen. As both Rheb- and Rag-integrated inputs are required for activation, this creates a situation of tight regulation which – in light of the pathological consequences of uncontrolled mTORC1 activation [16] – is critically important. Before discussing the inputs of ER stress on mTORC1 activation, we will next give an overview of specific "growth" conditions that elicit UPR signaling.

Upstream of the UPR

A growing body of evidence places the UPR downstream of physiological stimuli that do not necessarily act via the accumulation of unfolded proteins in the ER [10]. For instance, very much like mTORC1, the UPR is sensitive to the availability of

nutrients and growth signals [1]. Indeed, the historically oldest way to elicit the UPR (at that time measured by the increased synthesis of "glucose regulated proteins") was glucose starvation [22], illustrating that the responsiveness of the ER to a low nutritional or energetic state has been recognized for decades. UPR induction results, at least in part, from a glucose-deprivation-induced decrease in cellular ATP, which affects the function of the ER calcium pump SERCA2b and ER calcium levels [23]. Physiological UPR activation can therefore be mediated by unfavorable growth conditions and be reciprocal to the activation of mTORC1.

Hypoxia represents another stress condition which activates UPR signaling and inhibits mTORC1 [24]. What is the trigger of the UPR under hypoxic conditions? One possible answer is given by the fact that Ero1 oxidases which support disulfide-bond formation in nascent proteins in the ER depend on oxygen [25]. Accordingly, lowered Ero1 activity under hypoxia could lead to hampered protein folding and ER stress. This simple model, however, is complicated by the facts that oxygen deprivation transcriptionally induces Ero1α [26] and increases its activation state [27, 28]. Indeed, ER redox readouts showed that hypoxia-activated Ero1α is operative in preventing ER hypo-oxidation [27]. Alternative mechanisms are hypoxia-mediated up-regulation of GSK3β, which leads to the induction of PERK signaling via destabilization of the nascent polypeptide-associated complex [29] and/or an increase in free fatty acids (FFAs) evoked by the hypoxia-inducible transcription factor HIF-2α [30].

The fact that circulating FFAs, prevalent during obesity, induce ER stress illustrates that the UPR, like mTORC1, can also be activated by a high nutritional state [31]. Underlying mechanisms likely include changes in ER membrane composition, fluidity, and curvature that inhibit SERCA2b as shown in pancreatic β-cells [32] and

mouse liver [33]. Other examples that document the responsiveness of the UPR to overnutrition are cholesterol-loaded macrophages [34] and β-cells exposed to a high glucose concentration [35].

In certain contexts, the UPR is also activated by growth stimuli. Thus, ER expansion and induction of ER chaperones in B lymphocytes in response to antigen [36] and in thyrocytes in response to thyroid-stimulating hormone [37] is mediated by the UPR. Along the same line, UPR-mediated lipogenesis in the liver is activated following a high-carbohydrate meal in an mTORC1-dependent manner [38]. This finding implies that mTORC1 and UPR can act jointly to stimulate cell growth and suggests a pathway by which mTORC1 can induce UPR signaling. Interestingly, the UPR-dependent proliferation of ER membranes during differentiation of B lymphocytes and thyrocytes precedes the massive synthesis of immunoglobulin and thyroglobulin, respectively [36, 37], indicating that a mechanism other than ER overload is responsible for UPR activation. Whether this mechanism involves mTORC1 has not yet been examined.

Taken together, UPR signaling is elicited by a variety of physiological inputs, which include both favorable and unfavorable growth conditions. Likewise, a functional interaction between UPR and mTORC1 has been observed. As discussed in the next section, bidirectional crosstalk between mTORC1 and UPR also occurs under pathological conditions of chronic activation.

Links between mTORC1 and UPR

The primary output of UPR signaling is homeostatic adaptation by a variety of mechanisms that aim at restoring ER function (box 3). As a secondary output,

however, the UPR can also switch to promote apoptotic cell death through multiple pathways that remain to be fully understood [5]. Notably, the UPR as a mediator of ER-stress-induced apoptosis plays a pivotal role in a host of pathological conditions including neurodegenerative misfolding diseases and oxidative injury, as reviewed elsewhere [9, 39, 40]. It is intriguing to note that recent studies have also highlighted pathological situations where cell toxicity by ER stress is coupled to the chronic activation of mTORC1 [41-48]. This implies the apparent paradox that under certain settings, mTOR – a bona fide positive regulator of cell growth and division – can also signal cell demise. Furthermore, as discussed in this section, UPR activation can occur both upstream and downstream of mTORC1, which designates mTORC1 – at least in certain contexts – as a component in the process of ER-stress-induced cell death.

The best-documented ER pathway downstream of mTORC1 is Ire1α–ASK1–JNK (box 3). Constitutive mTORC1 activation by loss of TSC1–TSC2 stimulates JNK, which contributes to ER-stress-induced apoptosis [41, 43, 48]. Furthermore, thus activated JNK can participate in the development of insulin resistance [43], which occurs in parallel to other mechanisms such as ER-stress-facilitated de novo lipogenesis [49], activation of PKR [50], and the mTORC1–S6K1–IRS1 negative feedback loop [51, 52]. Activation of the Ire1α–JNK branch downstream of mTORC1 appears to be privileged over other arms of the UPR [41], and it has been suggested that it is in fact the induction of an incomplete UPR by chronic mTORC1 activation that kills the cell [53]. Consistent with apoptotic signaling through mTORC1–Ire1α–ASK1–JNK, over-expression or knockdown of Rheb enhances or antagonizes apoptotic stimuli in an ASK1-dependent fashion [54].

The finding that ER stress can also act upstream of mTORC1 adds a further layer of complexity. Pharmacological induction of the UPR rapidly activates the PI3K–Akt–mTORC1 signaling axis [41, 44, 45], which depends on the ATF6α branch of the UPR [55]. Prolonged treatment with ER-stress-inducing agents, however, inhibits Akt [45, 55-58] and mTORC1 [42, 59], which has – at least in part – been attributed to the mTORC1–S6K1–IRS1 negative feedback loop [41, 52]. Furthermore, PERK–CHOP-mediated induction of the Akt inhibitor TRB3 [60, 61] and GSK3β-mediated inactivation of mTORC2 (see section "mTORC2 and ER") may also contribute to the inhibition of Akt upon advanced ER stress.

The suppression of Akt following an extended period of ER stress apparently plays a central role in the activation of Ire1α–ASK1–JNK downstream of mTORC1, possibly by derepression of the ASK1 adaptor protein TRAF2 [41]. Such specific mTORC1-to-ER signaling in our opinion better explains the selective activation of Ire1α–JNK upon chronic activation of mTORC1 than a general mTORC1-mediated increase in protein synthesis and ER load. Additional modulation of UPR–mTORC1 crosstalk is provided by ATF6α-dependent up-regulation of Rheb [62] and by Akt-catalyzed suppressive phosphorylation of PERK [63]. The currently identified links between mTORC1 and UPR both upon acute and chronic stimulation are depicted in Figure 2A.

Given the interdependence of mTORC1 and UPR during pathological programs, it is also interesting to consider common target processes as well as antagonizing outputs of the two pathways during normal physiology. This interplay which is summarized in Figure 2 is likely fundamental for cell health. Synergism between mTORC1 and UPR occurs in the regulation of hepatic lipid synthesis [64-66], angiogenesis [67, 68], NFκB signaling [1, 69], and insulin resistance [43, 51]. By contrast, the two pathways

emit conflicting signals as to the control of ribosome biogenesis [70, 71], translation [4, 10], apoptosis [5, 72], and autophagy [4, 73].

Collectively, convincing evidence exists that ER stress, through stimulation of the "survival kinase" Akt, initially causes activation of mTORC1, which itself, in a later phase, contributes to Akt inhibition to activate the ER–JNK "death kinase" pathway. We therefore suggest that bi-phasic regulation of Akt by ER stress is a critical determinant of apoptotic UPR signaling. As mTORC2 also participates in the activation of Akt (box 2), the relationship between ER stress and mTORC2 is discussed in the next section.

mTORC2 and the ER

In contrast to mTORC1, much less is known about pathways operating upstream of mTORC2. However, ER stress also impacts mTORC2 signaling. Pharmacological induction of ER stress for several hours leads to GSK3β-catalyzed phosphorylation of the mTORC2 component rictor, which suppresses Akt activation [74]. Thus, together with the negative regulation of mTORC2 by the mTORC1 effector S6K1 [75], this mechanism likely contributes to the chronic UPR–mTORC1 apoptosis pathway described above.

Does mTORC2 also participate in Akt activation in an early phase of ER stress? At present, there is no compelling evidence in favor or against this notion. However, as demonstrated by immunofluorescence microscopy and subcellular fractionation, a significant fraction of mTORC2 resides on ER membranes – even following the stimulation of cells with growth factors [76]. It is therefore possible that phosphorylation of Akt by mTORC2 occurs on the surface of the ER. Upstream

regulation of mTORC2 critically relies on its binding to ribosomes, which is induced upon growth factor stimulation [77]. Whether such activation preferentially occurs on ER-associated ribosomes and depends on the physiological state of the ER remains to be explored. We would like to posit though that such regulation would be in line with the emerging view that mTOR complexes are controlled by association with specific membranes, as exemplified by mTORC1, which couples the sensing of amino acids in the lysosomal lumen with its activation on the surface of lysosomes [19].

Therapeutic implications and future directions

The connections and interdependencies between mTORC1 and UPR during chronic responses are associated with various pathologies [43, 45-48]. As a consequence, a number of new clues for combined therapy ensue, which warrant detailed preclinical evaluations and are discussed in this section.

A first example is TSC [16], a multisystem disorder which is caused by constitutive activation of mTORC1 and includes the activation of ER stress pathways [41, 43, 45, 53]. The most frequent medical symptom associated with TSC is epileptic seizure, which is widely believed to be caused by cerebral cortical tubers [16] but has also been proposed to be associated with mTORC1-dependent ER and oxidative stress through the ATF4–CHOP pathway [45]. Accordingly, antioxidant therapy or treatment with ER-stress-alleviating “chemical chaperones” such as 4-phenylbutyric acid (PBA) possibly combined with a low dosage of an mTORC1 inhibitor such as Everolimus (RAD001; approved for TSC patients who are not suitable for surgical intervention [7]) might inhibit epileptic seizures in TSC. A concern with systemic inhibition of mTORC1 in TSC, however, is that elimination of the S6K1–IRS1

feedback loop would lead to hyper-activation of Akt, which could potentially convert hamartomas into malignant tumors.

A deadly interplay between mTORC1 and ER stress has also been identified as a causal factor in renal syndromes such as diabetic nephropathy [47, 78] and so-called minimal change disease [46], which are characterized by the damage of glomerular podocytes. Similarly, pancreatic β-cell demise under glucolipotoxic conditions that occur in type 2 diabetes mellitus is supported by the same pathways [48]. In both cases, however, the functioning of mTORC1 is essential for cell viability so that the therapeutic value of mTOR inhibitors is restricted. It has therefore been suggested that a low dosage of rapamycin or alternative mTORC1 inhibitor [7] that would lower but not abolish mTORC1 signaling combined with PBA to inhibit ER stress could be used for treatment of nephropathies [47]. A similar strategy could also be considered for the treatment of type 2 diabetes, since combined inhibition of ER stress and mTORC1 is expected to improve the survival of β-cells [48] and also increase peripheral insulin sensitivity [43]. Furthermore, mTORC1 inhibition could counteract the pathological consequences of diabetes-linked obesity in white adipose tissue [79]. It is important to emphasize though that despite the fact that both rapamycin and PBA are FDA-approved drugs, such combined formulations for the treatment of TSC, renal failures, or diabetes still require preclinical assessment to identify possible pleiotropic effects. An alternative therapeutic approach to restore hepatic insulin sensitivity is the stimulation of the deacetylase SIRT1, as adenovirus-mediated over-expression of SIRT1 in the liver ameliorates the metabolic symptoms of obese animals through inhibition of mTORC1 and ER stress [44]. In addition, clinically approved ASK1 or JNK inhibitors would potentially add an important tool to mTORC1/ER-stress-targeting combination therapies [9].

As exemplified in TSC cells which are particularly sensitive to ER-stress-induced cell death [41, 43, 45, 53] the targeted activation of this pathway could be used to selectively kill tumors with hyperactive PI3K–Akt–mTORC1 axis. For instance, the approved anti-tumor drug Velcade/Bortezomib which indirectly induces ER stress by inhibiting the proteasome [80] could be considered. The Bcl-2 family tumor suppressor Mcl-1 provides another promising link between mTORC1- and ER-stress-based anti-tumor therapy. The inhibition of 4EBP-controlled translation of Mcl-1 is a central element in the treatment of mTORC1-hyperactive cancers [72]. Since – with the notable exception of melanoma cells [81] – the UPR also downregulates the translation of this key antiapoptotic protein through PERK–eIF2α [82, 83], a combination of PERK induction [84] and mTOR inhibition [7] could produce synergistic effects.

Although most of the links between mTOR and the ER have been uncovered by examination of chronic responses, the two signaling nodes almost certainly also interact under healthy conditions. Currently, this is best illustrated by the postprandial up-regulation of Ire1α signaling through mTORC1 in the liver [38]. As this up-regulation is an acute anabolic response to mTORC1, it is reasonable to assume that it does not result from inhibition of Akt, which elicits apoptotic Ire1α signaling during chronic response. A question for future investigation is, which factors make a cell sensitive to the channeling of initially anabolic mTORC1 signals into the apoptotic Ire1α–ASK1–JNK or ATF4–CHOP pathways. Moreover, it is important to stress that the current mechanistic understanding of UPR–mTORC1 as well as of mTORC1–UPR signaling is fragmentary (Fig. 2A). The linkage between UPR and the activation of mTORC1 apparently involves the ATF6α branch, which somehow elicits PI3K–Akt signaling upstream of mTORC1 [42, 55] as well as the induction of Rheb [62].

The stimulation of Ire1α by mTORC1, on the other hand, has been correlated with the up-regulation of TRAF2 through depletion of activated Akt [41]. How Akt lowers the levels of TRAF2, and whether there are additional mechanisms operating between mTORC1 and the UPR machinery in parallel remains to be worked out.

In summary, a number of recent reports have shed light on new connections that link two hitherto separated areas of modern cell biology. During the course of this review, we have discussed both physiological and therapeutic implications of these findings. Taken into consideration that the field of combined mTOR/UPR research is new, significant progress is likely still ahead.

Box 1

mTORC1

The catalytic subunit of mTORC1 (and mTORC2; box 2) is the PI3K-related protein kinase family member mTOR, which associates with Raptor and mLST8 to form the mTORC1 core complex [3] (Fig. I). Direct substrates of mTORC1 are 4E-BP1, S6K1, ATG13, ULK1, and Lipin 1 [4, 8] (Fig. I). 4E-BP1 inhibits translation initiation unless it is phosphorylated by mTORC1. Phosphorylated S6K1 positively regulates mRNA translation and ribosome biosynthesis. Conversely, mTORC1 phosphorylates and inhibits ATG13 and ULK1, thereby inhibiting autophagosome assembly. The mechanism by which mTORC1 enhances lipid synthesis through SREBP transcription factors has been elucidated only recently; Lipin 1, a negative regulator of SREBP transcriptional activity, is prevented from entering the nucleus when phosphorylated by mTORC1 [66]. Activation of mTORC1 also increases the transcription of HIF-1α by an unknown mechanism, which stimulates glycolytic gene expression and angiogenesis.

Box 2

mTORC2

The core components shared by mTORC2 and mTORC1 are mTOR and mLST8. In addition, mTORC2 contains Rictor, mSIN1, which exists in several isoforms, and PRR5 (also known as Protor1) (Fig. II). mTORC2 phosphorylates a subset of AGC kinase family members on a conserved serine residue in the hydrophobic motif [4]. Identified substrates are the AGC kinases Akt, SGK1, and PKC (Fig. II). Ablation of mTORC2 does not equally affect all Akt substrates; for example, phosphorylation of the FOXO1 and 3 transcription factors is affected, whereas phosphorylation of TSC2, which is upstream of mTORC1, is not affected [85]. By phosphorylating PKC, mTORC2 also regulates the actin cytoskeleton and cell polarity.

Box 3

The Unfolded Protein Response

UPR signaling in vertebrates depends on three types of ER membrane-embedded sensor proteins that impart distinct, but partially overlapping cell fate signals [1, 10] (Fig. III). (i) After UPR activation, the Ire1α RNase initiates the unconventional splicing of a specific mRNA on the surface of the ER, which then encodes the transcription factor Xbp1s. Alternatively, probably depending on the nature or severity of the triggering insult, Ire1α can both, degrade select ER-associated mRNAs (through a process called regulated Ire1α-dependent decay; RIDD) to attenuate protein import into the ER and initiate a MAP kinase signaling cascade that leads to JNK activation. (ii) By directly phosphorylating the eIF2α translation initiation factor, the ER stress-sensor PERK is part of an "integrated stress response", which lowers overall translation, while increasing the cellular antioxidant capacity by selectively stimulating the translation of the transcription factor ATF4 among others. In addition, PERK directly activates the antioxidant response transcription factor Nrf2. (iii) The activation of ATF6α and an increasing number of tissue-specific ATF6-like ER-resident transcription factors occurs by yet another mechanism; on sensing ER stress, these proteins travel to the Golgi complex where they are subjected to intramembrane proteolysis thus liberating their DNA-binding domain (p50) for nuclear translocation. Transcriptional targets of Xbp1s, ATF4, and ATF6α include genes encoding ER chaperones and oxidoreductases, ER-associated degradation factors, phospholipid biosynthesis enzymes, and proteins involved in metabolic control.

While the UPR primarily aims at lowering the burden of folding substrates and increasing the capacity of the ER's folding machinery, it can also mediate apoptotic cell death through many different pathways [5]. One key pathway to ER stress-

induced apoptosis is the transcriptional induction of CHOP by ATF4 (Fig. III). CHOP is an important proapoptotic transcription factor that, for instance, upregulates proapoptotic members of the Bcl-2 family. Likewise, the activation of the intrinsic apoptosis pathway via ER stress-induced ER-to-mitochondria calcium transmission and activation of Bax/Bak on mitochondria is of particular importance [5].

Figure legends

Figure 1

Upstream regulation of mTORC1

Activation of mTORC1 is controlled by TSC1–TSC2 and by amino acids (AA). (Upper part) By acting as a GTPase-activating protein on Rheb, TSC1–TSC2 inhibits mTORC1 signaling. TSC1–TSC2 loss-of-function systems are frequently used to study the consequences of chronic mTORC1 hyper-activity. Multiple pathways positively or negatively impact mTORC1 signaling through modulation of TSC1–TSC2. Growth factors and hormones signal via PI3K–Akt or Raf–Mek–Erk to phosphorylate and inhibit TSC1–TSC2. Conversely, GSK3 activates TSC2 by phosphorylation, as does AMPK in response to low intracellular energy levels [86]. Similarly, a cytokine-induced pathway where IKKβ phosphorylates and suppresses TSC1 exists to varying degrees in different cell types [87]. In addition to these phosphorylation-dependent events, the stability of TSC1–TSC2 is negatively regulated through association with the gluconeogenic (i.e. anabolic) transcription factor FOXO1 [88]. Furthermore, an inhibitory complex between TSC2 and 14-3-3 proteins is dissociated by the action of REDD1 in response to hypoxia [89], which adds to several different mechanisms for mTORC1 inhibition by hypoxia [24]. Where applicable, the principal phosphorylation sites of a given pathway in TSC1–TSC2, which either enhance or suppress its activity, are indicated. (Lower part) Different mechanisms by which AA talk to mTORC1 exist in parallel. (1) In mammals, AA stimulation induces influx of extracellular Ca^{2+}, which activates calmodulin (CaM) to bind and activate the class III PI3K mVps34. This complex in turn positively regulates mTORC1 by a mechanism that remains to be fully elucidated [21]. (2) AA

in the lysosomal lumen modulate the binding of the vacuolar proton pump (v-ATPase) to the trimeric p14–p18–MP1 complex (also called the Ragulator), which anchors Rag GTPases to the surface of lysosomes. Thus, v-ATPase promotes the translocation of mTORC1 to lysosomes where it is activated by Rheb–GTP [19]. (3) Phosphorylation of MAP4K3 on Ser170 in response to AA activates mTORC1 by an unknown mechanism [20].

Figure 2

Interplay between UPR and mTORC1

(A) The known signaling pathways linking UPR and mTORC1 activation can be subdivided into acute phase (black arrows) and chronic phase (grey arrows) pathways. ER stress/UPR can activate mTORC1 via ATF6α, which triggers the PI3K pathway and increases the levels of Rheb by unknown mechanisms (question marks). The former pathway leads to activation of Akt during an early phase of ER stress. Acute activation of the UPR by mTORC1 presumably occurs through increased protein synthesis, which elevates the demand on the ER machinery for protein folding. Chronic activation of mTORC1 and UPR causes inactivation of Akt through at least four mechanisms: (1) Suppressive phosphorylation of IRS1 and (2) mTORC2 by S6K1 downstream of mTORC1, (3) inhibition of mTORC2 by GSK3β-catalyzed phosphorylation downstream of ER stress, and (4) PERK–CHOP-mediated induction of TRB3, which directly binds to Akt and blocks its activation. Lowered Akt activation precipitates higher levels of TRAF2, which triggers the Ire1α–ASK1–JNK branch of the UPR. Question marks denote where molecular mechanisms remain to be identified.

(B) The diverse outputs of the UPR and mTORC1 pathways can be synergistic or antagonistic. Joint positive regulation occurs in the case of de novo lipogenesis, angiogenesis, insulin resistance, and activation of the NFκB pathway (light brown boxes). By contrast, autophagy is stimulated by the UPR and inhibited by mTORC1, whereas inverse signaling outputs regulate the processes of ribosome biogenesis and translation (light green boxes). Relating to apoptosis, the interplay between UPR and mTORC1 is context-dependent. While in an initial ("physiological") phase of ER stress, the output of the UPR may be homeostatic/antiapoptotic, the UPR will promote apoptosis upon chronic activation (represented by a dashed activation arrow). Likewise, on unknown stimulus and/or prolonged ER stress, mTORC1, which usually promotes cell survival, can also contribute to apoptotic signaling by the UPR (dashed grey arrow; see main text for details).

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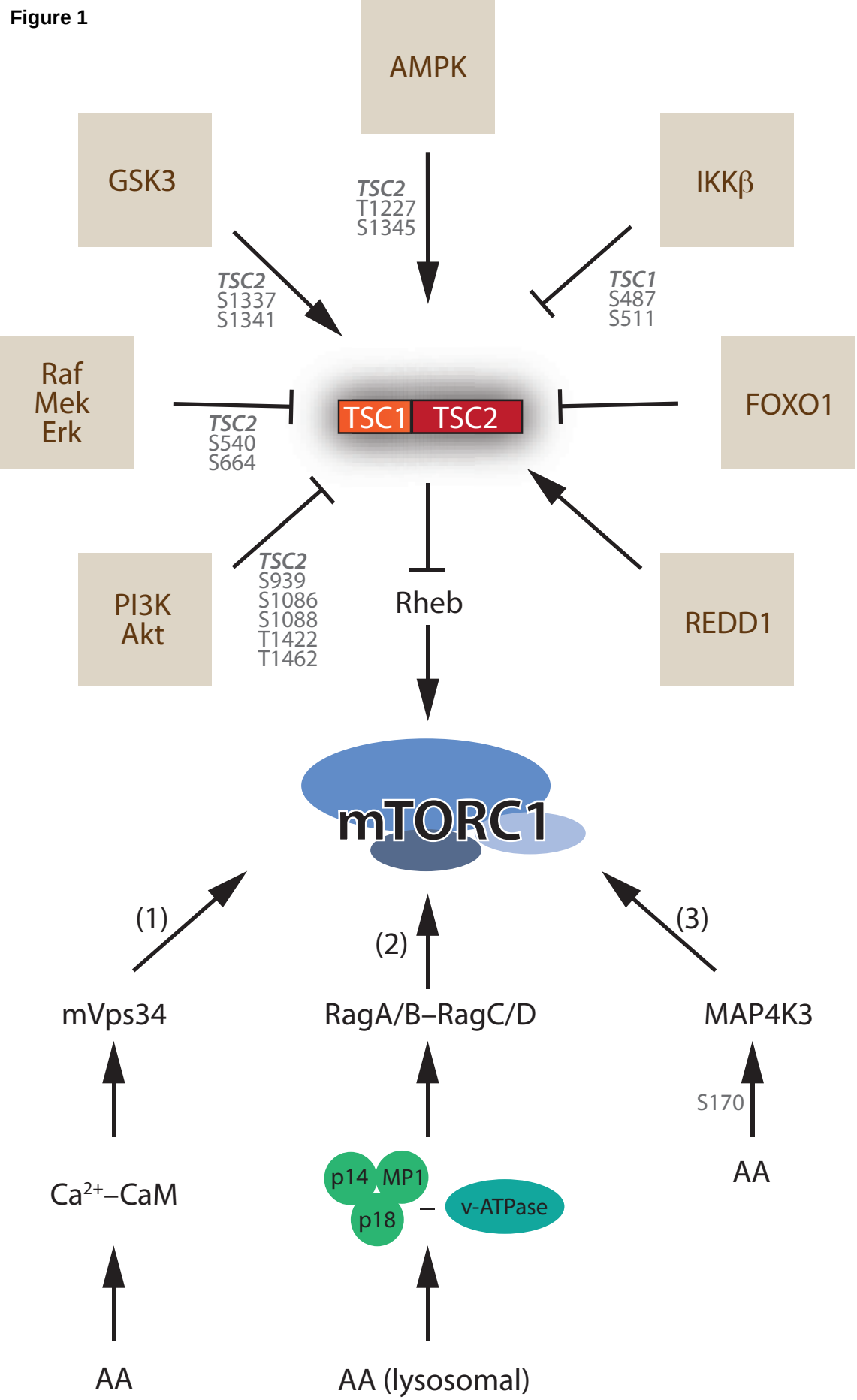
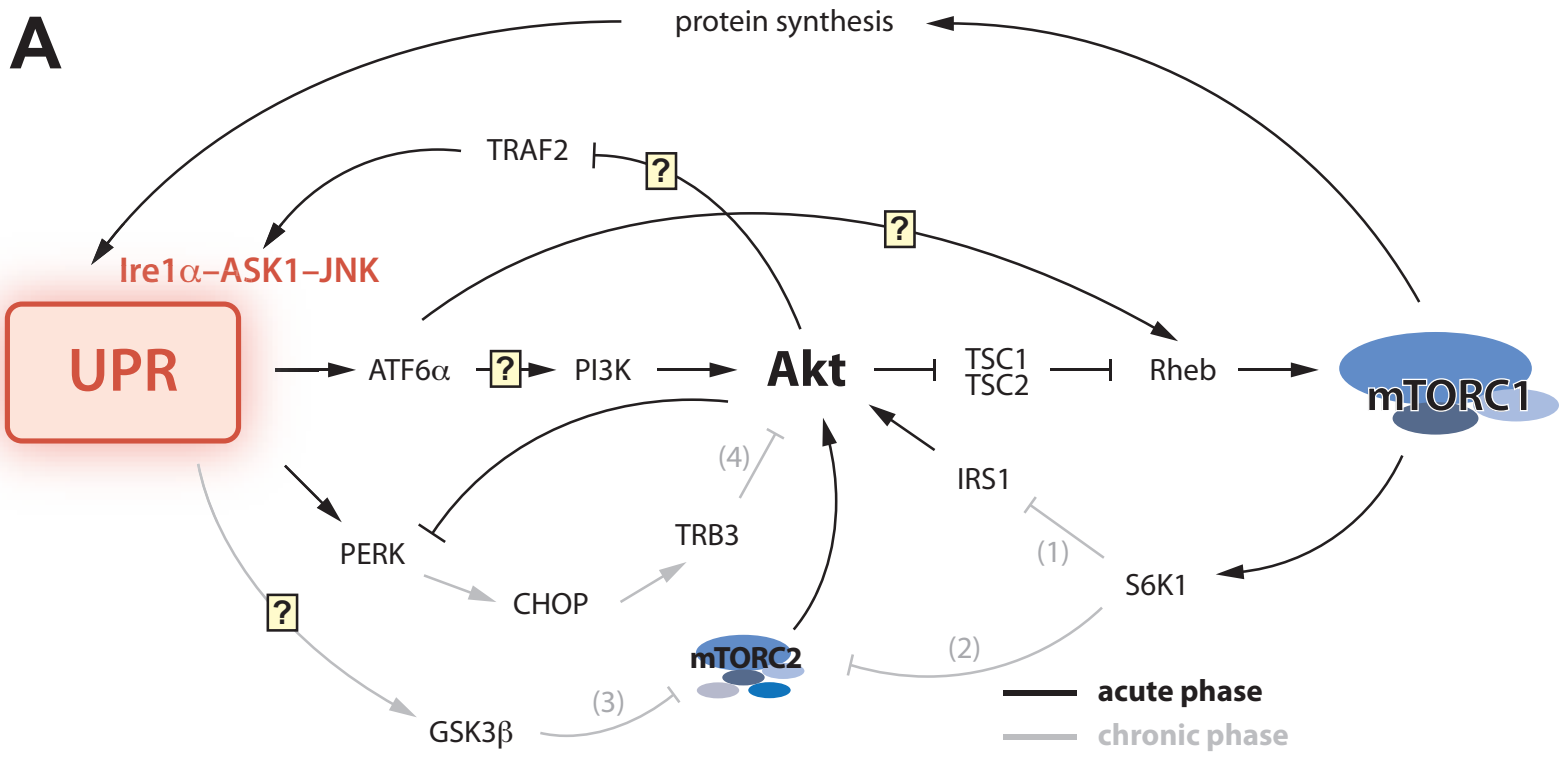
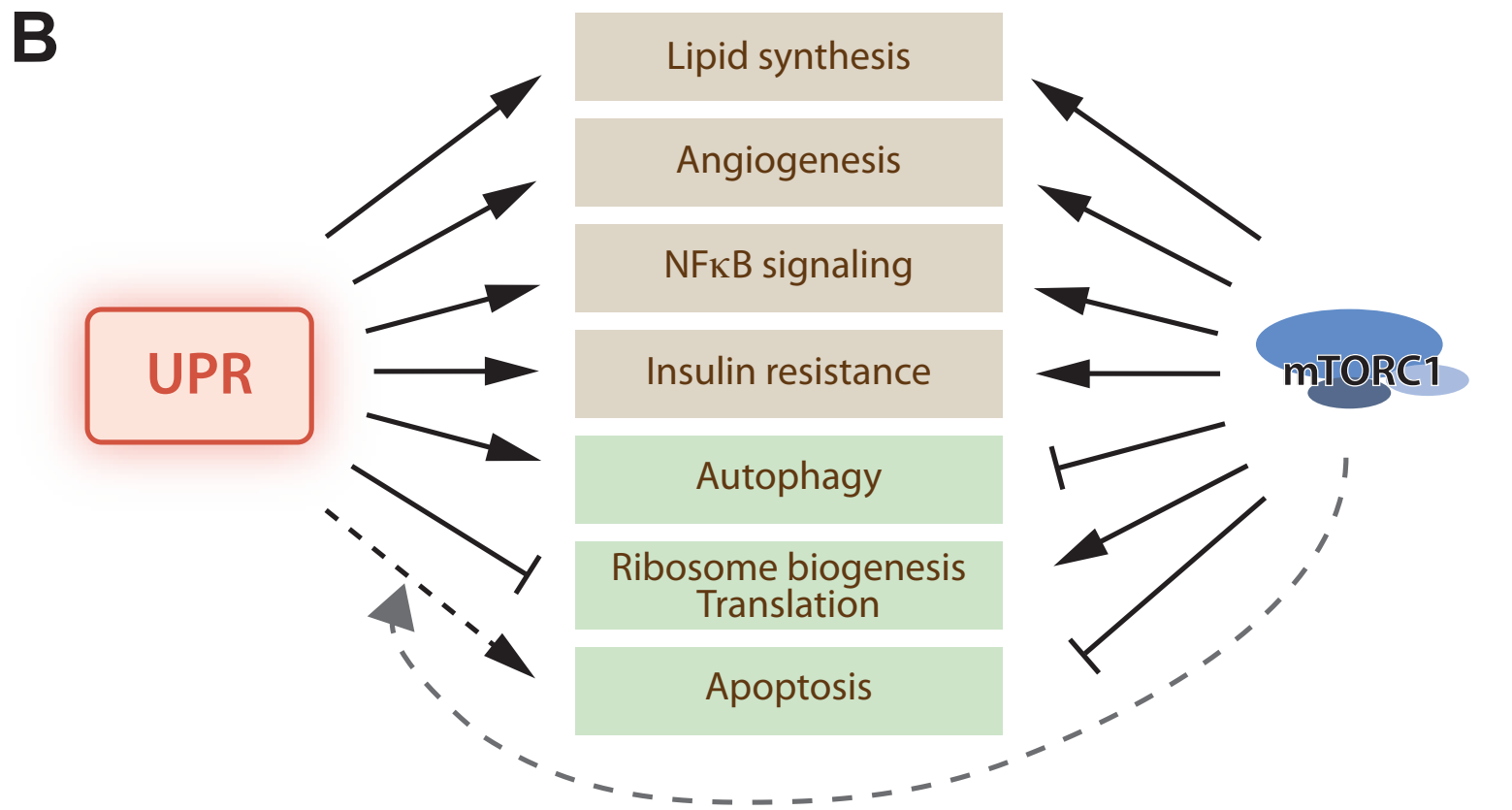
Figure 1



Appenzeller-Herzog Figure 1

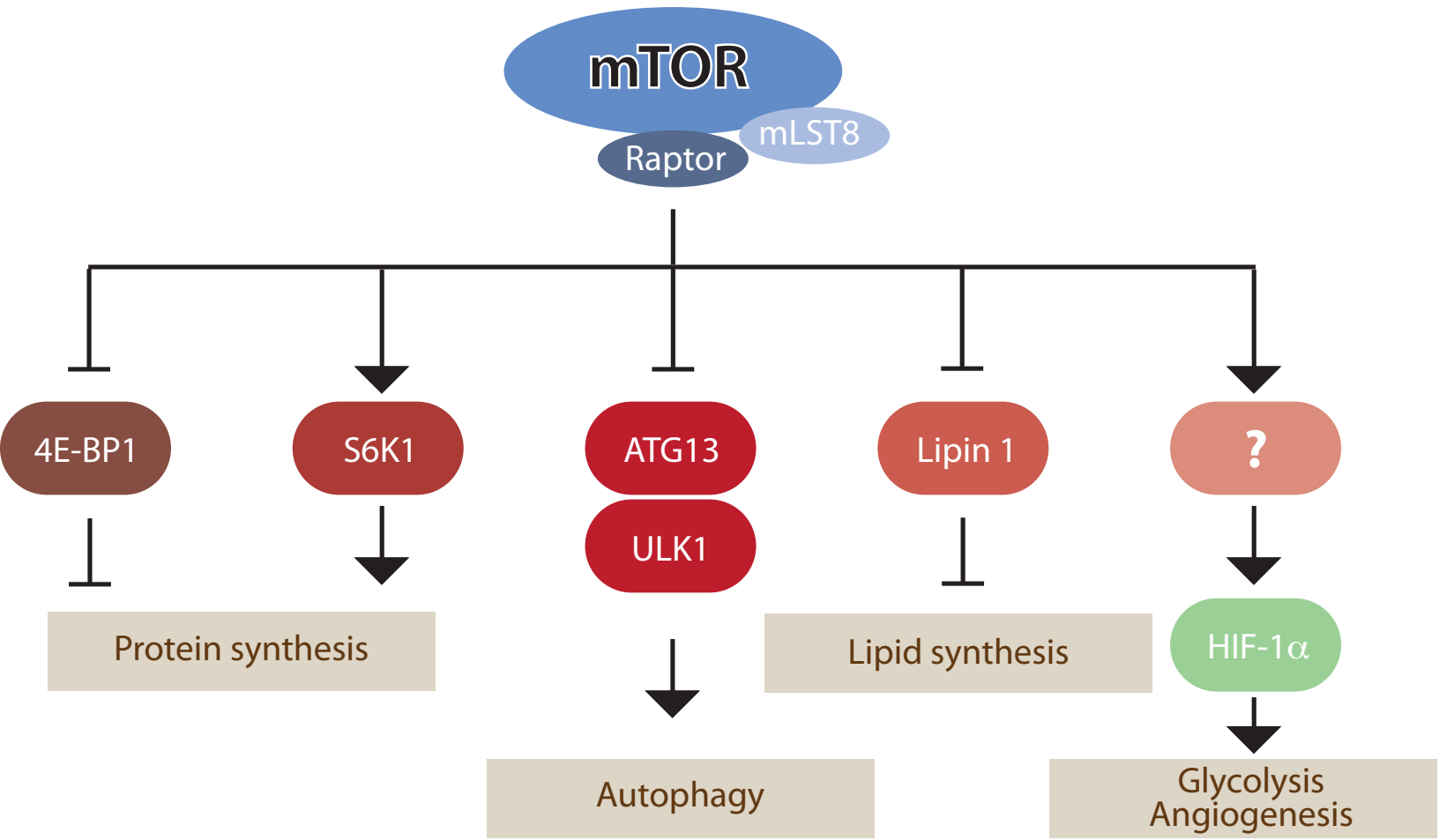
Figure 2





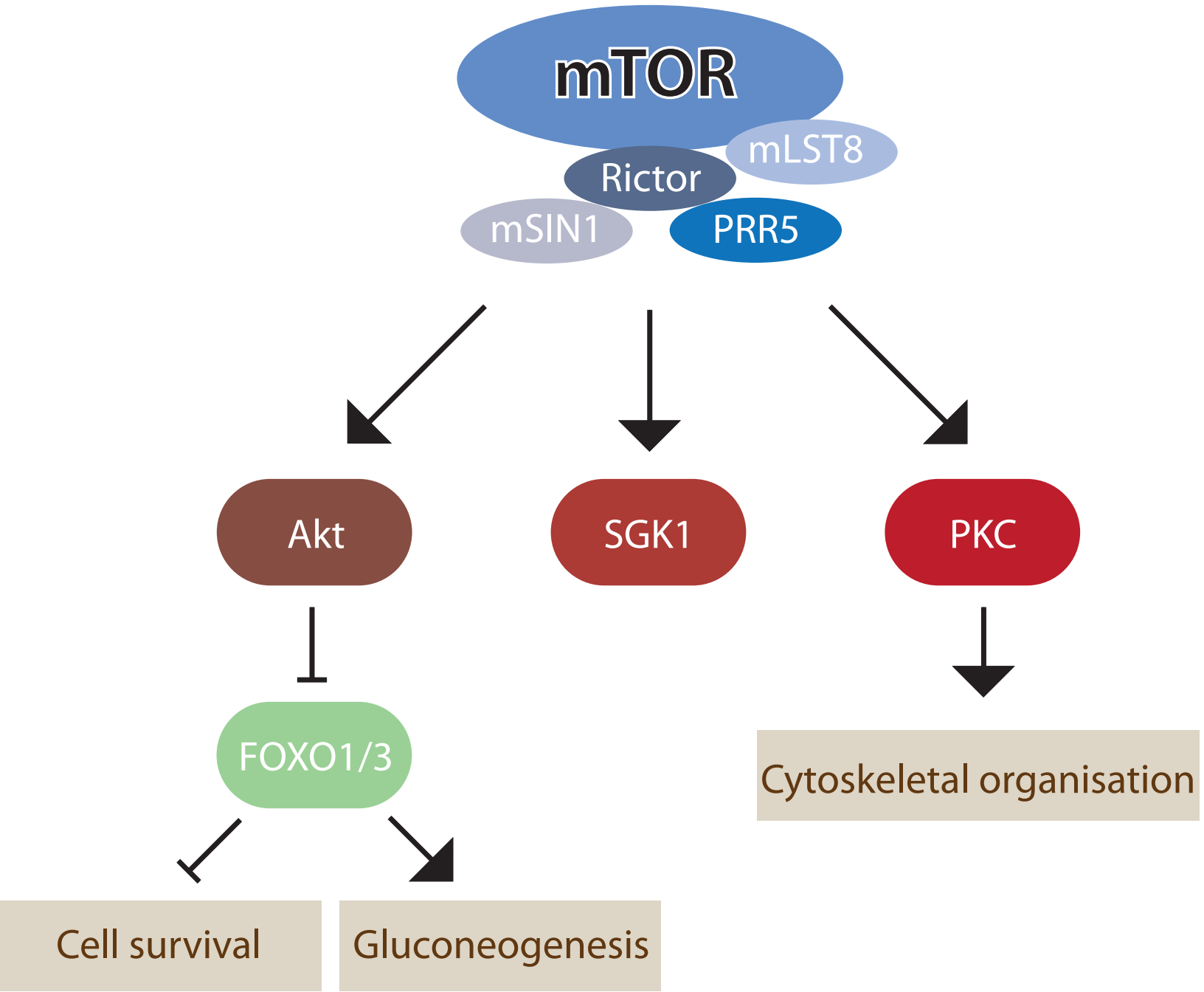
Appenzeller-Herzog Figure 2

Figure I



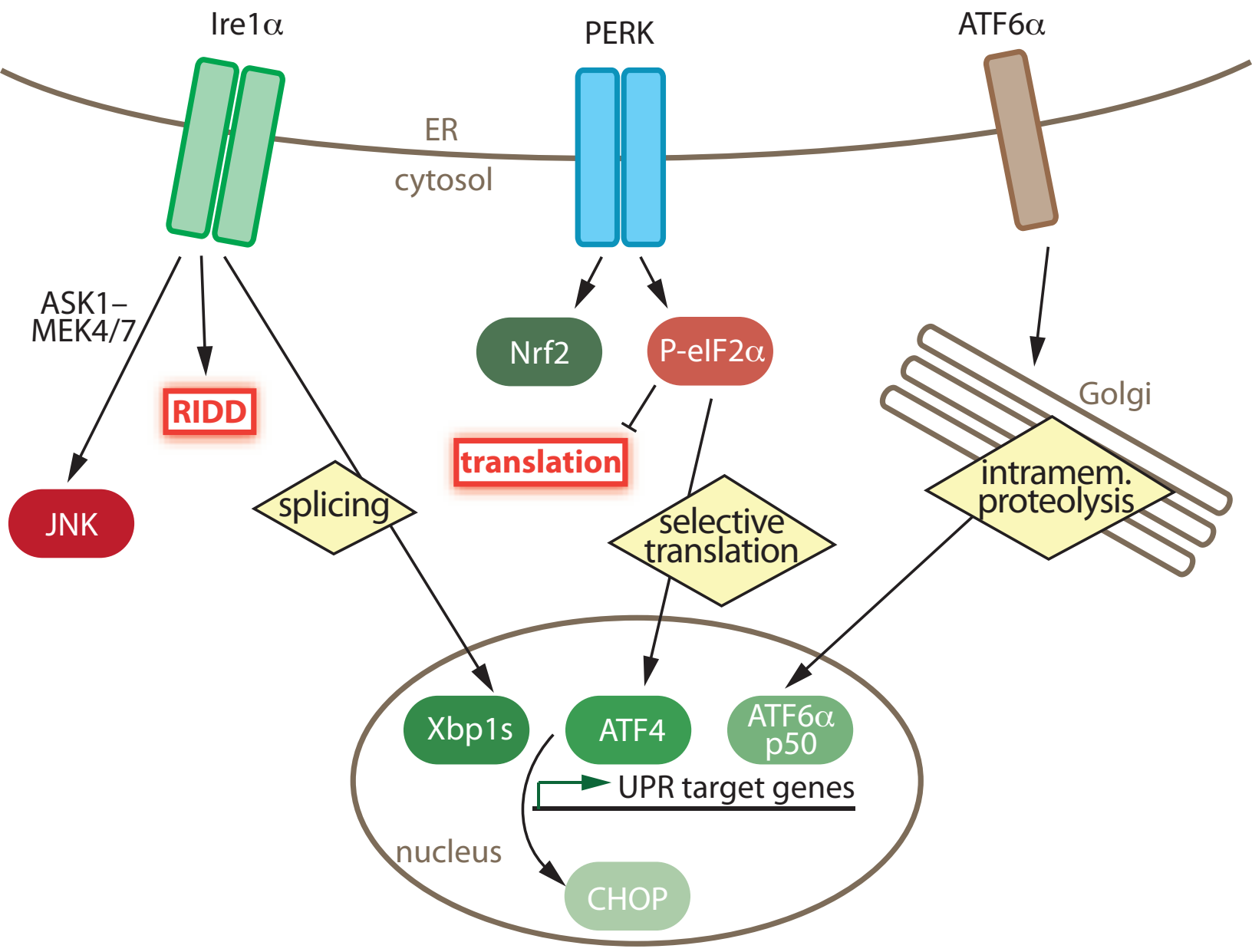
Appenzeller-Herzog Figure I

Figure II



Appenzeller-Herzog Figure II

Figure III



Appenzeller-Herzog Figure III