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Bidirectional crosstalk between endoplasmic reticulum stress and mTOR signaling

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Bidirectional crosstalk

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between endoplasmic reticulum stress and mTOR

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19

response, anabolism, apoptosis

20

21 **Abstract**

22

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

35

36 **Introduction**

37

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

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

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

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

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

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

87

88 **Signal integration by mTORC1**

89

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

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

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

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

122 Collectively, activation of mTORC1 signaling is a multi-step process that depends on
123 the inputs of cellular signaling cascades and the availability of nutrients, energy, and
124 oxygen. As both Rheb- and Rag-integrated inputs are required for activation, this
125 creates a situation of tight regulation which – in light of the pathological
126 consequences of uncontrolled mTORC1 activation [16] – is critically important.

127 Before discussing the inputs of ER stress on mTORC1 activation, we will next give
128 an overview of specific “growth” conditions that elicit UPR signaling.

129

130 **Upstream of the UPR**

131

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

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

143 Hypoxia represents another stress condition which activates UPR signaling and
144 inhibits mTORC1 [24]. What is the trigger of the UPR under hypoxic conditions?
145 One possible answer is given by the fact that Ero1 oxidases which support disulfide-
146 bond formation in nascent proteins in the ER depend on oxygen [25]. Accordingly,
147 lowered Ero1 activity under hypoxia could lead to hampered protein folding and ER
148 stress. This simple model, however, is complicated by the facts that oxygen
149 deprivation transcriptionally induces Ero1 α [26] and increases its activation state [27,
150 28]. Indeed, ER redox readouts showed that hypoxia-activated Ero1 α is operative in
151 preventing ER hypo-oxidation [27]. Alternative mechanisms are hypoxia-mediated
152 up-regulation of GSK3 β , which leads to the induction of PERK signaling via
153 destabilization of the nascent polypeptide-associated complex [29] and/or an increase
154 in free fatty acids (FFAs) evoked by the hypoxia-inducible transcription factor HIF-
155 2 α [30].

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

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

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

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

180

181 **Links between mTORC1 and UPR**

182

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

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

197 The best-documented ER pathway downstream of mTORC1 is Ire1 α –ASK1–JNK
198 (box 3). Constitutive mTORC1 activation by loss of TSC1–TSC2 stimulates JNK,
199 which contributes to ER-stress-induced apoptosis [41, 43, 48]. Furthermore, thus
200 activated JNK can participate in the development of insulin resistance [43], which
201 occurs in parallel to other mechanisms such as ER-stress-facilitated de novo
202 lipogenesis [49], activation of PKR [50], and the mTORC1–S6K1–IRS1 negative
203 feedback loop [51, 52]. Activation of the Ire1 α –JNK branch downstream of mTORC1
204 appears to be privileged over other arms of the UPR [41], and it has been suggested
205 that it is in fact the induction of an incomplete UPR by chronic mTORC1 activation
206 that kills the cell [53]. Consistent with apoptotic signaling through mTORC1–Ire1 α –
207 ASK1–JNK, over-expression or knockdown of Rheb enhances or antagonizes
208 apoptotic stimuli in an ASK1-dependent fashion [54].

209 The finding that ER stress can also act upstream of mTORC1 adds a further layer of
210 complexity. Pharmacological induction of the UPR rapidly activates the PI3K–Akt–
211 mTORC1 signaling axis [41, 44, 45], which depends on the ATF6 α branch of the
212 UPR [55]. Prolonged treatment with ER-stress-inducing agents, however, inhibits Akt
213 [45, 55-58] and mTORC1 [42, 59], which has – at least in part – been attributed to the
214 mTORC1–S6K1–IRS1 negative feedback loop [41, 52]. Furthermore, PERK–CHOP-
215 mediated induction of the Akt inhibitor TRB3 [60, 61] and GSK3 β -mediated
216 inactivation of mTORC2 (see section “mTORC2 and ER”) may also contribute to the
217 inhibition of Akt upon advanced ER stress.

218 The suppression of Akt following an extended period of ER stress apparently plays a
219 central role in the activation of Ire1 α –ASK1–JNK downstream of mTORC1, possibly
220 by derepression of the ASK1 adaptor protein TRAF2 [41]. Such specific mTORC1-
221 to-ER signaling in our opinion better explains the selective activation of Ire1 α –JNK
222 upon chronic activation of mTORC1 than a general mTORC1-mediated increase in
223 protein synthesis and ER load. Additional modulation of UPR–mTORC1 crosstalk is
224 provided by ATF6 α -dependent up-regulation of Rheb [62] and by Akt-catalyzed
225 suppressive phosphorylation of PERK [63]. The currently identified links between
226 mTORC1 and UPR both upon acute and chronic stimulation are depicted in Figure
227 2A.

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

234 emit conflicting signals as to the control of ribosome biogenesis [70, 71], translation
235 [4, 10], apoptosis [5, 72], and autophagy [4, 73].

236 Collectively, convincing evidence exists that ER stress, through stimulation of the
237 “survival kinase” Akt, initially causes activation of mTORC1, which itself, in a later
238 phase, contributes to Akt inhibition to activate the ER–JNK “death kinase” pathway.

239 We therefore suggest that bi-phasic regulation of Akt by ER stress is a critical
240 determinant of apoptotic UPR signaling. As mTORC2 also participates in the
241 activation of Akt (box 2), the relationship between ER stress and mTORC2 is
242 discussed in the next section.

243

244 **mTORC2 and the ER**

245

246 In contrast to mTORC1, much less is known about pathways operating upstream of
247 mTORC2. However, ER stress also impacts mTORC2 signaling. Pharmacological
248 induction of ER stress for several hours leads to GSK3 β -catalyzed phosphorylation of
249 the mTORC2 component rictor, which suppresses Akt activation [74]. Thus, together
250 with the negative regulation of mTORC2 by the mTORC1 effector S6K1 [75], this
251 mechanism likely contributes to the chronic UPR–mTORC1 apoptosis pathway
252 described above.

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

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

266

267 **Therapeutic implications and future directions**

268

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

273 A first example is TSC [16], a multisystem disorder which is caused by constitutive
274 activation of mTORC1 and includes the activation of ER stress pathways [41, 43, 45,
275 53]. The most frequent medical symptom associated with TSC is epileptic seizure,
276 which is widely believed to be caused by cerebral cortical tubers [16] but has also
277 been proposed to be associated with mTORC1-dependent ER and oxidative stress
278 through the ATF4-CHOP pathway [45]. Accordingly, antioxidant therapy or
279 treatment with ER-stress-alleviating “chemical chaperones” such as 4-phenylbutyric
280 acid (PBA) possibly combined with a low dosage of an mTORC1 inhibitor such as
281 Everolimus (RAD001; approved for TSC patients who are not suitable for surgical
282 intervention [7]) might inhibit epileptic seizures in TSC. A concern with systemic
283 inhibition of mTORC1 in TSC, however, is that elimination of the S6K1-IRS1

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

286 A deadly interplay between mTORC1 and ER stress has also been identified as a
287 causal factor in renal syndromes such as diabetic nephropathy [47, 78] and so-called
288 minimal change disease [46], which are characterized by the damage of glomerular
289 podocytes. Similarly, pancreatic β -cell demise under glucolipotoxic conditions that
290 occur in type 2 diabetes mellitus is supported by the same pathways [48]. In both
291 cases, however, the functioning of mTORC1 is essential for cell viability so that the
292 therapeutic value of mTOR inhibitors is restricted. It has therefore been suggested that
293 a low dosage of rapamycin or alternative mTORC1 inhibitor [7] that would lower but
294 not abolish mTORC1 signaling combined with PBA to inhibit ER stress could be used
295 for treatment of nephropathies [47]. A similar strategy could also be considered for
296 the treatment of type 2 diabetes, since combined inhibition of ER stress and mTORC1
297 is expected to improve the survival of β -cells [48] and also increase peripheral insulin
298 sensitivity [43]. Furthermore, mTORC1 inhibition could counteract the pathological
299 consequences of diabetes-linked obesity in white adipose tissue [79]. It is important to
300 emphasize though that despite the fact that both rapamycin and PBA are FDA-
301 approved drugs, such combined formulations for the treatment of TSC, renal failures,
302 or diabetes still require preclinical assessment to identify possible pleiotropic effects.

303 An alternative therapeutic approach to restore hepatic insulin sensitivity is the
304 stimulation of the deacetylase SIRT1, as adenovirus-mediated over-expression of
305 SIRT1 in the liver ameliorates the metabolic symptoms of obese animals through
306 inhibition of mTORC1 and ER stress [44]. In addition, clinically approved ASK1 or
307 JNK inhibitors would potentially add an important tool to mTORC1/ER-stress-
308 targeting combination therapies [9].

309 As exemplified in TSC cells which are particularly sensitive to ER-stress-induced cell
310 death [41, 43, 45, 53] the targeted activation of this pathway could be used to
311 selectively kill tumors with hyperactive PI3K–Akt–mTORC1 axis. For instance, the
312 approved anti-tumor drug Velcade/Bortezomib which indirectly induces ER stress by
313 inhibiting the proteasome [80] could be considered. The Bcl-2 family tumor
314 suppressor Mcl-1 provides another promising link between mTORC1- and ER-stress-
315 based anti-tumor therapy. The inhibition of 4EBP-controlled translation of Mcl-1 is a
316 central element in the treatment of mTORC1-hyperactive cancers [72]. Since – with
317 the notable exception of melanoma cells [81] – the UPR also downregulates the
318 translation of this key antiapoptotic protein through PERK–eIF2 α [82, 83], a
319 combination of PERK induction [84] and mTOR inhibition [7] could produce
320 synergistic effects.

321 Although most of the links between mTOR and the ER have been uncovered by
322 examination of chronic responses, the two signaling nodes almost certainly also
323 interact under healthy conditions. Currently, this is best illustrated by the postprandial
324 up-regulation of Ire1 α signaling through mTORC1 in the liver [38]. As this up-
325 regulation is an acute anabolic response to mTORC1, it is reasonable to assume that it
326 does not result from inhibition of Akt, which elicits apoptotic Ire1 α signaling during
327 chronic response. A question for future investigation is, which factors make a cell
328 sensitive to the channeling of initially anabolic mTORC1 signals into the apoptotic
329 Ire1 α –ASK1–JNK or ATF4–CHOP pathways. Moreover, it is important to stress that
330 the current mechanistic understanding of UPR–mTORC1 as well as of mTORC1–
331 UPR signaling is fragmentary (Fig. 2A). The linkage between UPR and the activation
332 of mTORC1 apparently involves the ATF6 α branch, which somehow elicits PI3K–
333 Akt signaling upstream of mTORC1 [42, 55] as well as the induction of Rheb [62].

334 The stimulation of Ire1 α by mTORC1, on the other hand, has been correlated with the
335 up-regulation of TRAF2 through depletion of activated Akt [41]. How Akt lowers the
336 levels of TRAF2, and whether there are additional mechanisms operating between
337 mTORC1 and the UPR machinery in parallel remains to be worked out.

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

343

344 **Box 1**

345 *mTORC1*

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

359

360 **Box 2**

361 *mTORC2*

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

371

372 **Box 3**

373 *The Unfolded Protein Response*

374 UPR signaling in vertebrates depends on three types of ER membrane-embedded
375 sensor proteins that impart distinct, but partially overlapping cell fate signals [1, 10]
376 (Fig. III). (i) After UPR activation, the Ire1 α RNase initiates the unconventional
377 splicing of a specific mRNA on the surface of the ER, which then encodes the
378 transcription factor Xbp1s. Alternatively, probably depending on the nature or
379 severity of the triggering insult, Ire1 α can both, degrade select ER-associated mRNAs
380 (through a process called regulated Ire1 α -dependent decay; RIDD) to attenuate
381 protein import into the ER and initiate a MAP kinase signaling cascade that leads to
382 JNK activation. (ii) By directly phosphorylating the eIF2 α translation initiation factor,
383 the ER stress-sensor PERK is part of an “integrated stress response”, which lowers
384 overall translation, while increasing the cellular antioxidant capacity by selectively
385 stimulating the translation of the transcription factor ATF4 among others. In addition,
386 PERK directly activates the antioxidant response transcription factor Nrf2. (iii) The
387 activation of ATF6 α and an increasing number of tissue-specific ATF6-like ER-
388 resident transcription factors occurs by yet another mechanism; on sensing ER stress,
389 these proteins travel to the Golgi complex where they are subjected to intramembrane
390 proteolysis thus liberating their DNA-binding domain (p50) for nuclear translocation.
391 Transcriptional targets of Xbp1s, ATF4, and ATF6 α include genes encoding ER
392 chaperones and oxidoreductases, ER-associated degradation factors, phospholipid
393 biosynthesis enzymes, and proteins involved in metabolic control.
394 While the UPR primarily aims at lowering the burden of folding substrates and
395 increasing the capacity of the ER’s folding machinery, it can also mediate apoptotic
396 cell death through many different pathways [5]. One key pathway to ER stress-

397 induced apoptosis is the transcriptional induction of CHOP by ATF4 (Fig. III). CHOP
398 is an important proapoptotic transcription factor that, for instance, upregulates
399 proapoptotic members of the Bcl-2 family. Likewise, the activation of the intrinsic
400 apoptosis pathway via ER stress-induced ER-to-mitochondria calcium transmission
401 and activation of Bax/Bak on mitochondria is of particular importance [5].
402

403 **Figure legends**

404

405 *Figure 1*

406 **Upstream regulation of mTORC1**

407 Activation of mTORC1 is controlled by TSC1–TSC2 and by amino acids (AA).
408 (Upper part) By acting as a GTPase-activating protein on Rheb, TSC1–TSC2 inhibits
409 mTORC1 signaling. TSC1–TSC2 loss-of-function systems are frequently used to
410 study the consequences of chronic mTORC1 hyper-activity. Multiple pathways
411 positively or negatively impact mTORC1 signaling through modulation of TSC1–
412 TSC2. Growth factors and hormones signal via PI3K–Akt or Raf–Mek–Erk to
413 phosphorylate and inhibit TSC1–TSC2. Conversely, GSK3 activates TSC2 by
414 phosphorylation, as does AMPK in response to low intracellular energy levels [86].
415 Similarly, a cytokine-induced pathway where IKK β phosphorylates and suppresses
416 TSC1 exists to varying degrees in different cell types [87]. In addition to these
417 phosphorylation-dependent events, the stability of TSC1–TSC2 is negatively
418 regulated through association with the gluconeogenic (i.e. anabolic) transcription
419 factor FOXO1 [88]. Furthermore, an inhibitory complex between TSC2 and 14-3-3
420 proteins is dissociated by the action of REDD1 in response to hypoxia [89], which
421 adds to several different mechanisms for mTORC1 inhibition by hypoxia [24]. Where
422 applicable, the principal phosphorylation sites of a given pathway in TSC1–TSC2,
423 which either enhance or suppress its activity, are indicated. (Lower part) Different
424 mechanisms by which AA talk to mTORC1 exist in parallel. (1) In mammals, AA
425 stimulation induces influx of extracellular Ca²⁺, which activates calmodulin (CaM) to
426 bind and activate the class III PI3K mVps34. This complex in turn positively
427 regulates mTORC1 by a mechanism that remains to be fully elucidated [21]. (2) AA

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

434

435 *Figure 2*

436 **Interplay between UPR and mTORC1**

437 (A) The known signaling pathways linking UPR and mTORC1 activation can be
438 subdivided into acute phase (black arrows) and chronic phase (grey arrows) pathways.
439 ER stress/UPR can activate mTORC1 via ATF6 α , which triggers the PI3K pathway
440 and increases the levels of Rheb by unknown mechanisms (question marks). The
441 former pathway leads to activation of Akt during an early phase of ER stress. Acute
442 activation of the UPR by mTORC1 presumably occurs through increased protein
443 synthesis, which elevates the demand on the ER machinery for protein folding.
444 Chronic activation of mTORC1 and UPR causes inactivation of Akt through at least
445 four mechanisms: (1) Suppressive phosphorylation of IRS1 and (2) mTORC2 by
446 S6K1 downstream of mTORC1, (3) inhibition of mTORC2 by GSK3 β -catalyzed
447 phosphorylation downstream of ER stress, and (4) PERK–CHOP-mediated induction
448 of TRB3, which directly binds to Akt and blocks its activation. Lowered Akt
449 activation precipitates higher levels of TRAF2, which triggers the Ire1 α –ASK1–JNK
450 branch of the UPR. Question marks denote where molecular mechanisms remain to be
451 identified.

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

464

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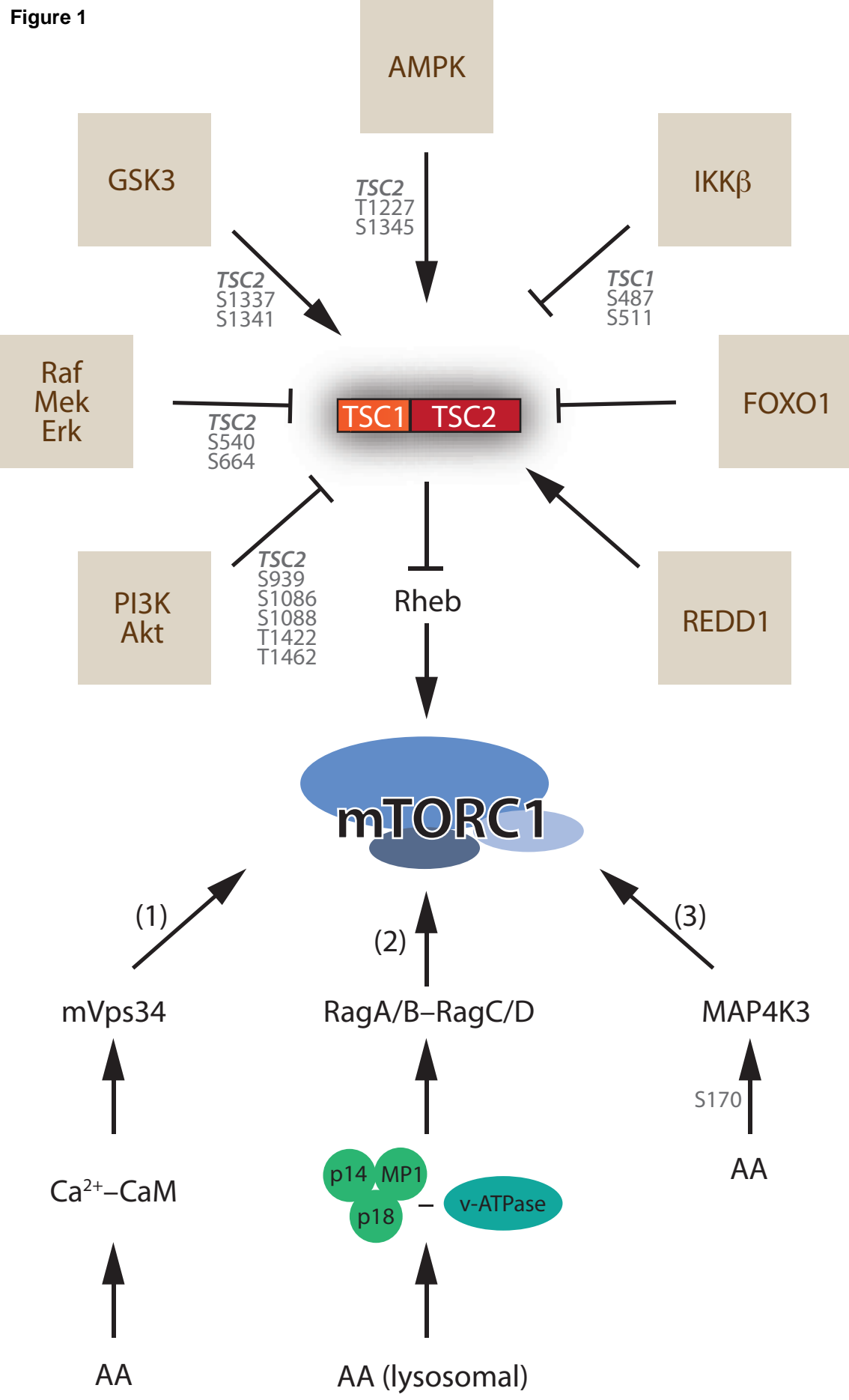
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Figure 1



Appenzeller-Herzog Figure 1

Figure 2

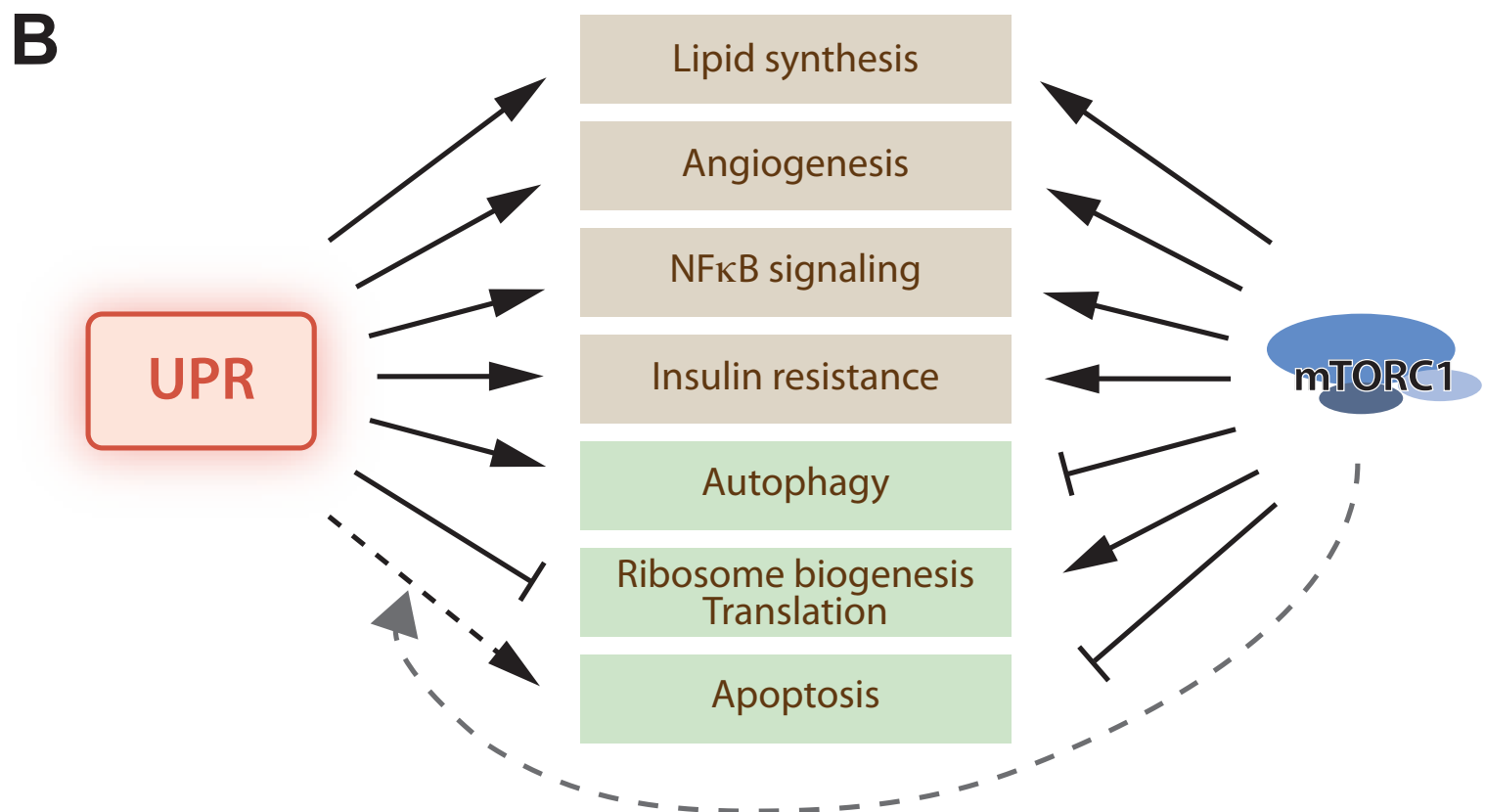
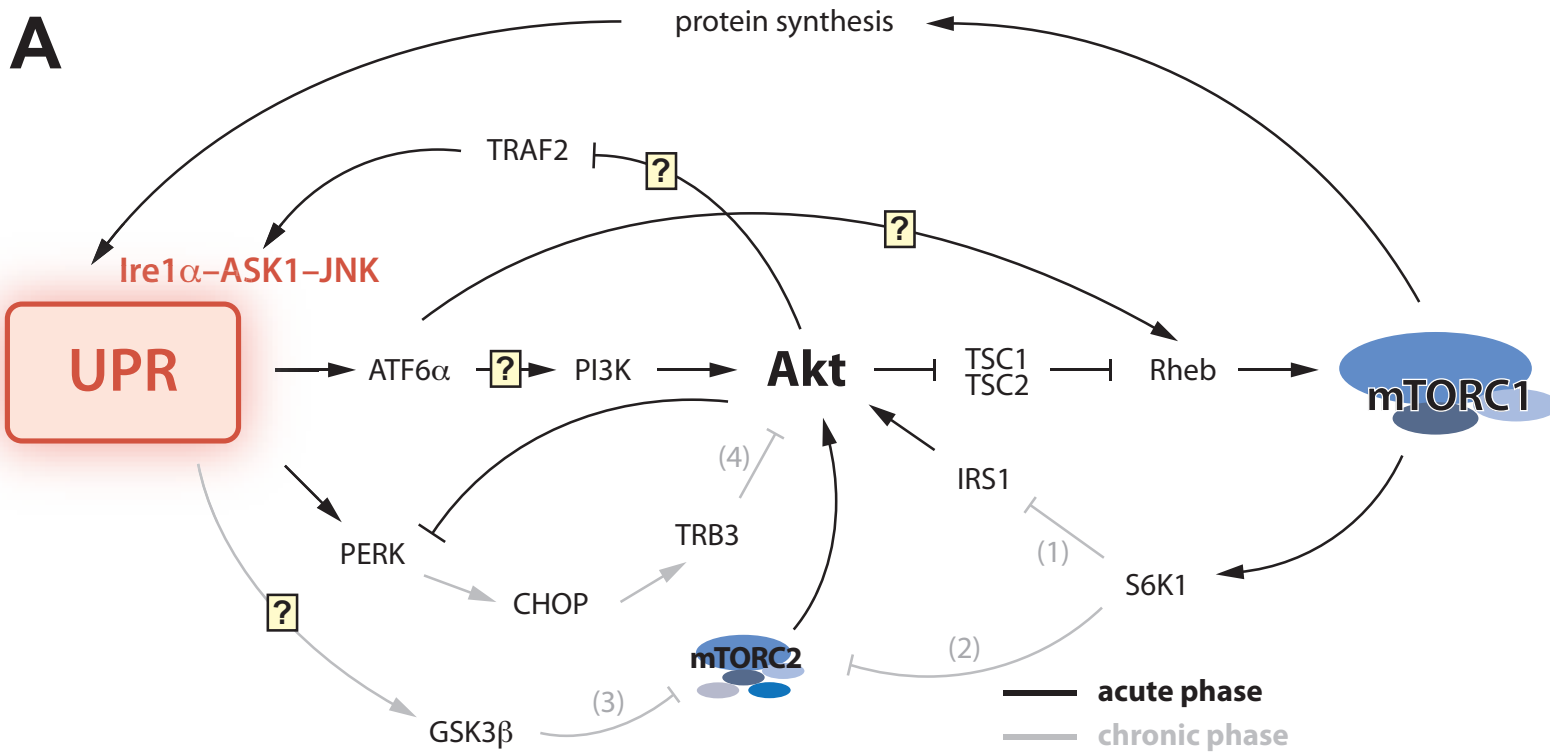


Figure I

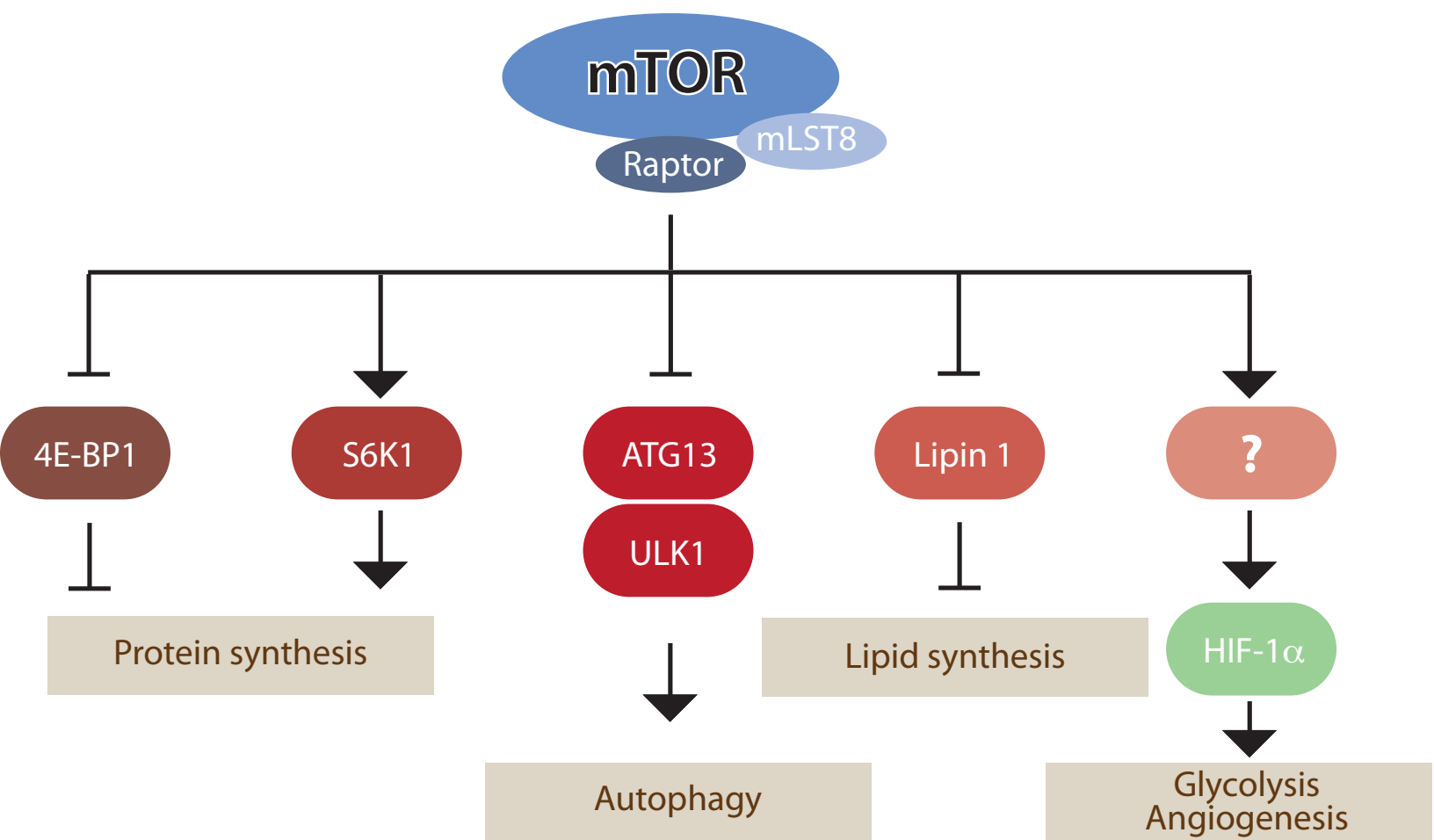


Figure II

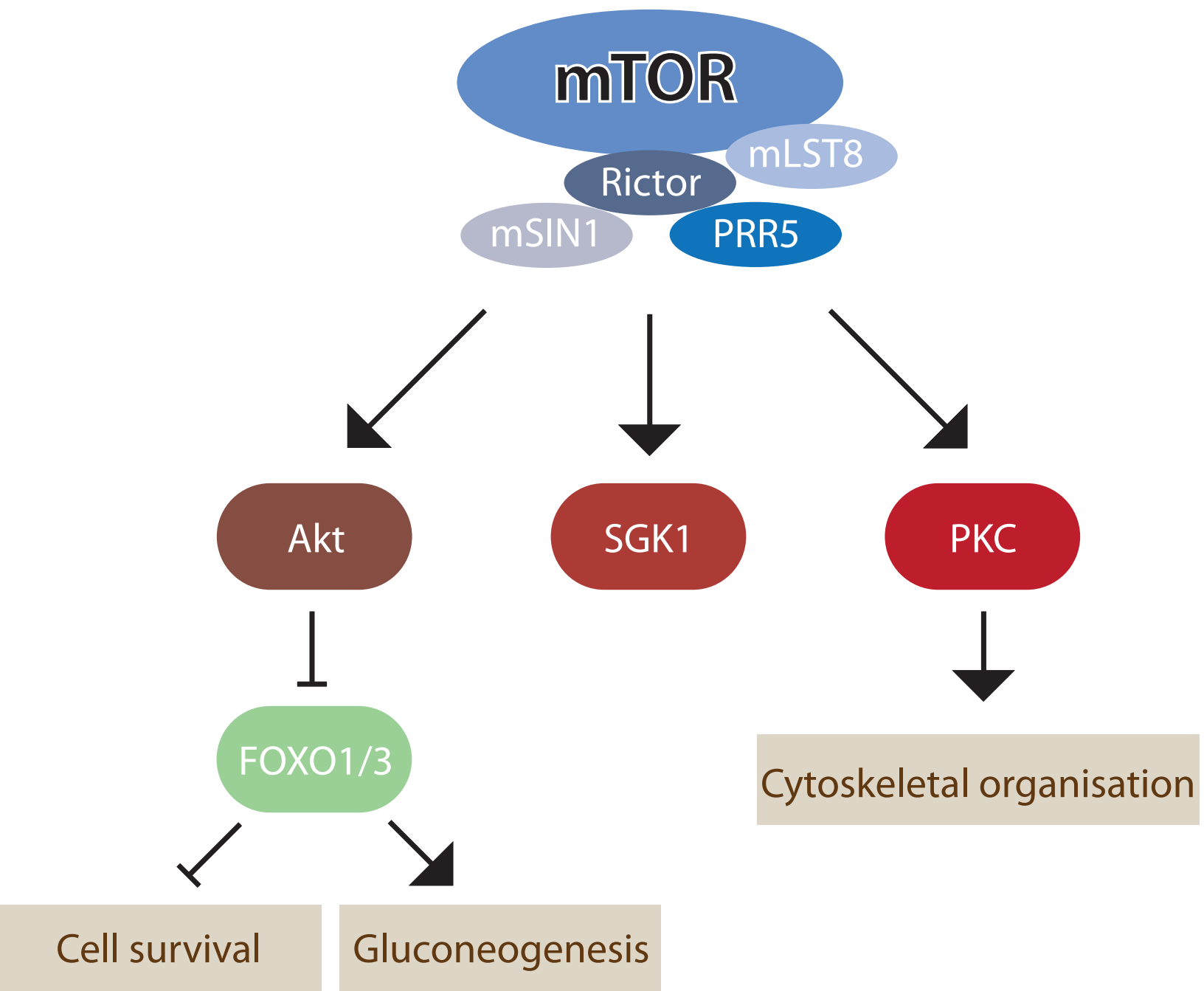
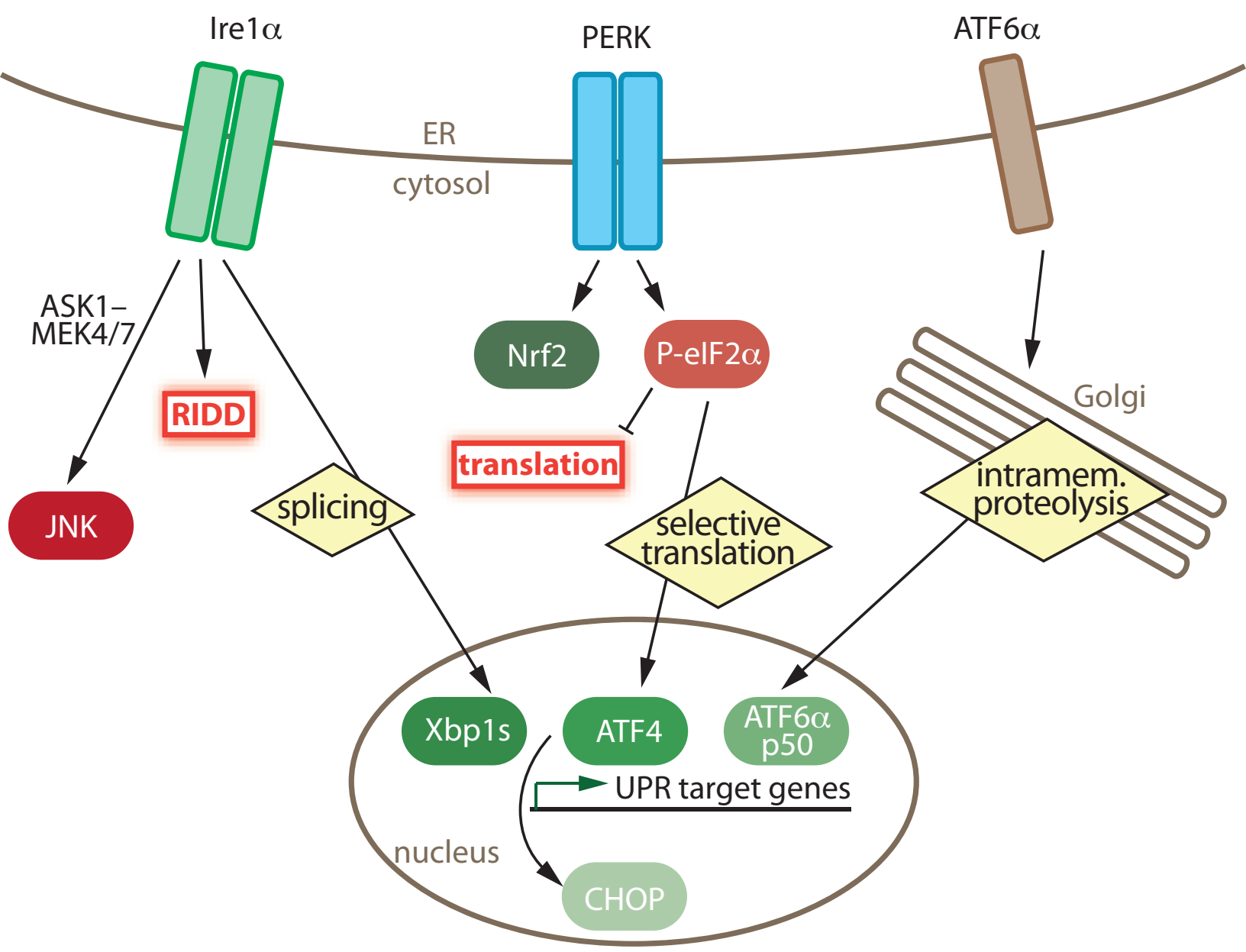


Figure III



Appenzeller-Herzog Figure III