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The mTOR Signaling Pathway and Regulation of Pancreatic Function

M. Dolors Sans and John A. Williams

University of Michigan Department of Molecular & Integrative Physiology

e-mail: mdsansg@umich.edu, jawillms@umich.edu

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1. General Functioning of the mTOR Pathway

The mTOR signaling pathway is a nutrient sensing mechanism coupled to mTOR, the mammalian or mechanistic target of rapamycin (15, 26, 39). mTOR is the target of the anti-fungal metabolite rapamycin. It is named after the island Rapa Nui (Easter Island) from whose soil it was first isolated and has broad antiproliferative and immunosuppresive properties (38). Genetic screens in the early 1990's in yeast identified two genes TOR1 and TOR2 that mediated the effects of rapamycin. Biochemical studies then led to the identification of the mammalian form (8, 32). mTOR is an atypical protein kinase related to phosphoinositide 3- kinase family although it is a Ser/Thr targeted kinase and not a lipid kinase. It is a large protein of about 2,500 amino acids with multiple domains including a C terminal kinase domain and a FKBP-rapamycin binding (FRB) domain.

mTOR is a component of two complexes, TORC1 and TORC2; each contains other proteins, some of which are shared. However, TORC1 uniquely contains the scaffolding protein Raptor (regulatory associated protein of mammalian target of rapamycin) (18) and PRAS40 (proline rich Akt substrate of 40 kDa) (45), whereas TORC2 contains, among other components, Rictor (rapamycin-insensitive companion of mTOR) (26). Both Raptor and PRAS40 are inhibitory proteins; phosphorylation blocks this inhibition. PRAS40 represents an essential component for insulin activation of TORC1. Raptor is an essential component and its genetic deletion leads to loss of TORC1 activity (3).

Much less is known as to the functions of TORC2. It is a regulator of the actin cytoskeleton in both yeast and mammalian cells (49). More recent studies have shown it to be active in phosphorylating various protein kinases especially Akt.

Only TORC1 is acutely sensitive to rapamycin which inhibits some, but not all, of TORC1 functions (30). This inhibition requires FKBP-12 (FK506 binding protein of 12 kDa). Much is known about the function of TORC1 which mediates the growth promoting effects including protein synthesis, lipid synthesis, inhibition of autophagy, ribosome and lysosome biogenesis, and energy metabolism. The effects on protein synthesis are mediated in part by activation of S6K1 (S6 Kinase-1) which phosphorylates ribosomal protein S6 and also activates initiation and elongation translation factors, the latter through elongation factor 2 kinase (30). In addition, TORC1 phosphorylates 4E-BP1, a binding protein which, upon phosphorylation, releases the initiation factor eIF4E which acts as a mRNA cap binding protein (46). The overall rate of protein synthesis also depends on the number of ribosomes, and TORC1 also enhances the synthesis of ribosomal proteins and RNA (30). TORC1 promotes lipid synthesis by activating SREBP (sterol responsive element binding protein) (38).

The action of TORC1 to inhibit autophagy is mediated by phosphorylation of ULK1 (unc-51 like autophagy activating Kinase-1) and Atg13 (autophagy related 13) which blocks the formation of the phagosome (40). Recently, TORC1 has also been shown to regulate the abundance of proteasomes; when TORC1 is inhibited, the ability of proteasomes to degrade proteins increases (31).

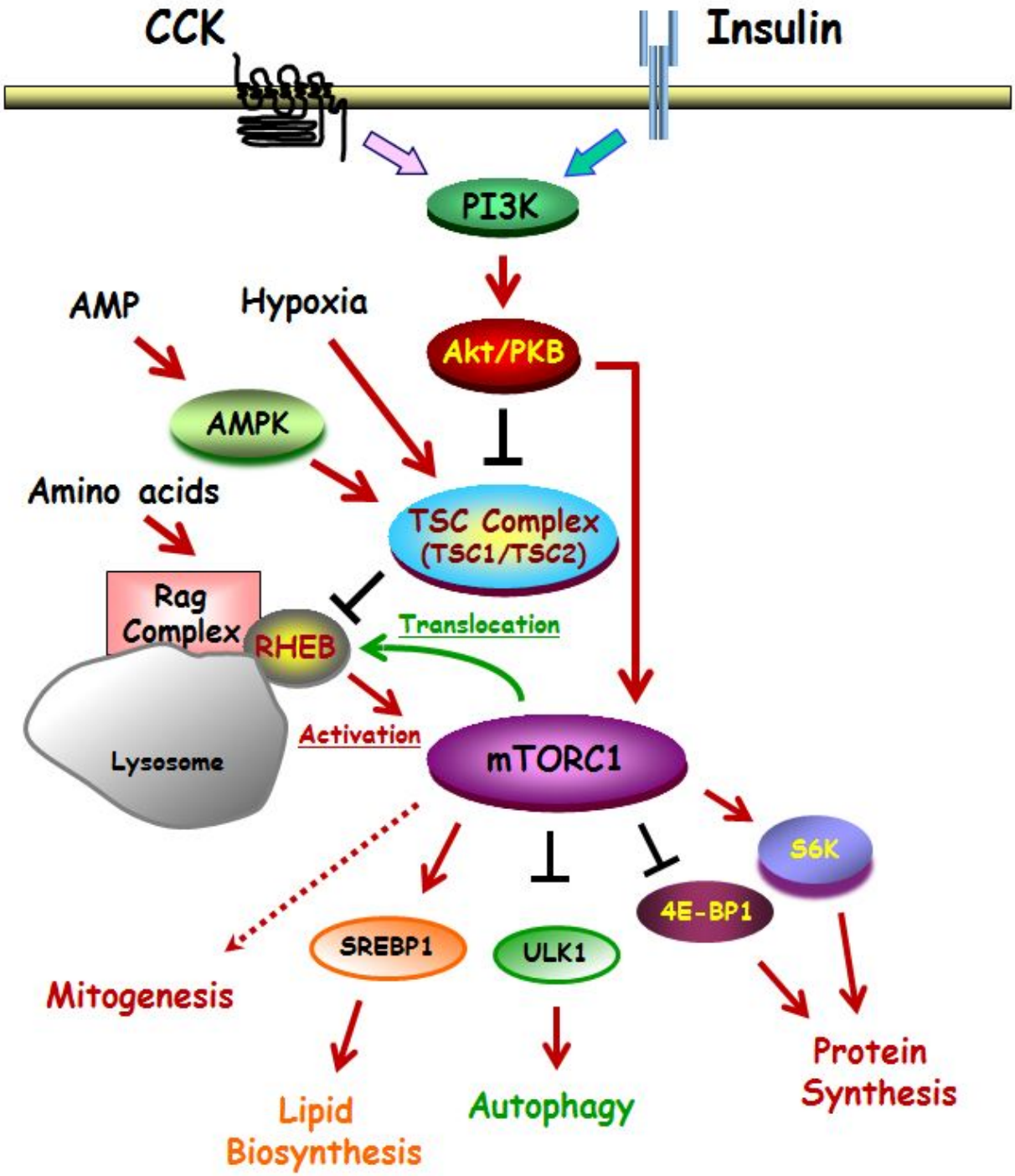

Figure 1. General and simplified diagram of mTORC1 pathway set in pancreatic acinar cell, where the pathway is regulated by CCK and insulin. Red arrows indicate activation; Black arrows indicate inhibition; Green arrow indicates translocation. Biological processes regulated by mTORC1 are shown at the bottom of the figure, along with the key proteins mediating the effect.

A variety of upstream signals regulate TORC1 including growth factors (and some hormones that stimulate growth of their target cells), amino acids, oxygen levels, and glucose **(Figure 1)**. Growth factors and insulin reflect the fed state of the organism and promote anabolic processes. Binding of insulin to its receptor activates PI3K and Akt that are upstream of TORC1, Akt phosphorylates and inhibits TSC1/2 the tuberosclerosus complex which acts as a tumor suppressor by acting as a GAP for Rheb (Ras homolog enriched in brain), a GTPase which is one of the major activators of TORC1. Akt also phosphorylates PRAS40 and thereby relieves its inhibition of mTOR in TORC1. TORC1 limits its own activation by the negative feedback of S6K1 on the early steps in PI3K activation especially by insulin.

Another major signal regulating TORC1 activity is the abundance of amino acids which tells the organism to undergo anabolic activity (1, 22). Conversely the absence of adequate amino acids is a stress which leads to the shutting down of biosynthetic pathways and the induction of autophagy. The major amino acids sensed are the branched chain amino acids, especially leucine, as well as arginine and glutamine. Amino acid sensing involves recruitment of TORC1 from cytoplasm to lysosomes where it interacts with proteins including the Rag GTPases (23), a protein complex termed the ragulator (33), amino acid transporters (17) and the lysosomal vacuolar ATPase (12). In the presence of amino acids, Rheb which is also localized on the lysosome, is activated and in turn activates TORC1. Furthermore, amino acids are necessary for almost all other mechanisms activating TORC1. Low oxygen or low glucose levels prevent TORC1 signaling through AMP Kinase and reduce the activity of the proteins REDD and BMIP3 (40, 48). DNA damage also inhibits TORC1. As a result of this network of interactions, growth and other anabolic activities can only take place in the presence of a supporting milieu.

2. mTOR Signaling in Pancreatic Cells

mTOR signaling in pancreas was first recognized and is most commonly followed through phosphorylation of the pathway's downstream mediators, ribosomal protein S6 and 4E-BP1. Phosphospecific antibodies are widely available for S6 and its upstream kinase, S6K1 which is activated by TORC1. 4E-BP1 resolves into multiple bands on Western blots with the higher band being most highly phosphorylated. Such measurements showed that in isolated rodent pancreatic acini CCK and similarly acting secretagogues (bombesin, carbachol) activated S6K1 (5), increased phosphorylation of S6, 4E-BP1 (5, 6, 42), and EF2K (elongation factor 1 Kinase (37) and that these effects were blocked by rapamycin, the TORC1 inhibitor. Moreover, rapamycin blocked the increase in protein synthesis stimulated by CCK in isolated acini. These studies showed that the primary cell type involved in the exocrine pancreas is the acinar cell and this has been reinforced in vivo where CCK injection increased phosphorylation of S6 and 4E-BP1 as well as the phosphorylation of eIF4E and the formation of the eIF4F initiation complex (7). Elevating endogenous CCK by feeding the trypsin inhibitor camostat (10) or diverting bile pancreatic juice (19) also led to similar effects. As discussed earlier, amino acids activate the TORC1 pathway and leucine and other branched chain amino acids activate protein synthesis and the TORC1 pathway both in pancreas in vivo and in isolated pancreatic acini (20, 36, 44). S6 and 4E-BP1 signaling in the pancreas are also affected by insulin and diabetes (29, 34, 43). TORC1 signaling is not required for secretion of digestive enzymes (5) but is required for protein synthesis and adaptive growth (6, 10, 11).

In addition to studies of acinar cells, the TORC 1 pathway appears to play a role in activated pancreatic stellate cells where it mediates effects of insulin to enhance collagen synthesis and

fibrosis (47). These in vitro effects of insulin were blocked by TOR inhibitors rapamycin and KU63794. TORC1 also plays a role in the endocrine pancreas where it is involved in islet development, beta cell growth and insulin processing and secretion (2, 4, 14, 27, 41).

The importance of the TORC1 pathway in the exocrine pancreatic response to feeding is shown by the activation of the downstream components when mice fasted overnight are refed (35). In this study, protein synthesis was also increased with feeding without a change in mRNA levels for digestive enzymes, indicating the importance of translational control primarily by the TORC1 pathway in synthesis of new digestive enzymes after secretion. Similar effects have also been seen in neonatal pigs (16). TORC1 was also shown to play a role in the hypertrophic response to feeding a high protein diet and this was independent of CCK (11). Conversely, pancreatic atrophy was seen in response to a loss of TORC1 signaling when mice were fed a protein free diet (9). These in vivo responses involve multiple hormones including CCK and insulin and nutrients acting directly, particularly amino acids.

3. mTOR Signaling and Pancreatic Disease

mTOR signaling has been implicated in a number of disease states with altered growth and metabolism including cancer and diabetes as well as aging where the life extending effect of low calorie diets is believed mediated by reduced TORC1 signaling (49). TORC1 activity is increased in many pancreatic ductal adenocarcinomas (PDAC) in part due to mutations in upstream regulatory molecules including PTEN, AKT and TSC1/2. Most PDAC cancers have RAS mutations leading to activation of the MEK/ERK pathway which can inactivate TSC1/2 and thereby activate TORC1. Rapamycin analogs have been considered as potential therapeutic agents for pancreatic and other cancers. However, these inhibitors have not shown significant effects in single agent clinical trials, though individual patients have shown responses (21). Currently attention has focused on dual agent therapy as well as identifying patients with specific patterns of gene activation that may be more responsive. In this context, genetically modified mice with Ras mutation and PTEN deficiency show sensitivity to TORC1 inhibition in contrast to those with Ras and p53 mutations which are not sensitive (28). Another study using a mouse model of decreased TSC1 by haploid sufficiency showed enhanced mTOR signaling and tumorigenesis could be blocked with dual inhibition of mTOR and MEK (25).

When TSC1 was completely ablated in the embryonic pancreas, mice developed pancreatic acinar adenocarcinoma superimposed on atrophy of the normal pancreas (13, 24). This is a rare form of pancreatic carcinoma in humans with the tumor cells having acinar characteristics and expressing amylase. It is generally slower growing and less malignant than pancreatic ductal adenocarcinoma.

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