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Previews

Raptor Swoops in on Metabolism

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The mammalian target of rapamycin (mTOR) serine/threonine kinase plays a central role in insulin signaling and cell growth, through two distinct complexes with its subunits raptor or rictor. In this issue, two reports utilizing tissue-specific knockout mice detail key roles for mTORC1 in skeletal muscle and adipose tissue function.

The mammalian target of rapamycin (mTOR) is a central regulator of cell growth in all eukaryotes. mTOR integrates environmental cues including growth factors and nutrients through a number of intersecting pathways that have been extensively decoded in the past decade (Wullschleger et al., 2006). Across eukaryotes, TOR assembles into two biochemically and functionally distinct multiprotein complexes. In mammals, the mTOR complex 1 (mTORC1) is composed of four known subunits: raptor (*r*egulatory *a*ssociated *p*rotein of m*TOR*), PRAS40, mLST8, and mTOR. The mTORC2 complex contains rictor (*r*apamycin *i*nsensitive *c*ompanion of m*TOR*), mSIN1, PRR5/Protor, mLST8, and mTOR (Guertin and Sabatini, 2007). Mouse embryos lacking mTOR or its key mTORC1 subunit raptor die early in development around the time of implantation (e5.5) (Guertin et al., 2006). Mice lacking mTORC2 subunit rictor or the common subunit mLST8 die at midgestation (e10.5) with vascular defects (Guertin et al., 2006; Shiota et al., 2006). Using tissue-specific deletion of the unique complex subunits raptor and rictor, two papers in this issue of *Cell Metabolism* examine the requirement for mTORC1 and/or mTORC2 in skeletal muscle (Bentzinger et al., 2008) and the requirement for mTORC1 activity in adipocytes (Polak et al., 2008).

Signaling from mTORC1 is nutrient sensitive, acutely inhibited by the bacterial macrolide rapamycin, and controls cell growth, angiogenesis, and metabolism. The best-known substrates of mTORC1 are the translational regulators 4EBP1 and ribosomal S6 kinase (S6K1). In contrast, mTORC2 is not sensitive to nutrients nor acutely inhibited by rapamycin, and its known substrates include the hydrophobic motif phosphorylation ("PDK2") sites in AGC kinases including Akt and PKC family members (Guertin and Sabatini, 2007). One of the key activators of the mTORC1 pathway is the PI3-kinase pathway, which plays a key role in promoting cell growth and insulin-mediated effects on metabolism. Conversely, the AMPK pathway suppresses mTORC1 and inhibits cell growth when nutrient conditions are limited and also plays key roles in glucose and lipid metabolism. Consistent with its dependence on growth factor and nutrient cues, mTORC1 hyperactivation is observed in a large number of cancers, as well as in key peripheral metabolic tissues in animals on a high-fat diet.

The study here from a collaborative effort between the Hall lab and the Ruegg lab examines a skeletal muscle-specific knockout of raptor, or rictor, or both. The muscle-specific loss of rictor has minimal impact on muscle physiology. In contrast, the mice lacking raptor in skeletal muscle exhibit progressive muscle degeneration, culminating in premature death between 4 and 7 months of age. The greatest defects were observed in the slow-twitch oxidative muscles such as the soleus and the diaphragm. Raptor-deficient soleus muscle from older mice exhibited a severe loss in the number of mitochondria, which was paralleled by an increase in glycogen content. Loss of raptor caused dramatically increased expression of slow myosin heavy chain and troponin in both slow- and fast-twitch muscle, which may be due to the observed increase in the myogenic transcription factor MEF2A. Indeed, functional assays revealed that the raptor-deficient muscle exhibited reduced aerobic capacity like fast-twitch fibers but contractile properties similar to slow-twitch oxidative muscle fibers. Consistent with these signs of dystrophy, the raptor muscle knockout (KO) mice performed only 60% as much voluntary wheel running as control mice.

Analysis of signaling downstream of mTORC1 and mTORC2 in soleus muscle reveals similarities with other cell types examined upon suppression of raptor or rictor, with some key distinctions. Deletion of raptor resulted in near complete loss of phosphorylation of mTORC1 substrates S6K and 4EBP1 with a dramatic increase in IRS-1 protein and Akt phosphorylation. Indeed, functional increases in Akt signaling were observed, consistent with the well-documented suppression of PI3K-Akt signaling downstream of mTOR C1 via downregulation of IRS proteins (Harrington et al., 2005).

In rictor-deficient muscle, phosphorylation of the hydrophobic motif sites in Akt and PKCα were greatly reduced, consistent with these sites being dependent on mTORC2 activity (Guertin and Sabatini, 2007). As observed previously, Akt signaling to mTORC1 is largely unaffected in rictor-deficient muscle (Kumar et al., 2008). However, muscle deficient for both raptor and rictor exhibited elevated Akt473 phosphorylation, similar to the raptor knockout alone (Bentzinger et al., 2008). These unexpected findings suggest that a kinase other than mTORC2 can phosphorylate Akt473 in skeletal muscle and that this kinase is upregulated when raptor is deficient. It will be interesting to identify the kinase responsible for this alternate muscle-"PDK2" activity and also to define under what physiological and pathological states mTORC2 and the mystery kinase contribute to the regulation of Akt signaling.

In the accompanying study, the Hall and Ruegg labs examine the requirement for mTORC1 in adipogenesis (Polak et al., 2008). The authors first repeat previous findings indicating that mTORC1 is required for adipogenesis in cell culture models and then further show that loss

of mTORC1 in mature adipocytes results in loss of triglycerides. To examine this in vivo, raptor was deleted in mature adipocytes, resulting in lean mice with a reduction in both adipocyte size and number. As seen in other lean mouse models, raptor adipose KO mice are resistant to diet-induced obesity and exhibit insulin hypersensitivity with improved glucose tolerance. The insulin sensitivity is due to increased insulin signaling in both adipose tissue and skeletal muscle. As observed in the mice lacking raptor in skeletal muscle, elevated insulin signaling through Akt in adipose tissue appears to be due to loss of the mTORC1 negative feedback loop on IRS-1/PI3K/Akt, paralleling previous findings from muscle and adipose tissue in whole-body S6K1 knockout mice on a high-fat diet (Um et al., 2004).

One molecular basis underlying the leanness of the mice appears to be elevated energy expenditure, which may be a consequence of increased mitochondrial uncoupling from a 25-fold elevated level of UCP1 mRNA expression in raptor-deleted white adipose tissue (WAT). In contrast to adipose tissue, decreases in PGC1α mRNA were observed in the raptor-deficient muscle, consistent with a previous study in C2C12 and mouse embryonic fibroblasts demonstrating that inhibition of mTORC1 reduces PGC1α mRNA through regulation of the YY-1 transcription factor (Cunningham et al., 2007). Other possible downstream effectors for mTORC1 in the control of muscle and adipose metabolism not explored in these studies are the key lipogenic transcription factor Srebp-1 and the lipodystrophy protein lipin, both of which have been previously shown to be regulated by mTORC1 (Porstmann et al., 2008; Huffman et al., 2002). Another well-described function for mTORC1 is in suppressing autophagy, and future studies will be needed to define any role for autophagy in the observed phenotypes reported here, as well as to further illuminate the precise downstream effectors of mTORC1 mediating control of metabolism, tissue homeostasis, and differentiation in different tissues.

Taken together, these studies define critical roles for mTORC1 in metabolic control in adipose tissue as well as muscle homeostasis. Given that rapamycin analogs are being examined for the treatment of a host of diseases ranging from cancer to neurological disease to facets of metabolic syndrome itself, further dissection of the roles of mTORC1 and mTORC2 in different tissues will provide future insights into the therapeutic promise this pathway holds.

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Regulating the Motor for GLUT4 Vesicle Traffic

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Insulin-triggered trafficking of GLUT4 glucose transporter-loaded vesicles and their fusion with the plasma membrane are mechanical processes involving multiprotein complexes that coordinate and facilitate vesicle movement. Now, Yip et al. (2008) link myosin-1c to insulin signaling by demonstrating direct CaMKII-driven phosphorylation of this critical motor protein.

Insulin signaling in its target tissues of fat and muscle is known to lead to the exocytosis of vesicles loaded with the glucose transporter GLUT4 from an intracellular reservoir compartment to the plasma membrane. Now, the challenge for researchers is to link early signaling to the vesicle-trafficking machinery. Early events in insulin signaling include the