Ribosomal Protein S6

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Gene Symbol: **RPS6**

1. General Information

Ribosomal protein S6 (rpS6) is one of 33 proteins which along with 18S ribosomal RNA make up the small (40S) subunit of the eukaryotic ribosome. It is located in the small head region of the 40S subunit and resides at the interface of the 40S and 60S (large) subunits where a groove is thought to be the site of new protein synthesis (35). Cross linking studies suggest rpS6 may interact with mRNA. rpS6 is an evolutionary conserved protein of 236 to 253 amino acids and is the first discovered and main phosphorylated ribosomal protein as shown originally by Gressner and Wool (13). The phosphorylation sites are located in the carboxyl terminus of the protein and have been mapped in mammals to Ser-235, -236, -240, -244, and -247 (21). It is believed that phosphorylation is ordered with Ser-236 the primary site (9) followed by Ser-235 and then the others. Yeast rpS6 has only two phosphorylation sites, Ser-232 and -233, that correspond to Ser-235 and -236 in mammals.

Numerous reports have shown that rpS6 is phosphorylated in multiple physiologic and pathophysiologic states with the best studied systems being hepatocytes in regenerating liver and serum stimulated mouse embryo fibroblasts (MEFs) (25). In a variety of specialized cell types including muscle, neurons and secretory cells, S6 phosphorylation is temporally correlated to the initiation of protein synthesis.

Considerable success has been achieved in understanding the role of different kinases in phosphorylating rpS6 (22). The first kinase identified was in Xenopus oocytes and is now known as p90 rpS6 Kinase (RSK) (8). This kinase has an apparent molecular mass of 90 kDa, is present in mammalian cells and is now known to be an effector of ERK. Avian and mammalian cells were then shown to also contain a distinct 70 kDa S6 kinase referred to as p70 S6K but now known simply as S6K which can phosphorylate all five sites on rpS6 (1, 18). This kinase has two highly related forms coded by two separate genes p70 S6K1 and p85 S6K2 both of which are required for full phosphorylation of rpS6. Deletion of these genes greatly reduced S6 kinase activity but revealed that phosphorylation of rpS6 could be carried out in part by other kinases including Protein kinase A (PKA) (7), protein kinase C (PKC) (23), RSK (8), and casein Kinase 1 (15). PKA and RSK only phosphorylated Ser-235 and -236 while
casein kinase 1 phosphorylated Ser-247; PKC phosphorylated at least 3 residues but they were not identified. Dephosphorylation of rpS6 is carried out primarily by a protein phosphatase 1 (15).

The major pathway signaling to rpS6 phosphorylation is the TORC1 (Target of rapamycin complex 1) pathway with TORC1 together with PDK1 (3'-phosphoinositide-dependent kinase 1) phosphorylating and activating S6K which then phosphorylates rpS6 (25). As TORC1 is activated by a variety of growth factors, hormones and nutrients (34), this explains the general correlation of rpS6 phosphorylation with protein synthesis and growth. However, rpS6 is not the only target of S6K and does not mediate all of its effects (22).

Much more poorly understood is the physiological consequence of rpS6 phosphorylation. Originally it was suggested to mediate initiation of translation but this has been disproved by multiple approaches. Most notably, replacing all five phosphorylatable serines in S6 with alanine failed to inhibit protein synthesis in MEFs derived from the knock in mice (27). The possibility of phospho rpS6 facilitating the translation of a subset of mRNA species has focused on those with a 5'-terminal polypyrimidine tract referred to as TOP mRNAs. These include all ribosomal proteins and many translation factors (26). Correlative data originally led to the hypothesis that phosphorylation of rpS6 facilitated the translation of these mRNAs (17). However, considerable evidence both biochemical and genetic is opposed to this hypothesis and is summarized by Ruvinsky & Meyuhas (26). S6K and phosphorylation of rpS6 has also been shown to be involved in controlling cell size (27). While some cell types including pancreatic beta cells and MEFs have smaller cells when rpS6 phosphorylation is prevented, in other cases cell size is dependent on the TORC1 pathway and on S6K activity involving other substrates. Other work has suggested S6K and possibly rpS6 phosphorylation is involved in cell proliferation and glucose homeostasis but may involve other S6K targets (24). Finally, there is some evidence that rpS6 might have effects outside the ribosome and protein synthesis. At present, the main experimental use of monitoring rpS6 phosphorylation is to show activation of the PI3K- AKT- mTOR-S6K signaling pathway which can be done by Western blotting or immunolocalization.

2. Ribosomal protein S6 in the Pancreas

Following the proposal by Paul Greengard that protein phosphorylation is a general mechanism by which hormones and neurotransmitters regulate cell function (12), pancreatologists began to evaluate changes in protein phosphorylation in the exocrine pancreas. Using isolated mouse acini incubated with $^{32}$P-orthophosphate to label intracellular ATP, Burnham and Williams (3) showed that carbachol, CCK and insulin altered the phosphorylation of 5 proteins including increased phosphorylation of a 32.5 kDa protein located in the particulate fraction as expected for rpS6. Similar results were obtained for rat and guinea pig pancreatic acini and lobules where VIP, secretin and dibutyrl cyclic AMP were shown to act similarly to CCK on a protein of 29-33 kDa (10, 16, 39). Latter studies using cell fractionation and 2-dimensional gel electrophoresis confirmed that the basic ribosomal protein behaved like rpS6 (11) and that cytosol contained a S6 kinase activity that was activated by insulin and CCK (36, 37). Following the development of specific peptide substrates and phosphospecific antibodies, the kinase was confirmed to be p70S6K (2). RpS6 was shown to be phosphorylated on Ser235, 236, 240/244 in isolated pancreatic acini stimulated with CCK, bombesin and carbachol and in intact animals after feeding (5, 30, 32). Studies using mice with genetic deletions of hormones or their receptors showed that both CCK and insulin participated in the response to feeding and that amino acids especially leucine can also induce rpS6 phosphorylation (6, 31).

There is good correlation between the stimulation of protein synthesis and rpS6 phosphorylation in
pancreatic acinar cells (30). All hormones and neurotransmitters that stimulate protein synthesis enhance rpS6 phosphorylation. In the converse, removing protein from the diet acutely partially reduces rpS6 phosphorylation and this affect increases in prolonged protein deprivation (4, 28). These studies were largely directed at showing the presence of the Akt – mTOR – S6K pathway in acini and its importance for regulating protein synthesis and cell growth (33); phosphorylation of rpS6 was used as a readout of pathway activation. Pancreatic acinar cell protein synthesis, stimulated by CCK, bombesin and carbachol, is blocked by inhibiting protein phosphatase 2B (calcineurin), but the inhibitors did not affect rpS6 phosphorylation (32). On in vivo experiments, endogenous release of CCK, by feeding mice trypsin inhibitor, stimulates pancreas growth and the phosphorylation of rpS6; blocking calcineurin with FK506, partially reduces this rpS6 phosphorylation on Ser 240/244 (38). Mice in which the 5 phosphorylatable Ser residues were mutated to Ala had smaller islet beta cells and reduced insulin secretion but acinar cells were of normal size (26). No acinar cell function studies have been performed to date on these mice without phosphorylatable rpS6 Ser residues.

Increased phospho S6 has also been noted to change in several pathological states. In acute cerulein-induced pancreatitis in mice, protein synthesis is inhibited but phospho S6 is increased after 1 hour (29). In a longer term study pS6 initially decreased but after several days it became increased during pancreatic regeneration (40). Here, pS6 appears to reflect the activity of the mTORC1 pathway. Increased phospho rpS6 has also been shown to be present in a precursor to pancreatic cancer, intraductal papillary mucinous neoplasms (IPMN) (14) where it is associated with glucose uptake and malignancy. The TORC1 pathway has been implicated in the development of PDAC in various mouse models. A role for rpS6 is indicated by studies of the non-phosphorylatable mutant where p53 suppression of tumorigenesis was attenuated (19).

3. Tools for the study of Ribosomal protein S6

a. Antibodies. A number of antibodies have been raised against phosphorylated peptide. In our hands the best antibody for Western Blotting is Cell Signaling #2215, a rabbit polyclonal prepared against p-Ser 24/244 which can be used at 1:5000 or higher dilution (5, 28, 30, 31). This gives a bigger response than the Cell Signaling Ab to pSer 235/236 which has a higher basal level of phosphorylation. We use an Ab from Santa Cruz for total S6. These antibodies were against multiple species. We use the same Cell Signaling antibody for IF but at 1:100 dilution. The Cell signaling D68F8 XP antibody has also been used for IF (20).

b. Genetic Models. Knockout of rpS6 in liver or thymus leads to organ hypoplasia or death. Replacement of the 5 phosphorylatable Ser residues with Ala has effects on cell size for some cell types (27).

4. References

1. Blenis J, Kuo CJ, and Erikson RL. Identification of a ribosomal protein S6 kinase regulated by transformation and growth-promoting stimuli. J Biol Chem 262: 14373-14376, 1987. PMID: 2822690.
2. Bragado MJ, Groblewski GE, and Williams JA. p70s6k is activated by CCK in rat pancreatic acini. Am J Physiol 273: C101-109, 1997. PMID: 9252447.
3. Burnham DB, and Williams JA. Effects of carbachol, cholecystokinin, and insulin on protein phosphorylation in isolated pancreatic acini. J Biol Chem 257: 10523-10528, 1982. PMID: 7050109.
4. Crozier SJ, D’Alecy LG, Ernst SA, Ginsburg LE, and Williams JA. Molecular mechanisms of pancreatic dysfunction induced by protein malnutrition. Gastroenterology 137: 1093-1101, 1101 e1091-1093, 2009. PMID: 19427311.
5. Crozier SJ, Sans MD, Guo L, D’Aleyc LG, and Williams JA. Activation of the mTOR signalling pathway is required for pancreatic growth in protease-inhibitor-fed mice. *J Physiol* 573: 775-786, 2006. PMID: 16613881.
6. Crozier SJ, Sans MD, Wang JY, Lentz SI, Ernst SA, and Williams JA. CCK-independent mTORC1 activation during dietary protein-induced exocrine pancreas growth. *Am J Physiol Gastrointest Liver Physiol* 299: G1154-1163, 2010. PMID: 20798356.
7. del Grande RW, and Traugh JA. Phosphorylation of 40-S ribosomal subunits by cAMP-dependent, cGMP-dependent and protease-activated protein kinases. *Eur J Biochem* 123: 421-428, 1982. PMID: 6281008.
8. Erikson E, and Maller JL. A protein kinase from Xenopus eggs specific for ribosomal protein S6. *Proc Natl Acad Sci U S A* 82: 742-746, 1985. PMID: 3856226.
9. Flotow H, and Thomas G. Substrate recognition determinants of the mitogen-activated 70K S6 kinase from rat liver. *J Biol Chem* 267: 3074-3078, 1992. PMID: 1737763.
10. Freedman SD, and Jamieson JD. Hormone-induced protein phosphorylation. I. Relationship between secretagogue action and endogenous protein phosphorylation in intact cells from the exocrine pancreas and parotid. *J Cell Biol* 95: 903-908, 1982. PMID: 6296160.
11. Freedman SD, and Jamieson JD. Hormone-induced protein phosphorylation. II. Localization to the ribosomal fraction from rat exocrine pancreas and parotid of a 29,000-dalton protein phosphorylated in situ in response to secretagogues. *J Cell Biol* 95: 909-917, 1982. PMID: 6296161.
12. Greengard P. Phosphorylated proteins as physiological effectors. *Science* 199: 146-152, 1978. PMID: 22932.
13. Gressner AM, and Wool IG. The phosphorylation of liver ribosomal proteins in vivo. Evidence that only a single small subunit protein (S6) is phosphorylated. *J Biol Chem* 249: 6917-6925, 1974. PMID: 4423396.
14. Hiroshita T, Hiroshita Y, Iwashita Y, Endo Y, Kiyonaga M, Matsumoto S, Hijiyama N, Moriyama M, Murakami K, and Inomata M. S6 ribosomal protein phosphorylation is associated with malignancy of intraductal papillary mucinous neoplasm of the pancreas. *Ann Gastroenterol Surg* 4: 571-579, 2020. PMID: 33005852.
15. Hutchinson JA, Shanware NP, Chang H, and Tibbetts RS. Regulation of ribosomal protein S6 phosphorylation by casein kinase 1 and protein phosphatase 1. *J Biol Chem* 286: 8688-8696, 2011. PMID: 21233202.
16. Jahn R, and Soling HD. Phosphorylation of the ribosomal protein S6 in response to secretagogues in the guinea pig exocrine pancreas, parotid and lacrimal gland. *FEBS Lett* 153: 71-76, 1983. PMID: 6298009.
17. Jefferies HB, Reinhard C, Kozma SC, and Thomas G. Rapamycin selectively represses translation of the "polypyrimidine tract" mRNA family. *Proc Natl Acad Sci U S A* 91: 4441-4445, 1994. PMID: 8183928.
18. Jeno P, Ballou LM, Novak-Hofer I, and Thomas G. Identification and characterization of a mitogen-activated S6 kinase. *Proc Natl Acad Sci U S A* 85: 406-410, 1988. PMID: 3257566.
19. Khalailah A, Dreaizen A, Khatib A, Apel R, Swisa A, Kidess-Bassir N, Maitra A, Meyuhas O, Dor Y, and Zamir G. Phosphorylation of ribosomal protein S6 attenuates DNA damage and tumor suppression during development of pancreatic cancer. *Cancer Res* 73: 1811-1820, 2013. PMID: 23361300.
20. Koehler JA, Baggio LL, Cao X, Abdulla T, Campbell JE, Secher T, Jelsing J, Larsen B, and Drucker DJ. Glucagon-like peptide-1 receptor agonists increase pancreatic mass by induction of protein synthesis. *Diabetes* 64: 1046-1056, 2015. PMID: 25277394.
21. Krieg J, Hofsteenge J, and Thomas G. Identification of the 40 S ribosomal protein S6 phosphorylation sites induced by cycloheximide. *J Biol Chem* 263: 11473-11477, 1988. PMID: 3403539.
22. Meyuhas O. Ribosomal Protein S6 Phosphorylation: Four Decades of Research. *Int Rev Cell Mol Biol* 320: 41-73, 2015. PMID: 26614871.
23. Parker PJ, Katan M, Waterfield MD, and Leader DP. The phosphorylation of eukaryotic ribosomal protein S6 by protein kinase C. *Eur J Biochem* 148: 579-586, 1985. PMID: 3158521.
24. Pende M, Kozma SC, Jaquet M, Oorschot V, Burcelin R, Le Marchand-Brustel Y, Klumperman J, Thoresen B, and Thomas G. Hypoinsulinaemia, glucose intolerance and diminished beta-cell size in S6K1-deficient mice. *Nature* 408: 994-997, 2000. PMID: 11140689.
25. Roux PP, and Topisirovic I. Signaling Pathways Involved in the Regulation of mRNA Translation. *Curr Opin Cell Biol* 38: 2018. PMID: 29610153.
26. Ruvinsky I, and Meyuhas O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. *Trends Biochem Sci* 31: 342-348, 2006. PMID: 16679021.
27. Ruvinsky I, Sharon N, Lerer T, Cohen H, Stolovich-Rain M, Nir T, Dor Y, Zisman P, and Meyuhas O. Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis. *Genes Dev* 19: 2199-2211, 2005. PMID: 16166381.
28. Sans MD, Crozier SJ, Vogel NL, D’Aleyc LG, and Williams JA. Dietary Protein and Amino Acid Deficiency Inhibit Pancreatic Digestive Enzyme mRNA Translation by Multiple Mechanisms. *Cell Mol Gastroenterol Hepatol* 11: 99-115, 2021. PMID: 32735995.
29. **Sans MD, DiMagno MJ, D’Aleyc LG, and Williams JA.** Caerulein-induced acute pancreatitis inhibits protein synthesis through effects on eIF2B and eIF4F. *Am J Physiol Gastrointest Liver Physiol* 285: G517-528, 2003. [PMID: 12773302]

30. **Sans MD, Lee SH, D’Aleyc LG, and Williams JA.** Feeding activates protein synthesis in mouse pancreas at the translational level without increase in mRNA. *Am J Physiol Gastrointest Liver Physiol* 287: G667-675, 2004. [PMID: 15117679]

31. **Sans MD, Tashiro M, Vogel NL, Kimball SR, D’Aleyc LG, and Williams JA.** Leucine activates pancreatic translational machinery in rats and mice through mTOR independently of CCK and insulin. *J Nutr* 136: 1792-1799, 2006. [PMID: 16772439]

32. **Sans MD, and Williams JA.** Calcineurin is required for translational control of protein synthesis in rat pancreatic acini. *Am J Physiol Cell Physiol* 287: C310-319, 2004. [PMID: 15044154]

33. **Sans MD, and Williams JA.** The mTOR signaling pathway and regulation of pancreatic function. *Pancreapedia: Exocrine Pancreas Knowledge Base* 2017. DOI: 10.3998/panc.2017.08

34. **Saxton RA, and Sabatini DM.** mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168: 960-976, 2017. [PMID: 28283069]

35. **Stewart MJ, and Thomas G.** Mitogenesis and protein synthesis: a role for ribosomal protein S6 phosphorylation? *Bioessays* 16: 809-815, 1994. [PMID: 7840758]

36. **Sung CK, and Williams JA.** Cholecystokinin stimulates a specific ribosomal S6 kinase in rat pancreatic acini. *Pancreas* 5: 668-676, 1990. [PMID: 2281080]

37. **Sung CK, and Williams JA.** Insulin and ribosomal protein S6 kinase in rat pancreatic acini. *Diabetes* 38: 544-549, 1989. [PMID: 2653925]

38. **Tashiro M, Dabrowski A, Guo L, Sans MD, and Williams JA.** Calcineurin-dependent and calcineurin-independent signal transduction pathways activated as part of pancreatic growth. *Pancreas* 32: 314-320, 2006. [PMID: 16628088]

39. **Vandermeers A, Vandermeers-Piret MC, Rathe J, Dehaye JP, Winand J, and Christophe J.** Phosphorylation of 3 particulate proteins in rat pancreatic acini in response to vasoactive intestinal peptide (VIP), secretin and cholecystokinin (CCK-8). *Peptides* 5: 359-365, 1984. [PMID: 6089135]

40. **Willet SG, Lewis MA, Miao ZF, Liu D, Radyk MD, Cunningham RL, Buralcliff J, Sibbel G, Lo HG, Blanc V, Davidson NO, Wang ZN, and Mills JC.** Regenerative proliferation of differentiated cells by mTORC1-dependent paligenosis. *EMBO J* 37: 2018. [PMID: 29467218]