RSK and MSK in MAP kinase signalling

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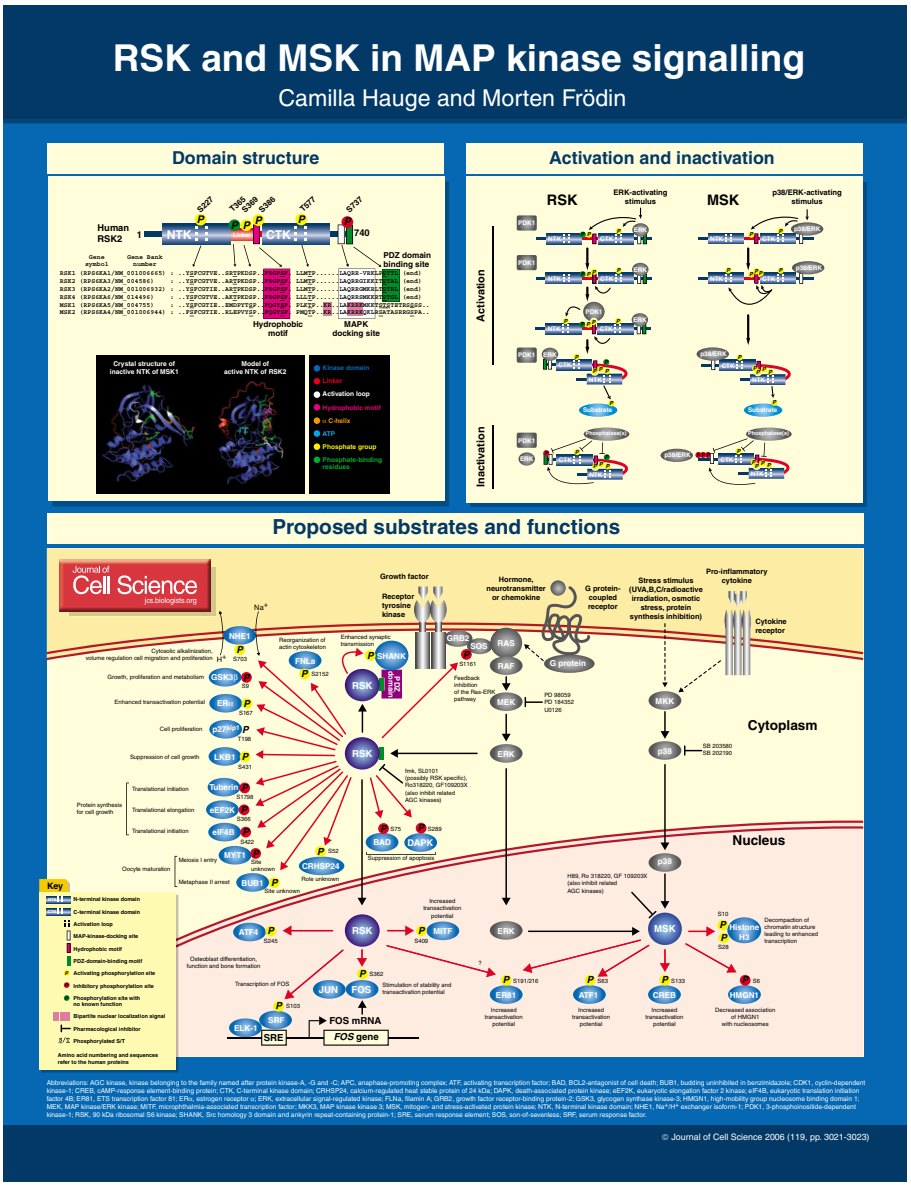
RSK [for 90 kDa ribosomal S6 kinase ($p90^{rsk}$); also known as MAPKAP-K1] and MSK (for mitogen- and stress-activated protein kinase) constitute a family of protein kinases that mediate signal transduction downstream of MAP kinase cascades. RSK is activated by MAP kinases of the extracellular signal-regulated kinase (ERK) family in response to growth factors, many polypeptide hormones, neurotransmitters, chemokines and other stimuli. MSK is also activated by ERK in response to such stimuli, but in addition, it can be activated by p38 family MAP kinases in response to various cellular stress stimuli and pro-inflammatory cytokines/factors. In mammals, four *RSK* genes (*RSK1-RSK4*) and two *MSK* genes (*MSK1* and *MSK2*) have been identified. *RSK* and *MSK* are ubiquitously expressed and many cell types express several members of each family. At present, little is known about specific and overlapping functions of the individual RSK/MSK family members. Orthologues of RSK and MSK have been described in *D. melanogaster* and *C. elegans*, but not in yeast or plants.

RSK and MSK each contain two kinase domains connected by a regulatory linker sequence. The N-terminal kinase domain (NTK) belongs to the AGC kinase family and is responsible for phosphorylation of substrates. The C-terminal kinase domain (CTK) belongs to the CamK family and its only known function is activation of NTK. The activation mechanism of RSK involves phosphorylation at four major sites (Dalby et al., 1998). First, ERK, bound to a C-terminal MAP-kinase-docking site, phosphorylates the linker at Ser369 and the activation loop of CTK at Thr577 (human RSK2 numbering). Phosphorylation of Thr577 activates CTK, which thereafter phosphorylates Ser386, which is located within a hydrophobic motif of aromatic residues in the linker. Phosphorylation of Ser386 generates a docking site that recruits 3-phosphoinositide-dependent protein kinase 1 (PDK1) and stimulates its activity five times, allowing PDK1 to phosphorylate NTK at Ser227 in the activation loop (Frodin et al., 2000). Thus, despite its name, PDK1 does not depend on phosphoinositide 3-kinase (PI3-K) activity for activation of RSK. After dissociation of PDK1, phosphoSer386 binds a phosphate-binding site in NTK, which enables the aromatic residues of the hydrophobic motif to bind and stabilize a nearby hydrophobic pocket, partly formed by the so-called αC-helix. The ordered αC-helix then cooperates with phosphoSer227 in the activation loop to stabilize NTK in an active conformation, resulting in synergistic stimulation of kinase activity (Frodin et al., 2002). The phosphorylation of Ser369 stimulates the catalytic activity of NTK two- to threefold, but the mechanism is unknown. Inactivation of RSK involves NTK-catalyzed phosphorylation Ser737. This event decreases the affinity of ERK for RSK, which helps prevent reactivation of RSK after dephosphorylation of the activating sites. RSK4 is somewhat unusual in that it has very low expression levels and appears to be fully phosphorylated and activated in unstimulated cells for reasons not entirely clear (Dümmler et al., 2005). The activation mechanism of MSK follows a similar scheme, except for some



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important differences (Deak et al., 1998; McCoy et al., 2005). First, the MAP-kinase-docking site of MSK can interact with both ERK and p38. Second, in addition to the serine in the hydrophobic motif, CTK also phosphorylates the activation loop of NTK as well a site in the linker, not conserved in RSK, which contributes to full activation. RSK is abundant in the cytoplasm and, upon activation, a fraction of the cytosolic RSK molecules appears to translocate to the nucleus by an unknown mechanism. MSK is restricted to the nucleus.

Identification of bona fide substrates and functions of RSK and MSK has been difficult owing to the lack of specific inhibitors. The poster shows a number of likely substrates, of which about 70% have been thoroughly validated by evidence from knockout/knockdown cells, semi-specific inhibitors and correlation between kinase activation and substrate phosphorylation, as exemplified by LKB1 (Sapkota et al., 2001). The poster also indicates cellular processes thought to be regulated by RSK or MSK via these substrates, but only in about 30% of the cases has the phosphorylation event been shown to be rate limiting. RSK and MSK phosphorylate serine residues (threonine phosphorylation has been reported only twice) in variations of the motifs R/KxRxxS and RRxS (or RKS), respectively, but these are not fully defined. The RSK-phosphorylation motif overlaps one of the phosphoSer motif recognized by 14-3-3 proteins, and in NHE1, BAD and $p27^{Kip1}$, 14-3-3 recruitment appears to be important for modulation of function resulting from phosphorylation by RSK. Experiments using overexpressed, constitutively active mutants of RSK and MSK have helped identify possible cellular functions regulated by these kinases, but must be supported by other evidence, because most AGC kinases promiscuously phosphorylate substrates of other AGC kinases when overexpressed. Recently, two potentially very specific inhibitors of RSK were reported: one (fmk) inhibits the CTK (and thereby also the NTK) of RSK1, RSK2 and RSK4 (Cohen et al., 2005); the other (SL0101), which is commercially available, inhibits the NTK of all four RSKs (Smith et al., 2005). These compounds, together with the use of RNA interference, should greatly help us to establish new and re-evaluate proposed cellular functions of RSK.

RSK is best described as a multi-functional ERK effector that participates in regulation of diverse cellular processes. ERK and RSK appear to cooperate in regulation of several proteins, including FOS, GSK3, MITF, ERα, ER81, SOS and tuberin. Perhaps this serves as a mechanism for detecting signal coincidence that enables substrates to be fully activated/inhibited selectively by the ERK-RSK pathway and not by any kinase that has a substrate specificity that overlaps that of either ERK or RSK. RSK is thought to stimulate cell cycle progression. Indeed, SL0101 inhibits proliferation of breast and prostate cancer cells and knocking down RSK in *Drosophila* cells impairs cell cycle progression. Proteins through which RSK may stimulate proliferation include the cyclin-dependent kinase inhibitor $p27^{Kip1}$, the kinases GSK3α and -β, the Na^+/H^+ exchanger NHE1 and the transcription factors ERα and MITF. Moreover, RSK2 contributes to transcriptional induction of *FOS*, probably via phosphorylation of SRF, and it also phosphorylates the FOS protein at Ser362, which increases its stability and its ability to promote proliferation and cell transformation.

RSK may also promote proliferation by stimulating growth-related protein synthesis in G1 phase, which may occur through phosphorylation of substrates such as GSK3, eEF2 kinase, tuberin and eIF4B. RSK may also act to inhibit proliferation. Thus, RSK phosphorylates the kinase LKB1 at a site required for its tumor suppressor activity and studies using RNA interference suggest that RSK4 is required for growth arrest induced by the p53 tumorsuppressor protein. During oocyte maturation in *Xenopus* (put apparently not in mammals), RSK appears to be the key effector of ERK-induced transition through the meiotic divisions and subsequent induction of metaphase II arrest, probably via phosphorylation of the kinases MYT1 and BUB1, as well as other substrates (reviewed in Maller et al., 2002; Schmitt and Nebreda, 2002).

RSK is thought to stimulate cell survival, for example, through phosphorylation and inactivation of the pro-apoptotic proteins BAD and DAPK or through phosphorylation of GSK3. Studies of knockout mice have shown that RSK2 stimulates osteoblast differentiation and bone formation, at least partly via the transcription factor ATF4. These findings agree with the observed decreased bone growth and skeletal dysmorphisms in humans afflicted with Coffin-Lowry syndrome, which is caused by mutation of the *RSK2* gene (reviewed by Hanauer and Young, 2002). RSK2 appears to be required for correct neuronal development and/or function, since Coffin-Lowry individuals frequently exhibit severe psychomotor retardation and RSK2-knockout mice display impaired learning and coordination. RSK may regulate synaptic transmission and plasticity via phosphorylation of the synaptic proteins SHANK and GRIP1 (or their binding partners), which contain a PDZ domain that recruits RSK via a class 1 PDZ-domain-binding motif located in its C-terminal tail. These findings raise the question of whether RSK targets other proteins that have a PDZ domain. RSK2-knockout mice, but not Coffin-Lowry individuals, exhibit an age-dependent 50-80% loss of white adipose tissue mass despite normal food intake. *RSK1*$^{-/-}$ *RSK2*$^{-/-}$ *RSK3*$^{-/-}$ triple-knockout mice are viable, but other details of their phenotype have not been reported. Although RSK2 is widely cited as the growth-factor-stimulated kinase that phosphorylates the transcription factor CREB and histone H3, conclusive evidence has shown that MSK fulfils these functions.

MSK has only one well-established function: regulation of gene expression by phosphorylation of transcription factors and chromatin-associated proteins. Evidence from studies using *MSK1*$^{-/-}$ *MSK2*$^{-/-}$ double-knockout mice and pharmacological inhibitors has established that MSK mediates ERK- or p38-induced phosphorylation of CREB at Ser133 (Wiggin et al., 2002) and thereby stimulates transcription of CREB-target genes, including immediate early genes such as *FOS, MKP1, NUR77* and *JUNB*. MSK is thought to stimulate production of pro-inflammatory cytokines and prostaglandins in response to various inflammatory stimuli, at least partly

through phosphorylation of CREB. Firm evidence based on knockout mice has established that MSK, particularly MSK2, mediates ERK- or p38-induced phosphorylation of histone H3 on Ser10 and Ser28 (Soloaga et al., 2003). These phosphorylation events occur in a limited set of genes and are thought to stimulate their transcription by altering chromatin structure locally by an unknown mechanism. In *Drosophila,* the MSK ortholog JIL-1 has a global role promoting a transcription-competent, euchromatic structure in chromosomes. *MSK1*$^{-/-}$ *MSK2*$^{-/-}$ double-knockout mice display no overt phenotype, but analysis of processes thought to be regulated by MSK, such as inflammation, remains to be reported for these mice. In *Drosophila*, inactivation of JIL-1 is lethal.

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