The RSK (90 kDa ribosomal S6 kinase) family comprises a group of highly related serine/threonine kinases that regulate diverse cellular processes, including cell growth, proliferation, survival and motility. This family includes four vertebrate isoforms (RSK1, RSK2, RSK3 and RSK4), and single family member orthologues are also present in Drosophila and Caenorhabditis elegans. The RSK isoforms are downstream effectors of the Ras/ERK (extracellular-signal-regulated kinase) signalling pathway. Significant advances in the field of RSK signalling have occurred in the past few years, including several new functions ascribed to the RSK isoforms, the discovery of novel protein substrates and the implication of different RSK isoforms in cancer. Collectively, these new findings increase the diversity of biological functions regulated by RSK, and highlight potential new directions of research. In the present paper, we review the structure, expression and activation mechanisms of the RSK isoforms, and discuss their physiological roles on the basis of established substrates and recent discoveries.

Key words: cell signalling, mitogen-activated protein kinase (MAPK), phosphorylation, proliferation, protein kinase, 90 kDa ribosomal S6 kinase (RSK).

INTRODUCTION

The Ras/ERK (mitogen-activated protein kinase) pathway (Figure 1) plays a central role in transducing extracellular signals to intracellular target proteins involved in cell growth and proliferation (reviewed in [1–3]). Inappropriate regulation of this pathway leads to a variety of disorders and diseases, including many types of cancers [4]. In this pathway, the GTPase Ras activates Raf isoforms (A-, B- and C-Raf), which are serine/threonine kinases frequently mutated in human cancers [5,6]. In turn, activated Raf phosphorylates and activates MEK [MAPK/ERK (extracellular-signal-regulated kinase)] kinase1/2, which are dual-specificity kinases capable of phosphorylating and activating the MAPKs ERK1/2. Once activated, ERK1/2 phosphorylate several substrates, including members of the RSK (90 kDa ribosomal S6 kinase) family [7,8]. This family consists of four human isoforms (RSK1–4) and two structurally related homologues, termed MSK1 [mitogen- and stress-activated kinase 1; also known as RLPK (RSK-like protein kinase)] and MSK2 [also known as RSK-B (RSK-like B)]. Despite being highly related to the RSKs, the MSKs have very different biological functions and have been reviewed extensively [9,10]. Whereas the RSKs are directly activated by ERK1/2 in response to various stimuli, the MSKs are activated by both the ERK1/2 and the p38 pathways, and are generally thought to be more responsive to cellular stresses [8]. The past few years have witnessed a wealth of new information on the biological roles of the RSKs, and in the present review we discuss these insights and also highlight novel biological pathways that are modulated by these kinases.

DISCOVERY

The regulated phosphorylation of rpS6 (ribosomal protein S6) has attracted much attention since its discovery in 1974 [11]. This is due to the temporal correlation of rpS6 phosphorylation with the initiation of protein synthesis and the suggestion that its phosphorylation facilitates polysome assembly [12]. Several laboratories deployed great efforts to identify the protein kinases responsible for rpS6 phosphorylation [13,14], and in 1985, the groups of Erikson and Maller purified an intracellular kinase activity, termed ribosomal S6K (S6 kinase), that phosphorylated rpS6 from unfertilized Xenopus laevis eggs [15]. Two protein kinases of 85–90 kDa (S6K1 and S6KII) were identified by biochemical purification, which led to the cloning of cDNAs encoding highly homologous proteins that were later renamed p90 RSKs or RSKs [16]. It was subsequently shown that S6KII was phosphorylated and activated by the insulin-stimulated MAP2
The Ras/MAPK pathway leads to activation of the RSK family of protein kinases

Growth factor binding to their cognate receptor tyrosine kinase promotes GTP-loading of the Ras isoforms, which subsequently activate isoforms of the Raf family of serine/threonine kinases. Activated Raf promotes activation of the MAPK pathway by direct phosphorylation of MEK1/2, which then phosphorylate and activate ERK1/2. The RSK protein kinases are directly phosphorylated by ERK1/2 and PDK1, resulting in their activation. This leads to phosphorylation of functionally diverse RSK substrates in the cytosol as well as nuclear translocation of RSK, where it phosphorylates transcription factors, including those of the IEG response. Whereas PD184352, U0126 and PD98059 are specific MEK inhibitors, BI-D1870, SL0101 and FMK specifically inhibit RSK activity. Grb2, growth-factor-receptor-bound protein 2; NF1, neurofibromin.

(microtubule-associated protein-2) kinase, which was renamed ERK2 [17] and provided the first evidence that S6KII (or RSK) is activated downstream of the ERK/MAPK cascade.

TO BE, OR NOT TO BE, AN S6 KINASE

As described above, RSK was originally identified as an in vitro rpS6 kinase [15,18]. However, two related protein kinases, termed 70 kDa ribosomal S6 kinases (S6K1 and S6K2), were later shown to be the predominant rpS6 kinases operating in somatic cells [19,20]. Inhibition of mTOR (mammalian target of rapamycin) activity and thus S6K1/2 activation using rapamycin was shown to completely prevent rpS6 phosphorylation induced by insulin [20,21], which suggested at the time that the RSKs played negligible roles in rpS6 phosphorylation. This conclusion was also suggested by experiments performed in cells derived from S6k1−/− S6k2−/− double-knockout mice, where absence of S6K1/2 is concomitant with an almost complete loss of rpS6 phosphorylation [22]. Interestingly, low levels of rpS6 phosphorylation were found to persist in S6K1/2-deficient cells and to depend on the MAPK pathway, suggesting some involvement on the part of the RSKs. The specific role of the RSK isoforms in rpS6 phosphorylation was later confirmed in vivo using RNAi (RNA interference) and pharmacological inhibitors [23]. Indeed, both RSK1 and RSK2 were found to contribute to rpS6 phosphorylation in response to agonists or oncogenes that activate the MAPK pathway. Interestingly, whereas S6K1/2 were found to phosphorylate all sites on rpS6 (Ser235, Ser236, Ser240 and Ser244), the RSKs were shown to specifically phosphorylate Ser235 and Ser240 [23].

The role of this specific regulation remains elusive, but growing evidence suggests that rpS6 phosphorylation may be involved in fine-tuning the cellular response elicited by certain growth signals [12]. The specific evaluation of the RSK- and S6K-specific phosphorylation events on rpS6 will be necessary to determine the complexities of its regulation and function.

STRUCTURE AND CONSERVATION

The vertebrate RSK family contains four isoforms, termed RSK1 [24], RSK2, RSK3 [25] and RSK4 [26]. The RSKs are 73–80% identical with each other and are mostly divergent in their N- and C-terminal sequences (Figure 2). The most striking feature of the RSK family is the presence of two functional and non-identical phosphotransferase domains within the same polypeptide [16,27]. It is thought that, during evolution, the genes for two distinct protein kinases have fused, generating a single protein kinase capable of receiving an upstream activating signal from ERK1/2 to its CTKD (C-terminal kinase domain) and transmitting, with high efficiency and fidelity, an activating input to its NTKD (N-terminal kinase domain). The kinase domains are connected by a linker region of approximately 100 aa (amino acids) containing essential regulatory domains, including hydrophobic and turn motifs, involved in activation of the NTKD (Figure 3). Because they possess two separate catalytic domains, the RSKs are part of two different protein kinase families. The NTKD belongs to the AGC (protein kinase A, G and C) family, which also
The RSK family of protein kinases

Figure 2  Alignment of the amino acid sequence of the RSK isoforms (RSK1–4)

Human RSK (hRSK) sequences comprising the NTKDs and CTKDs of RSK1–4 are indicated by blue and dark brown lines respectively. The activation loop (T-loop) of each kinase domain is indicated with a yellow line. The conserved ERK1/2-binding sequence and PDZ-binding motifs are indicated by orange and mid-brown lines respectively. The conserved phosphorylation sites involved in RSK activation are indicated by black circles.

includes Akt (also known as protein kinase B), SGK (serum- and glucocorticoid-induced protein kinase) and S6K1/2. In that respect, the NTKD of RSK is most similar to the kinase domain of S6K1, with 57% aa identity. The CTKD of RSK belongs to the CAMK (Ca\(^{2+}\)/calmodulin-dependent protein kinase) family which also includes AMPK (AMP-activated protein kinase), MARK (MAP-regulating kinase/microtubule affinity-regulating kinase) and DAPK (death-associated protein kinase). In addition, the RSKs also belong to a functional subgroup of CAMKs termed the MAPKAPK (MAPK-activated protein kinase) family [8], which comprises MSK1/2 [9], MNKs (MAPK-interacting kinases) [28], MK2/3 (MAPK-activated protein kinase 2/3) [29] and MK5 [30]. This group is characterized by the fact that they are activated by different MAPKs, and also display substantial homology in their kinase domains [7]. Whereas the NTKD of the RSKs is responsible for downstream substrate phosphorylation, the only known function of the CTKD is to activate the NTKD via autophosphorylation of the hydrophobic motif [27,31,32], and no exogenous substrates have yet been ascribed to this domain [33]. Although this is true, the idea that the CTKD could phosphorylate heterologous substrates in trans remains an interesting possibility.
RSK is characterized by the presence of two functional domains, the NTKD and the CTKD, which are connected by a linker region of approximately 100 aa. Activation of RSK is associated with increased phosphorylation at six sites (each shown as black or white circles). These phosphorylation sites are highly conserved in RSK1–4 and were shown to be either essential (black circles) or accessory (white circles) for RSK activation. The C-terminal end of the protein contains an ERK1/2-docking domain resembling a KIM motif, and the extreme C-terminus comprises a PDZ-binding motif. Amino-acid numbering refers to human RSK1.

All RSK isoforms interact with ERK1/2 through their C-terminally located docking domain (Figure 3). This domain differs from classical D-type ERK1/2 docking domains as it does not match the best-characterized consensus sequence for such motifs [34]. Instead, docking domains found in RSKs appear to fit the KIM (kinase interaction motif) consensus sequence [35]. This D domain-related motif corresponds to a hydrophobic residue closely followed by two positively charged lysine or arginine residues (Leu-Xaa-Lys/Arg-Xaa-Leu). A similar motif has also been reported in several other MAPK substrates, including the protein tyrosine phosphatase PTP-SL [36] and the cAMP-specific phosphodiesterase PDE4D [35]. This domain is required for ERK1/2 binding to RSKs [37,38], and was shown to be essential for ERK1/2-mediated activation [39]. D domains usually increase the affinity of MAPKs for their substrates and thus the efficiency of their phosphorylation. In some cases, D domains are responsible for pre-existing complexes between inactive MAPKs and their substrates. In the case of RSK1 and RSK2, these isoforms were shown to have higher affinity towards ERK1/2 when in their inactive state than following mitogen stimulation [39]. Indeed, a complex between RSK and ERK was found to transiently dissociate for the duration of ERK1/2 activation and to reassemble upon signal termination [39]. Autophosphorylation of a serine residue near the ERK1/2 docking domain of RSK1 was found to promote its dissociation from ERK1/2 [39], providing a mechanism by which active ERK and RSK dissociate to find their respective substrates. These findings also suggest that an unknown serine/threonine phosphatase dephosphorylates the C-terminal domain of RSK, thereby promoting ERK1/2 binding. In addition to a KIM motif, the C-terminal tails of all four RSK isoforms contain a type 1 PDZ domain binding motif (Ser-Thr-Xaa-Leu). Although this motif is not conserved in RSK orthologues from Drosophila and Caenorhabditis elegans, it was shown to be functional and probably helps in bringing RSK closer to certain of its targets [40].

ADULT AND EMBRYONIC TISSUE EXPRESSION

With the exception of RSK4, expression of the RSK mRNAs has been shown to be ubiquitous in every human tissue tested [41]. This is consistent with approximate expression patterns inferred from EST (expressed sequence tag) sources from both mouse and human origins, which suggest that RSK4 is in low abundance in both embryonic and adult tissues (Figure 4A). Although these results support functional redundancy, available Affymetrix microarray data demonstrate important tissue variations between the RSK isoforms (BioGPS) [42], suggesting at least some isoform-specific functions (Figure 5). Consistent with this, more targeted studies have shown that the RSK1 mRNA is predominantly found in the lung, kidney and pancreas, whereas both RSK2 and RSK3 mRNAs are more abundant in skeletal muscle, heart and pancreas [41,43,44]. In the brain, the RSK1 mRNA was found to be expressed in the granular cell layer of the cerebellum, whereas RSK2 was more present in the neocortex, hippocampus and cerebellum. RSK3 is also very abundant in the central nervous system, with high mRNA expression in the cerebral cortex, the dentate gyrus and the amygdala [41,45]. With regards to RSK4, its expression is much lower than RSK1–3, but Northern blot analysis has revealed the presence of the RSK4 mRNA in the brain, heart, cerebellum, kidney and skeletal muscle, whereas other tissues such as lung, liver, pancreas and adipose tissue showed no detectable RSK4 expression [46]. Developmental expression of the RSK isoforms has also been determined. The mouse RSK2 mRNA was found to be expressed at a very low level compared with RSK3, whose mRNA is very abundant in fetal tissues [47]. RSK3 expression can be
detected in the ventricular zone, a site of high proliferative activity. Conversely, RSK1 expression is strongest in the neuroepithelium of the forming neural tube, whereas at later stages it decreases dramatically and becomes undetectable in the nervous system. These results are consistent with a temporal regulation of RSK1 and RSK3 expression and support the requirement of RSK1 in early development and RSK3 in later development of the nervous system. These results are also corroborated with approximate expression patterns inferred from EST sources (Figure 6). At late stages of development, RSK1 is highly expressed in regions harbouring highly proliferating cells. These include liver, lung, thymus, olfactory and gut epithelia. RSK4 was found to be ubiquitously expressed throughout development [47], and approximate expression patterns from EST sources suggest that RSK4 is more highly expressed in specific phases of embryogenesis (Figure 6). Whereas both Rsk1 and Rsk3 genes give rise to only one transcript, Northern blot analysis of Rsk2 expression revealed the alternative use of two different polyadenylation sites giving rise to two transcripts of 3.5 and 8.5 kb [41]. Similarly, two secondary Rsk4 transcripts (5 and 9 kb) also exist, but whether they result from alternative splicing or alternative polyadenylation remains unknown [48].

**SUBCELLULAR LOCALIZATION**

At the subcellular level, RSK1–3 are usually present in the cytoplasm of quiescent cells, but upon stimulation, a significant proportion of these proteins translocates to the nucleus (Figure 1) [49–53]. Within minutes of stimulation, RSK1 was shown to accumulate transiently at the plasma membrane, where it presumably receives additional inputs necessary for activation before nuclear translocation [52]. RSK4 appears to be predominantly cytoplasmic [46], and contrary to other RSK isoforms, RSK4 does not significantly accumulate in the nucleus following mitogenic stimulation. The mechanisms involved in RSK translocation to the nucleus remain elusive, but the small death effector domain protein PEA-15 (phosphoprotein enriched in astrocytes, 15 kDa) has been shown to interact with RSK2 and inhibit its nuclear translocation [53]. Interestingly, RSK2 was recently found to associate with TIA-1 (T-cell-restricted intracellular antigen-1) and localize to stress granules upon oxidative stress [54], suggesting the possibility that PEA-15 may also localize to these structures. RSK3 is the only isoform to possess a classical NLS (nuclear localization signal), consisting of Lys-Lys-Xaa10-Leu-Arg-Arg-Lys-Ser-Arg, but the functionality of this domain has never been tested. Compared with other isoforms, RSK3 is often found associated to detergent-resistant cellular fractions, indicating that this isoform may localize to distinct cellular compartments (P. P. Roux, unpublished work). At the moment, very little is known about isoform-specific functions of the RSKs, but apparent differences in their subcellular localization probably determine their respective roles.

**CANONICAL PATHWAY OF ACTIVATION**

Understanding the mechanisms underlying RSK activation was several years in the making, mostly due to the complex nature by which several kinases induce sequential phosphorylation events (Figure 7). All RSK isoforms, including C. elegans and Drosophila melanogaster RSK orthologues, contain the four essential phosphorylation sites (Ser272, Ser363, Ser380 and Thr358) in human RSK1 responsive to mitogenic stimulation [55]. Phosphorylation of Ser272 is mediated by PDK1 (3′-phosphoinositide-dependent protein kinase 1) [56,57], a constitutively active serine/threonine kinase that regulates several other AGC family members, including Akt, SGK and S6K [58]. Consistent with this, mitogens do not stimulate RSK1–3 activity in PDK1-deficient cells [59], and PI3K (phosphoinositide 3-kinase) inhibitors do not affect RSK1–3 activation. Although PDK1-dependent activation of Akt was shown to require a functional PH (pleckstrin homology) domain on PDK1, activation of RSK1–3 can still occur in the absence of this domain [56,60], indicating that RSK activation is not restricted to PIP2 (phosphatidylinositol 3,4,5-trisphosphate)-containing membrane domains. Interestingly, whereas PDK1 is required for mitogenic stimulation of RSK1–3, RSK4 does not appear to require PDK1 to maintain its high basal activity [46]. Ser363 and Ser380 are located in the linker region within sequences that are conserved among most AGC family kinases. These sequences are termed the ‘turn motif’ and the ‘hydrophobic motif’ respectively [61]. Ser363 is followed by a proline residue and has been shown to be phosphorylated by ERK1/2, but whether it also undergoes autophosphorylation and/or phosphorylation by a heterologous kinase remains unclear [57]. The hydrophobic motif containing Ser380 is phosphorylated by the CTKD [32], but the involvement of the NTKD or a heterologous kinase in Ser380 phosphorylation cannot be excluded. The phosphorylated hydrophobic motif of RSK serves as a docking site for PDK1 by binding to the PIF (PDK1-interacting fragment) pocket of PDK1 [62]. For RSK2, this interaction has been shown to increase the catalytic activity of PDK1 severalfold, indicating that this motif functions to both recruit and activate PDK1 [62]. PDK1 binding promotes its ability to phosphorylate the RSK NTKD activation loop (Ser272), a phosphorylation event that leads to full activation of this kinase domain in collaboration with binding of the phosphorylated hydrophobic motif to the PIF pocket in the RSK NTKD [63].
Figure 5  Expression pattern of the RSK1–4 mRNAs across human and mouse tissues

Relative expression of the RSK1–4 mRNAs derived from fluorescence intensities of multiple probes for each transcript arrayed on Affymetrix microarray chips. Intensity values were summarized using the data-processing algorithm gcrma, and data were retrieved from the BioGPS database.
The RSK family of protein kinases

Figure 6 Approximate expression patterns of the RSK isoforms during mouse development

Relative expression of RSK1–4 is indicated using approximate expression patterns inferred from EST sources from mouse embryonic tissues. Data were retrieved from the UniGene database (http://www.ncbi.nlm.nih.gov/unigene) and are shown as transcripts per million.

Thr573 is also followed by a proline residue and is located in the activation loop of the CTKD. This residue is phosphorylated by ERK1/2 [37,64] and might contribute to RSK translocation to the plasma membrane [57], where it can be further activated by a membrane-associated kinase. This hypothesis is supported by the discovery that a myristoylated RSK1 mutant that cannot associate with ERK1/2 displays increased phosphorylation on Ser363 and Ser380 [57], which suggests that membrane translocation of RSK1 might have an important role in its activation.

The use of phosphomimetic mutations introducing a negative charge that functionally mimicked phosphorylation of Ser363, Ser380 and Thr573 residues led to the prediction of a hierarchical phosphorylation cascade for the activation of RSK [55]. The current model of RSK activation is that ERK1/2 (and potentially ERK5) phosphorylate the activation loop of the CTKD on Thr573 [37,65,66]. This phosphorylation event requires ERK docking to the KIM domain of RSK and a proline-directed ERK consensus sequence in the RSK activation loop. ERK1/2 also phosphorylate Thr579/Ser363 in the linker region [55].

Phosphorylation of Ser363 stimulates the NTKD catalytic activity by an unknown mechanism. Phosphorylation of Thr573 activates CTKD autophosphorylation on Ser380, which is located within the hydrophobic motif of aromatic residues in the linker [32]. Phosphorylation of Ser380 then creates a docking site for PDK1 [62], which, in turn, phosphorylates Ser221 in the activation loop of the NTKD [56,57]. Once PDK1 dissociates from RSK, phosphorylated Ser380 binds a phosphate-binding site in the NTKD, resulting in a stable association between the hydrophobic motif and a proximal hydrophobic pocket within the NTKD. The αC-helix then collaborates with phosphorylated Ser221 to stabilize NTKD in an active conformation, resulting in synergistic full activation of RSK. Phosphorylation of RSK at its C-terminus releases ERK1/2 and thereby allows active RSK to translocate to its many cytoplasmic and nuclear substrates (Figure 7).

ALTERNATIVE MECHANISMS OF ACTIVATION

Recent evidence indicates that RSK2 is also phosphorylated on tyrosine residues in response to FGF (fibroblast growth factor) receptor [67] and Src activation [68]. These phosphorylation
to phosphorylate Ser380 in several cell types, which may explain [33,39]. Interestingly, the related MK2/3 enzymes were found only partially inhibits activation of the NTKD of RSK1 manner [52,69], since mutational inactivation of the CTKD ability of cAMP to stimulate PKA. RSK inactivation may require interact with the PKA catalytic subunit, thereby decreasing the normal PKA function, whereas activated RSK1 was shown to interact with the PKA regulatory subunit and thereby stimulate cAMP-dependent PKA activation [33,39].

Ser380 phosphorylation may also occur in a CTKD-independent manner [52,69], since mutational inactivation of the CTKD only partially inhibits activation of the NTKD of RSK1 [33,39]. Interestingly, the related MK2/3 enzymes were found to phosphorylate Ser380 in several cell types, which may explain how various p38-mediated stresses lead to RSK activation [70]. In addition to phosphorylating RSK, ERK1/2 may also promote RSK1 activation by facilitating its recruitment to the plasma membrane, as suggested by the apparent constitutive activation of the myristoylated RSK mutant [52].

A new point of cross-talk between PKA (protein kinase A) and ERK1/2 signalling pathways was recently identified and shown to be involved in both PKA activity and the cellular distribution of active RSK1 [71,72]. Inactive RSK1 was found to interact with the PKA regulatory I subunit and thereby stimulate cAMP-dependent PKA function, whereas activated RSK1 was shown to interact with the PKA catalytic subunit, thereby decreasing the ability of cAMP to stimulate PKA. RSK inactivation may require PP2Cδ (protein phosphatase 2Cδ), which was shown to associate with RSK1–4 [73]. Inactivation of RSK activity may also involve its autophosphorylation at Ser321, which was found to promote ERK/RSK dissociation and correlate with reduced RSK kinase activity [39].

**PHARMACOLOGICAL INHIBITORS**

RSK activation is closely linked to ERK1/2 activity, and therefore MEK1/2 inhibitors (U0126, PD98059 and PD184352) have been and are still widely used to study RSK function (Figure 1). To date, three different classes of RSK inhibitors (Figure 8) have been identified [74]. The dihydropteridinone BI-D1870 is a reversible inhibitor that competes with ATP by binding to the NTKD ATP-interacting sequence. BI-D1870 is remarkably selective for RSK relative to other AGC kinases [75], and its *in vitro* IC50 was shown to be approximately 15–30 nM at an ATP concentration of 100 μM [76]. *In vivo* results indicate that to completely inhibit the phosphorylation of RSK substrates in cells, a concentration of 10 μM BI-D1870 is required [76]. The kaempferol glycoside SL0101 is another ATP-competitive inhibitor of the NTKD, and was in fact the first identified specific inhibitor of RSK [77]. SL0101 is a natural product obtained from the tropical plant *Fosteronia refracta* and was reported to inhibit RSK2 with an IC50 of 90 nM at an ATP concentration of 10 μM. The EC50 of SL0101 was found to be approximately 50 μM in intact cells [77], indicating that the activity of SL0101 in intact cells is significantly weaker than *in vitro*. [39]. Like BI-D1870, SL0101 was tested against a panel of purified kinases and was found to be relatively specific to the RSK [75]. In contrast, the pyrrolopyrimidine FMK (Z-VAD-FMK, benzoyloxy carbonyl-Val-Ala-Asp-fluoromethylketone) is an irreversible inhibitor that covalently modifies the CTKD of RSK1, RSK2 and RSK4 [78]. FMK is a potent and specific inhibitor of RSK, and was shown to inhibit RSK2 with an *in vitro* IC50 of 15 nM and an EC50 of 200 nM. FMK contains a reactive electrophile in its fluoromethylketone motif and, in this instance, derives its potent kinase activity from the covalent addition of its chloromethylketone functionality to the thiol group of Cys526 located in the ATP-binding pocket of the RSK2 CTKD. Despite having a mechanism of action involving covalent addition to the molecular target, FMK was shown to be remarkably RSK-specific. In human epithelial cell lysate, containing thousands of proteins, biotin-labelled FMK was found to only react with RSK1 and RSK2, which were identified using quantitative immunodepletion with specific antibodies. Therefore, there are now three potent and highly specific inhibitors of RSK, which can be used as chemical probes for dissecting the complex signalling events associated with RSK, and additionally, their potential as therapeutic candidates.

**SUBSTRATE RECOGNITION**

The substrate specificity of RSK1 for target phosphorylation was originally determined using synthetic peptides and found to require the minimum motifs Arg/Lys-Xaa-Arg-Xaa-Xaa-pSer/Thr or Arg-Arg-Xaa-pSer/Thr [79]. Recently, we have used an arrayed positional scanning peptide library to determine the consensus phosphorylation motif of RSK1 and also found a stronger requirement for the −3 position arginine residue compared with the −5 position residue (P. P. Roux, unpublished work). In addition, RSK1 appeared to greatly prefer arginine residues to lysine, which is in agreement with the majority of RSK substrates in cells.

Ala-DL-Asp-fluoromethylketone) is an irreversible inhibitor that covalently modifies the CTKD of RSK1, RSK2 and RSK4 [78]. Alkylating agents like BI-D1870 are remarkable inhibitors of RSK [77]. SL0101 is a natural product obtained from the tropical plant *Fosteronia refracta* and was reported to inhibit RSK2 with an IC50 of 90 nM at an ATP concentration of 10 μM. The EC50 of SL0101 was found to be approximately 50 μM in intact cells [77], indicating that the activity of SL0101 in intact cells is significantly weaker than *in vitro*. [39]. Like BI-D1870, SL0101 was tested against a panel of purified kinases and was found to be relatively specific to the RSK [75]. In contrast, the pyrrolopyrimidine FMK (Z-VAD-FMK, benzoyloxy carbonyl-Val-Ala-Asp-fluoromethylketone) is an irreversible inhibitor that covalently modifies the CTKD of RSK1, RSK2 and RSK4 [78]. FMK is a potent and specific inhibitor of RSK, and was shown to inhibit RSK2 with an *in vitro* IC50 of 15 nM and an EC50 of 200 nM. FMK contains a reactive electrophile in its fluoromethylketone motif and, in this instance, derives its potent kinase activity from the covalent addition of its chloromethylketone functionality to the thiol group of Cys526 located in the ATP-binding pocket of the RSK2 CTKD. Despite having a mechanism of action involving covalent addition to the molecular target, FMK was shown to be remarkably RSK-specific. In human epithelial cell lysate, containing thousands of proteins, biotin-labelled FMK was found to only react with RSK1 and RSK2, which were identified using quantitative immunodepletion with specific antibodies. Therefore, there are now three potent and highly specific inhibitors of RSK, which can be used as chemical probes for dissecting the complex signalling events associated with RSK, and additionally, their potential as therapeutic candidates.

Although a certain number of RSK functions can be deduced from the nature of its substrates, accumulating evidence suggests that the RSK isoforms regulate nuclear signalling, cell-cycle...
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<td>GSK3β</td>
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<td>c-Fos</td>
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<td>NHE-1</td>
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<tr>
<td>eEF2K</td>
<td>RV^I^L^S^G^R^P^P^P^P (h)</td>
<td>Inhibits its kinase activity</td>
<td>RSK1</td>
<td>[146]</td>
</tr>
<tr>
<td>elf4B</td>
<td>R^E^F^F^F^L^G^S^S^T^S^T^S^T (h)</td>
<td>Enhances interaction to elf3</td>
<td>RSK1/2</td>
<td>[147]</td>
</tr>
<tr>
<td>Hsp90α</td>
<td>L^S^P^K^P^P (h)</td>
<td>Promotes translation of the C/EBPβ protein</td>
<td>RSK1/2</td>
<td>[129]</td>
</tr>
<tr>
<td>Mad2</td>
<td>NR^P^P^P^P^P^P^P^P^P^P^P (h)</td>
<td>Inhibits the translation of the C/EBPβ protein</td>
<td>RSK1/2</td>
<td>[129]</td>
</tr>
<tr>
<td>Myc</td>
<td>Serine residues (x)</td>
<td>Inactivating</td>
<td>RSK1</td>
<td>[121]</td>
</tr>
<tr>
<td>HSP90α</td>
<td>L^S^P^K^P^P (h)</td>
<td>Promotes its kinase activity</td>
<td>RSK1</td>
<td>[127]</td>
</tr>
<tr>
<td>TSC2</td>
<td>RK^R^R^S^T^P^S^E^D^F (h)</td>
<td>Inhibits TSC2 activity</td>
<td>RSK1</td>
<td>[140,181]</td>
</tr>
<tr>
<td>METTL1</td>
<td>RO^R^R^A^I^S^T^P^N^M^A^D (h)</td>
<td>Reduces its RNA methylase activity</td>
<td>RSK1</td>
<td>[182]</td>
</tr>
<tr>
<td>Bad</td>
<td>R^S^R^H^S^S^T^Y^P^A^T^P^ (h, r)</td>
<td>Suppresses Bad-mediated apoptosis</td>
<td>RSK1/2</td>
<td>[150,153]</td>
</tr>
<tr>
<td>DAPK</td>
<td>LS^R^R^S^A^3^S^P^A^M^E (h)</td>
<td>Promotes its kinase activity</td>
<td>RSK1</td>
<td>[146]</td>
</tr>
<tr>
<td>C/EBPβ</td>
<td>PS^R^K^R^S^P^S^P^H^G^Y^S^V^ (h, m)</td>
<td>Reduces its pro-apoptotic function</td>
<td>RSK1/2</td>
<td>[155]</td>
</tr>
<tr>
<td>mNOS</td>
<td>K^V^R^B^B^R^S^7^A^A^Y^S^S^Y^S^S (y)</td>
<td>Stimulates hepatocyte proliferation</td>
<td>RSK1</td>
<td>[152]</td>
</tr>
<tr>
<td>CREB</td>
<td>LS^R^R^S^A^3^S^P^A^M^E (h, m)</td>
<td>Blocks toxic accumulation of NO</td>
<td>RSK1</td>
<td>[161]</td>
</tr>
<tr>
<td>SRF</td>
<td>L^S^R^R^S^A^3^S^P^A^M^E (h, m)</td>
<td>Stimulates its transcriptional activity</td>
<td>RSK1</td>
<td>[82–84,150]</td>
</tr>
<tr>
<td>ERB1</td>
<td>FR^Q^L^Q^L^S^P^E^P^C^N^S^E^ (h, m)</td>
<td>Enhances its affinity for the SRE motif</td>
<td>RSK1</td>
<td>[81,87]</td>
</tr>
<tr>
<td>Nur77</td>
<td>RG^L^P^G^S^P^K^P^Q^D^ (m)</td>
<td>Enhances its transcriptional potential</td>
<td>RSK1</td>
<td>[96]</td>
</tr>
<tr>
<td>le^Bat</td>
<td>DD^R^M^S^G^L^D^L^S^M^ (h)</td>
<td>No clear function</td>
<td>RSK2</td>
<td>[27,89,90]</td>
</tr>
<tr>
<td>NFATc4</td>
<td>GR^A^R^R^G^S^P^I^T^G^S^F^ (h, m)</td>
<td>Increases NF-κB activity</td>
<td>RSK1</td>
<td>[99,100]</td>
</tr>
<tr>
<td>NFATc3</td>
<td>Serine residues (h, m)</td>
<td>Promotes DNA binding</td>
<td>RSK1</td>
<td>[183]</td>
</tr>
<tr>
<td>TIF-1A</td>
<td>LY^M^G^P^S^4^S^4^S^P^L^ (h)</td>
<td>Induces NFATc3 nuclear accumulation</td>
<td>RSK1</td>
<td>[184]</td>
</tr>
<tr>
<td>AT-F4</td>
<td>P^S^P^G^S^S^R^S^S^S^R^S^S^ (m)</td>
<td>Enhances cellular pre-rRNA synthesis</td>
<td>RSK2</td>
<td>[93]</td>
</tr>
<tr>
<td>SHP2</td>
<td>AV^R^T^L^S^S^5^S^E^N^D^ (h)</td>
<td>Promotes collagen synthesis</td>
<td>RSK2</td>
<td>[107,185]</td>
</tr>
<tr>
<td>AT-F1</td>
<td>L^A^B^R^P^S^5^R^Y^K^I^L^K^I^L^ (h)</td>
<td>Promotes cell motility</td>
<td>RSK1</td>
<td>[159]</td>
</tr>
<tr>
<td>p38</td>
<td>L^A^B^R^P^S^5^R^Y^K^I^L^K^I^L^ (h)</td>
<td>Induces AT-F1 transcriptional activity</td>
<td>RSK2</td>
<td>[186]</td>
</tr>
<tr>
<td>MEKc2</td>
<td>L^A^B^R^P^S^5^R^Y^K^I^L^K^I^L^ (h)</td>
<td>Stimulates MEKc2 transcriptional activity</td>
<td>RSK2</td>
<td>[187]</td>
</tr>
<tr>
<td>Filamin A</td>
<td>P^R^R^R^P^S^S^P^A^V^N^V^G^V^ (h, r)</td>
<td>Controls actin cytoskeletal dynamics</td>
<td>RSK1/2</td>
<td>[156]</td>
</tr>
<tr>
<td>AS160</td>
<td>R^R^R^R^R^A^S^P^P^S^H^S^V^T^G^V^G^O^G^ (h)</td>
<td>14-3-3 binding and inactivation of its GAP activity</td>
<td>RSK1</td>
<td>[165]</td>
</tr>
<tr>
<td>Raptor</td>
<td>T^R^H^L^L^S^I^S^5^I^S^2^I^S^2^Y^G^N^T (h)</td>
<td>Increases mTORC1 activity</td>
<td>RSK1/2</td>
<td>[142]</td>
</tr>
<tr>
<td>CCTβ</td>
<td>LY^R^V^P^S^S^4^T^K^A^V^R^K^A^ (h)</td>
<td>Plays a role in cytoskeletal reorganization</td>
<td>RSK1/2</td>
<td>[188]</td>
</tr>
<tr>
<td>CH25H</td>
<td>R^R^R^R^R^S^S^S^P^A^V^R^ (h)</td>
<td>No clear function</td>
<td>RSK2</td>
<td>[189]</td>
</tr>
<tr>
<td>SHANK</td>
<td>Not determined</td>
<td>Enhances synaptic transmission</td>
<td>RSK2</td>
<td>[40]</td>
</tr>
</tbody>
</table>

Figure 9  WebLogo frequency plot of the target sequences in known RSK substrates

The RSK family of protein kinases

Table 1  Phosphorylation substrates of the RSK isoforms

Phosphorylated residues are in bold. * indicates the end of the sequence and underlined residues indicate basic residues forming the RSK consensus sequences. Abbreviations: CCT, chaperonin containing TCP-1 (T-complex polypeptide 1); β subunit; CRHSP24, calcium-regulated heat-stable protein of apparent molecular mass 24 kDa; GAP, guanine nucleotide-activating protein; h, human; m, mouse; MEF2c, myocyte enhancer factor 2C; METTL1, methyltransferase-like protein 1; mTORC1, mTOR complex 1; NFAT3, nuclear factor of activated T-cells 3; r, rat; SHANK, Src homology 3 domain and ankyrin repeat-containing protein-1; SRE, sterol-regulatory element; x, Xenopus; YB-1, Y-box-binding protein-1.

* The Authors Journal compilation © 2012 Biochemical Society
progression and cell proliferation, cell growth and protein synthesis, cell migration and cell survival (Table 1). RSK2 has been extensively studied and, to date, most substrates have been identified for this isoform. Nevertheless, most studies have not determined isoform selectivity. Therefore many known RSK2 substrates may be shared by other RSK family members and vice versa. Thus more effort will be necessary to assess potential overlapping functions between the RSK isoforms. In addition, the RSK consensus phosphorylation motif is shared by other AGC family members, such as Akt and S6K1, suggesting that an important functional overlap may exist between these related basophilic protein kinases. Indeed, several RSK substrates have been shown to be also targeted by Akt [TSC2 (tuberous sclerosis complex 2), Bad (Bcl-2/Bcl-xL-antagonist, causing cell death), GSK3 (glycogen synthase kinase-3) and p27kip1 (cyclin-dependent kinase inhibitor 1B)] and S6K1 [eEF2K (eukaryotic elongation factor-2 kinase), rpS6, GSK3 and ef4B (eukaryotic initiation factor 4B)]. This needs to be kept in mind when analysing the phenotype of knockout mice as compensation may occur between these highly related kinases.

Transcriptional regulation

Global profiling analysis of RSK has been reported and identified RSK as an important effector of ERK in global transcriptional regulation [80]. RSK was found to regulate ∼20% of mRNAs controlled by ERK in MDCK cells (Madin–Darby canine kidney cells) through direct and indirect mechanisms, such as induction of transcription factors. Indeed, activated RSK was shown to phosphorylate several transcription factors, some of which contribute to the IEG (immediate-early gene) response or are IEG products themselves. Two studies using human cells from CLS (Coffin–Lowry Syndrome) patients and primary fibroblasts isolated from Rsk2−/− mice showed that RSK2 mediates mitogen-induced c-Fos transcription by activating both the Elk1 [ETS (E-twenty-six)-like kinase 1/SRF (serum-response factor) complex-induced c-Fos transcription by activating both the Elk1 [ETS (E-twenty-six)-like kinase 1)/SRF (serum-response factor) complex] and CREB (cAMP-response-element-binding protein) [81,82]. Although RSK2 was shown to phosphorylate CREB at Ser133 [83,84], more recent evidence indicated that the related MSK1/2 protein kinases are the predominant CREB kinases following mitogenic stimulation or stresses in somatic cells [85]. Residual CREB phosphorylation in cells derived from Msk1−/− Msk2−/− mice suggested that RSK might co-operate with MSK1/2 for the phosphorylation of CREB induced by mitogens, but not cellular stresses, although more work will be needed to strengthen this hypothesis. Consistent with this idea, histone H3 was also initially found to be regulated by RSK, but convincing evidence indicated that MSK1/2 are the predominant histone H3 kinases operating in response to stress or mitogenic stimulations [86]. In addition, RSK1 was shown to participate in the post-translational modification of the IEG products SRF [87], c-Fos [88] and Nur77 [27,89,90]. Regulation of c-Fos by RSK2 was shown to play an important role in bone homeostasis and tumorigenesis [91,92].

Data from several groups point towards a role for RSK in regulating growth-related transcription initiation. Upon serum stimulation, RSK2 phosphorylates one serum residue important for the function of TIF-1A, a transcription initiation factor required for RNA polymerase I transcription and rRNA synthesis [93]. Growth factor-activated RSK1 also associates with and phosphorylates ERα (oestrogen receptor α) on Ser164, which increases ERα-mediated transcription [94,95]. RSK may also be responsible for transcriptional control of genes involved in homeostasis, signalling response and development, as RSK interacts with the ETS transcription factor ER81 and enhances ER81-dependent transcription by phosphorylating Ser391 and Ser216 [96]. The RSKs were also found to phosphorylate the microphthalmia (mi) transcription factor [97], which has been linked to malignant melanoma [98].

RSK regulates the activity of nuclear proteins involved in the inflammatory response, such as the transcription factor NF-κB (nuclear factor κB). RSK1 phosphorylates the inhibitory proteins IκBα (inhibitor of NF-κB α) and IκBβ on sites that promote their degradation, thereby stimulating NF-κB activity [99–101]. Related to this, RSK was also suggested to promote p65 phosphorylation at Ser536 [102,103], but the involvement of RSK in Ser65 phosphorylation is still unclear [104]. Activated RSK1 binds to the transcriptional co-activators CBP (CREB-binding protein) and p300 [105,106], but the exact outcome of this association remains to be determined. Interestingly, CBP also interacts with transcription factors that are phosphorylated by RSK1 and RSK2, such as CREB, c-Fos, ER81, ERα and NF-κB, suggesting that RSK may help in the recruitment of CBP and p300 cofactors to promoters regulated by these transcription factors. The CREB family member ATF4 (activating transcription factor-4) is a transcription factor required for the timely onset of osteoblast differentiation during development. RSK2 was found to phosphorylate and thereby control ATF4 activity [107], suggesting a mechanism by which loss of RSK2 may lead to CLS-associated skeletal abnormalities.

Cell-cycle progression and cell proliferation

RSK has been directly involved in the regulation of cell-cycle progression and cell proliferation. Treatment with the SL0101 inhibitor or RNAi against RSK1 and RSK2 was shown to inhibit proliferation of cells derived from human prostate and breast tumours, where both isoforms are overexpressed (P. P. Roux, unpublished work) [77,108]. These data indicate that RSK1 and RSK2 positively regulate cancer-cell proliferation, and consistent with this affirmation, approximate expression patterns inferred from EST sources indicate that RSK1 is often found in higher abundance in tumours compared with normal tissues (Figure 4B). Although this is not the case for RSK2, it is also interesting to note that RSK3 and RSK4 are usually found in lower abundance in tumour specimens (Figure 4B).

RSK controls cell proliferation through the regulation of mediators of the cell cycle. RSK2 was shown to promote cell-cycle progression by phosphorylating c-Fos, a transcription factor that positively regulates expression of cyclin D1 during G1/S transition. RSK2-mediated phosphorylation of c-Fos at Ser256 promotes its stability and oncogenic properties, and was found to be essential for osteosarcoma formation in mice [91,109,110]. RSK1 and RSK2 may also regulate G1 phase progression by controlling the activity of the CDK2 (cyclin-dependent kinase 2) inhibitor p27kip1 [111]. RSK-mediated phosphorylation of p27kip1 on Thr180 prevents its translocation to the nucleus by promoting its association with 14-3-3 [111,112]. RSK also mediates cell-cycle progression by negatively regulating GSK3 [113], which targets c-Myc and cyclin D1 for degradation [114]. More recently, Mad1 (mitotic arrest-deficient protein 1), a suppressor of Myc-mediated cell proliferation and transformation, was shown to be phosphorylated by RSK on Ser145 [115]. This phosphorylation event results in increased degradation of Mad1 by the ubiquitin–proteasome system, leading to enhanced Myc-dependent transcription and cell proliferation. Inhibition of RSK activity was shown to prevent the kinetochore localization of Mad1 and Mad2, suggesting that RSK may participate in the spindle assembly checkpoint [116]. In addition, RSK2-mediated phosphorylation on Ser161 of the tumour suppressor LKB1 (liver kinase B1), a kinase found mutated in the cancer-prone
Immature oocytes are arrested in late G2 phase of the first meiotic cell division. Upon progesterone exposure, the synthesis of the MAPKK (MAPK kinase kinase) c-Mos activates the MEK1/ERK/RSK cascade leading to M phase entry and subsequent maturation to an unfertilized egg. M phase entry is controlled in part by Cdc2 (cell division cycle 2 kinase), a CDK that is regulated through phosphorylation on both Thr14 and Tyr15 by the inhibitory kinase Myt1 (myelin transcription factor 1). Among the RSK isoforms, RSK2 is the most highly expressed in *Xenopus* oocytes [119] and was shown to participate in the control of critical stages of the meiotic cell cycle [120], including G2/M progression during meiosis I by phosphorylating and inhibiting the Myt1 kinase [121–123]. In fact, Myt1 was the first identified substrate of RSK [121], providing a direct link between RSK and the cell cycle. RSK2 was also found to promote Cdc2 activation by directly phosphorylating and activating Cdc25C, an enzyme involved in the activation of Cdc2 complexed to cyclin B [124]. RSK also modulates the meiotic cell cycle in *Xenopus* through ERK-mediated metaphase II arrest, an activity known as CSP (cystostatic factor) [125, 126]. RSK1 is thought to participate until metaphase II arrest in part by phosphorylating and activating the kinase Bub1, a mediator of APC (anaphase-promoting complex) inhibition [127, 128]. More recently, Emi2 [early mitotic inhibitor 2, also called Erp1 (Emi1-related protein)], another APC inhibitor, has been identified as a RSK substrate. Phosphorylation of Emi2 by RSK promotes Emi2–PP2A (protein phosphatase 2A) association, facilitating Emi2 dephosphorylation at specific Cdc2 phosphorylation sites, which in turn enhances Emi2 stability and function [129–131].

RSK1 and RSK2 recently emerged as essential regulators of tumorigenesis. Ectopic expression of RSK2 was demonstrated to increase proliferation as well as anchorage-independent transformation [132]. In addition, RSK2 is thought to mediate FGFR3-dependent transformation of haemopoietic cells, as FGFR3 was shown to activate RSK2 by facilitating both ERK–RSK2 interaction and subsequent phosphorylation of RSK2 by ERK in these cells [67]. In contrast to what was initially believed [51], RSK3 was recently shown to act as a potential tumour suppressor in ovarian cancer [133]. RSK3 expression was found to be downregulated in ovarian cancer, and cells overexpressing RSK3 displayed reduced proliferation due to G1 arrest and increased apoptosis. At the moment, the mechanisms by which RSK3 negatively regulates cell proliferation are still unknown. The role of RSK4 on proliferation is even more enigmatic. As for RSK3, several lines of evidence suggest that RSK4 behaves as a negative regulator of cell proliferation. Exogenous expression of RSK4 resulted in decreased breast cancer cell proliferation and increased accumulation of cells in G0/G1 phase of the cell cycle [134]. In addition, RSK4 was found to play an inhibitory role during embryogenesis by suppressing RTK (receptor tyrosine kinase) signalling [135], as well as to participate in p53-dependent cell growth arrest [136] and in oncogene-induced cellular senescence [137]. Clearly, RSK3 and RSK4 appear to play separate functions to their brethren, and more in-depth studies will be required to fully understand the underlying mechanisms.

**Cell growth and protein synthesis**

The first evidence linking RSK to translational control came from the identification of RSK1 as an *in vitro* ribosomal protein (rp) S6 kinase [15, 18]. However, S6K1 and S6K2 were later shown to be the predominant rpS6 kinases operating in somatic cells [20]. A few years later, RSK was found to associate with polyamines [138], which are ribosomes that are actively translating mRNAs. More recently, studies using *S6k1−/− S6k2−/−* [22] or rapamycin-treated [23] cells demonstrated that the RSK isoforms participate in rpS6 phosphorylation *in vivo*. Upon Ras/MAPK pathway activation, RSK1/2-mediated phosphorylation of rpS6 at both Ser235 and Ser236 was found to promote assembly of the cap-binding complex and to correlate with increased cap-dependent translation [23]. These findings strongly support RSK as an important regulator of mRNA translation that provides an additional oncosine- and mitogen-regulated input into the regulation of translation initiation. These data also indicate that rpS6 phosphorylation can occur in an mTOR-independent manner.

mTOR is an essential regulator of ribosome biogenesis, mRNA translation and cell growth, and its activity is controlled by several growth-regulating pathways. Activated RSK promotes mTOR signalling through the phosphorylation of TSC2 on Ser795, which prevents its GAP (guanine nucleotide-activating protein) activity towards the small GTPase Rheb [139–141]. More recently, RSK was shown to phosphorylate Raptor, an important mTORC1 (mTOR complex 1) scaffolding protein, providing another link between the Ras/MAPK and mTOR signalling pathways [142]. It is becoming apparent that certain cell types are more dependent on RSK activity for the regulation of mTOR signalling than others, such as cancer cells harbouring activating mutations in components of the Ras/MAPK pathway (P. P. Roux, unpublished work).

RSK may also regulate mRNA translation through the phosphorylation of GSK3β [113, 138]. Phosphorylation by RSK on Serθ inhibits GSK3β kinase activity and thereby releases inhibition of the translation-initiation factor eIF2B [143]. Interestingly, activated GSK3β and the LKB1-activated kinase AMPK also stimulates TSC2 activity [144, 145], suggesting that RSK regulates TSC2 directly or indirectly. In addition, RSK phosphorylates eEF2K [146] and the translation-initiation factor eIF4B [147]. RSK-mediated phosphorylation of eIF4B stimulates its recruitment to the translation-initiation complex and thereby probably promotes translation of mRNAs encoding proteins involved in cell growth and survival [148]. These findings also suggest that RSK may control several biological processes by altering the pattern of mRNA translation. Recently, RSK was found to play a role in mRNA translation mediated by type III IFN (interferon) (IFNλ) receptors, a novel family of cytokines involved in certain viral infections and malignancies [149]. An inactive form of RSK1 was found to associate with 4E-BP1 (eIF4E-binding protein 1), a protein that binds eIF4E and thereby prevents initiation of mRNA translation. IFNλ-mediated activation of RSK1 was found to promote 4E-BP1 phosphorylation on Thr37/Thr46, thereby promoting selective cap-dependent translation of growth-inhibitory genes whose transcription is induced during engagement of the IFNλ receptor [149].

**Cell survival**

RSK1 and RSK2 have been shown to positively regulate cell survival through transcription-dependent mechanisms. RSK2 was shown to promote survival of primary neurons by increasing CREB-dependent transcription of survival-promoting genes, including Bcl-2, Bcl-xL and Mcl1 (myeloid cell leukaemia sequence 1) [84, 150]. More recently, RSK1 was found to promote survival through the activation of the transcription factor NF-κB [99, 100, 151]. RSK also promotes cell survival...
through post-translation mechanisms. Indeed, RSK1-mediated phosphorylation of C/EBPβ (CCAAT/enhancer-binding protein β) promotes survival of hepatocytic stellate cells in response to the hepatotoxin CCl4 [152]. Phosphorylation on Thr217 was suggested to create a functional XEVD caspase-inhibitory box that binds and inhibits caspases 1 and 8. In addition, RSK1 and RSK2 phosphorylate the pro-apoptotic protein Bad on Ser112, thereby enhancing its ability to bind, and be inactivated by, cytosolic 14-3-3 proteins [150,153]. More recently, RSK1 and RSK2 were shown to inactivate DAPK. DAPK behaves as a tumour suppressor and its expression is commonly silenced in cancer through DNA methylation [154]. RSK-mediated phosphorylation on Ser299 was found to prevent DAPK pro-apoptotic activity, resulting in increased cell survival in response to mitogenic stimulation [155].

**Cell migration**

A potential role for RSK in cell migration was first suggested by the identification of the cytoskeleton-associated protein filamin A as a phosphorylation substrate of both RSK1 and RSK2 [156]. Indeed, these RSK isoforms were suggested to play a role in membrane ruffling by phosphorylating filamin A on Ser152 [156], a site previously shown to be phosphorylated by PAK1 (p21-activated kinase-1) and to be necessary for membrane ruffling in response to PAK1 activation [157]. More recently, RSK1/2 were identified as key effectors of Ras/ERK-mediated EMT (epithelial–mesenchymal transition) [80]. RSK1/2 were found to stimulate motility and invasion by activating a transcriptional programme that co-ordinately modulates the extracelluar environment, the intracellular motility apparatus and receptors mediating communication between these compartments. Therefore RSK1 and RSK2 were shown to be important ERK1/2 effectors of Raf1-dependent migration in MDCK cells [80]. Accordingly, a genome-wide RNAi screen highlighted a prominent role for RSK as a common effector for multiple migratory stimuli [158]. Pharmacological inhibitors of all RSK isoforms were used in this study, suggesting that several RSK isoforms could be involved in the migratory phenotype [158]. More recently, RSK was shown to phosphorylate the SH3 (Src homology 3) domain-containing protein SH3P2 on a residue that inhibits its function [159]. This protein appears to be a negative regulator of cell motility, indicating a new mechanism by which RSK activation promotes cell migration.

**Other substrates and functions**

In addition to their role in cell growth, proliferation, survival and migration, the RSK isoforms have been shown to phosphorylate many more substrates involved in diverse cellular processes. Indeed, RSK2 was linked to neurite outgrowth, since it phosphorylates the cell adhesion molecule L1 on Ser105 [160], a protein that becomes hyperphosphorylated during periods of high neuronal activity. RSK may also be involved in the regulation of nitric oxide function in the brain, since EGF exposure of hippocampal and cerebellar neurons leads to RSK1-mediated phosphorylation on Ser47 of nNOS [neuronal NOS (nitric oxide synthase)] and inhibition of NOS activity [161]. Activated RSK2 was shown to phosphorylate NHE-1 (Na+/H+ exchanger isofrom-1) on Ser70 [162], thereby regulating mitogen-dependent Na+/H+ exchange and intracellular pH [162].

RanBP3 (Ran-binding protein 3) phosphorylation by RSK modulates nucleocytoplasmic protein transport. Both RSK and Akt phosphorylate Ser8 of RanBP3, and this phosphorylation event was shown to contribute to the formation of a Ran gradient essential for nucleocytoplasmic transport, kinetochore function, spindle assembly, microtubule dynamics and other mitotic events [163]. RSK-mediated phosphorylation of the transcription/translation factor YB-1 (Y-box binding protein-1) on Ser102 promotes its nuclear accumulation [164], suggesting that RSK signalling may contribute to the oncogenic functions of YB-1. RSK has been shown to phosphorylate AS160 (Akt substrate of 160 kDa), a protein implicated in the translocation of GLUT4 (glucose transporter 4) to the plasma membrane in response to insulin [165]. Deregulation of GLUT4 translocation occurs early in the pathophysiology of insulin resistance and Type 2 diabetes, providing a potential mechanism by which insulin signalling is altered.

In addition, accumulating evidence suggests the existence of a RSK-initiated negative-feedback loop that restricts activation of the Ras/ERK signalling cascade. More than a decade ago, RSK2 was shown to phosphorylate SOS (son of sevenless) in vitro, a guanine-nucleotide-exchange factor for Ras [166]. Although these results suggested that RSK may inhibit SOS function and thereby reduce Ras/ERK pathway activation, the impact of SOS phosphorylation and the exact sites of phosphorylation have not been addressed. Interestingly, a subsequent study demonstrated increased ERK1/2 phosphorylation in skeletal muscle tissue from RSK2-deficient mice [167], suggesting that RSK is indeed implicated in a feedback loop that limits ERK1/2 activation. More recently, inhibition of RSK using the BI-D1870 inhibitor was shown to increase ERK1/2 phosphorylation in several cell types [76], suggesting that a normal function of RSK may be to prevent hyperactivation of the MAPK pathway.

**Physiological functions**

The involvement of the RSK isoforms in physiological functions was highlighted by the finding that inactivating mutations in the Rps6ka3 gene (which encodes RSK2) were the cause of CLS [168]. CLS is an X-linked mental retardation syndrome characterized in male patients by severe psychomotor retardation, and facial, hand and skeletal malformations [169]. Female patients are usually mildly and variably affected, and thus more difficult to diagnose. In addition, individuals with CLS consistently displayed markedly reduced total brain volume, with the cerebellum and hippocampus being particularly affected [170]. Rps6ka3 mutations are heterogeneous and most often result in truncated forms of RSK2 that are devoid of phosphotransferase activity [171]. RSK2-knockout mice have been generated and extensively studied. These mice exhibit deficient learning and cognitive functions, as well as poor coordination compared with wild-type littermates, suggesting that RSK2 may be required for proper neuronal development and function [167,172]. Consistently, a recent study demonstrated that shRNA (small hairpin RNA)-mediated RSK2 depletion perturbs the differentiation of neural precursor cells into neurons and maintains them as proliferating radial precursor cells [173]. Although these findings indicated that other RSK isoforms do not compensate for the loss of RSK2, the developmental role of RSK2 in the nervous system remains unknown and will require further investigation. Deletion of the single Drosophila RSK isoform leads to learning and conditioning defects [174], but whether these defaults result from the specific loss of RSK activity or deregulated ERK activation or function remains to be determined. RSK2-deficient mice also develop osteopenia, a progressive skeletal disease caused by cell-autonomous defects in osteoblast activity [91]. Findings have suggested that the ATF4
and c-Fos transcription factors may be in vivo RSK2 substrates responsible for osteosarcoma development [91,107]. Accordingly, ATF4 deletion in the mouse was found to partly phenocopy the loss of RSK2 [107]. In addition, RSK2-knockout mice are approximately 15% smaller than their wild-type littermates, with a specific loss of white adipose tissue that is accompanied by reduced serum levels of the adipocyte-derived peptide, leptin [175]. Although RSK1/RSK2/RSK3 triple-knockout mice are viable, no other information regarding their phenotype has yet been reported [176]. The Rps6ka6 gene, which encodes RSK4, is located on chromosome X and was suggested to be involved in non-specific X-linked mental retardation [48]. Recent evidence suggests that Rps6ka6 deletion perturbs early embryonic development [177], but further experiments will be required to validate and characterize these preliminary results concerning the role of RSK4 during development.

CONCLUDING REMARKS

On the basis of recent advances in our understanding of its biological functions, RSK is emerging as a multifunctional effector of the Ras/MAPK signalling cascade. These discoveries have been facilitated by the use of small-molecule inhibitors and RNAi, which have expanded the repertoire of biological functions linked to the RSK family. As critical regulators of several substrates involved in cell growth, proliferation, survival and motility, it is becoming increasingly important to address the role of individual RSK isoforms in these biological processes. Towards this end, the development of knockout animals for the other RSK isoforms will probably help to resolve their exact biological and physiological functions. Recently, a large-scale proteomics approach identified a large number of potential RSK substrates [178], and such high-throughput approaches will help define the exact targets and/or partners specific to each RSK isoform.

Considering the growing number of cancers that exhibit deregulated RSK expression and/or activity, the roles played by each RSK family member in human diseases will also require further investigation. In addition, the discovery of RSK-specific inhibitors [74] will allow the testing of RSK as a potential therapeutic target in pre-clinical cancer studies. Research in this area will hopefully expand our knowledge of RSK signalling in cancer and reveal novel therapeutic approaches for treatment. Indeed, targeting RSK in cancer may be a valuable and more specific alternative to the inhibition of upstream components of the Ras/MAPK pathway [179], such as MEK1/2, but this interesting hypothesis will have to be directly evaluated.

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The RSK family of protein kinases


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