Bacterial expression, purification and in Vitro phosphorylation of full-length ribosomal S6 kinase 2 (RSK2)

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Abstract

Ribosomal S6 kinases (RSK) play important roles in cell signaling through the mitogen-activated protein kinase (MAPK) pathway. Each of the four RSK isoforms (RSK1-4) is a single polypeptide chain containing two kinase domains connected by a linker sequence with regulatory phosphorylation sites. Here, we demonstrate that full-length RSK2 - which is implicated in several types of cancer, and which is linked to the genetic Coffin-Lowry syndrome - can be overexpressed with high yields in Escherichia coli as a fusion with maltose binding protein (MBP), and can be purified to homogeneity after proteolytic removal of MBP by affinity and size-exclusion chromatography. The purified protein can be fully activated in vitro by phosphorylation with protein kinases ERK2 and PDK1. Compared to full-length RSK2 purified from insect host cells, the bacterially expressed and phosphorylated murine RSK2 shows the same levels of catalytic activity after phosphorylation, and sensitivity to inhibition by RSK-specific inhibitor SL0101. Interestingly, we detect low levels of phosphorylation in the nascent RSK2 on Ser386, owing to autocatalysis by the C-terminal domain, independent of ERK. This observation has implications for in vivo signaling, as it suggests that full activation of RSK2 by PDK1 alone is possible, circumventing at least in some cases the requirement for ERK.

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