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**EXPRESSION OF VACCINIA VIRUS PROTEIN K3L_p IN
YEAST INHIBITS eIF-2 KINASE GCN2 AND THE GENERAL
CONTROL PATHWAY**

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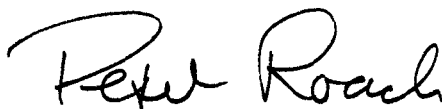
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Abstract

Phosphorylation of the α subunit of eukaryotic initiation factor-2 (eIF-2) is a well characterized mechanism regulating total protein synthesis in mammalian cells. In the yeast *Saccharomyces cerevisiae*, phosphorylation of eIF-2 α has also been shown to regulate translation, although this mechanism is adapted to control gene-specific expression. Viral and cellular proteins have been identified that regulate the activity of the eIF-2 α kinases. An example of such a regulatory protein is the K3Lp from vaccinia virus. K3Lp is homologous to the amino terminal portion of eIF-2 α and is thought to inhibit the activity of the double-stranded-RNA-dependent kinase, PKR, suppressing the antiviral mechanism mediated by this kinase. I investigated whether K3Lp can inhibit the activity of the yeast eIF-2 α kinase GCN2. Expression of K3Lp in yeast reduced the level of eIF-2 α phosphorylation by GCN2. This reduction in phosphorylation blocked the ability of yeast cells to stimulate the general amino acid control pathway in response to starvation conditions. Accompanying in vitro studies showed that recombinant K3Lp reduced GCN2 autophosphorylation and phosphorylation eIF-2 α . To assess the importance of the lysine-45 residue in K3Lp that aligns with the phosphorylation site of eIF-2 α , I expressed and purified mutant forms of recombinant K3Lp in which this residue was altered to serine or glutamate. Both mutant versions of K3Lp inhibited GCN2 phosphorylation of eIF-2 α in vitro, although a greater concentration of K3L-K45Ep was required to block GCN2 activity compared to wild-type or K3L-K45Sp proteins. In agreement with the hypothesis that K3Lp inhibits eIF-2 α kinases by functioning as a pseudosubstrate, I observed that K3Lp fused to a GST-tag directly interacted with GCN2 kinase and

sequences in the catalytic domain of GCN2 mediated this protein-protein contact. Together, these results indicate that K3Lp is a specific inhibitor of eIF-2 α kinases from mammals and yeast and suggest that the kinases contain common structural features important for recognition of their substrate eIF-2 α . These studies also illustrate the versatility of the yeast model system to study in vivo the inhibitors of eIF-2 α kinases.

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