

## Identification of sites phosphorylated by the vaccinia virus B1R kinase in viral protein H5R

### ABSTRACT

We have identified the first phosphorylation site for the vaccinia virus B1R protein kinase. This gives important information about the substrate-specificity of the enzyme, which differs from that of other known protein kinases. It remains to be seen whether the same site is phosphorylated in vivo.

### INTRODUCTION

Background Vaccinia virus is a large DNA virus that replicates in the host cell cytoplasm in granular sites called viroosomes. It encodes at least two protein kinases belonging to the cellular family of serine/threonine protein kinases, the products of the B1R and F10L genes. The F10L kinase is encapsidated in the virion and plays an essential role in virion morphogenesis. The B1R protein kinase is expressed early in infection, is found in the viroosomes, and is also packaged into virions. It appears to be an essential viral protein, and temperature-sensitive mutations that map to the B1R gene produce virus that cannot replicate its DNA at the restrictive temperature. The B1R kinase does not appear to have a broad substrate specificity, and, although it has some activity against the acidic protein, casein, this is a poor substrate compared with the enzyme's known physiological substrates. Three proteins which become phosphorylated during infection of cells with vaccinia virus have been shown to be substrates of the B1R protein kinase in vitro. Two of these are the ribosomal proteins Sa and S2 and the third is the product of the H5R open reading frame of the vaccinia virus genome. The fact that the B1R kinase phosphorylates these proteins in vitro does not, of course, prove that it is responsible for their phosphorylation during infection by virus in vivo. However, one piece of evidence consistent with this possibility is that all these proteins have multiple phosphorylation sites predominantly involving threonine (rather than the more usual serine) residues, and it is threonine residues on the proteins that the B1R kinase phosphorylates in vitro. In the case of protein H5R - the subject of the current work - there is further reason to believe that the B1R kinase contributes to the phosphorylation in vivo. In a mutant strain of virus, temperature-sensitive for B1R, the proportion of underphosphorylated H5R decreases at the restrictive temperature (G. Beaud and R. Beaud, unpublished). In cells infected with a temperature-sensitive mutant of the B1R gene, the proportion of underphosphorylated H5R protein decreases at the restrictive temperature, showing that the B1R protein kinase controls the phosphorylation state of the H5R protein synthesized at the early stage of vaccinia virus infection. It has recently been shown that most of the H5R protein is found in viroosomes, although some of the more highly phosphorylated forms of the protein appear to be cytoplasmic, suggesting multiple roles in vaccinia virus development. Kovacs and Moss have demonstrated that the H5R protein is, in fact, equivalent to the late stage-specific transcription factor VLTF-4, and Black et al. have showed that it associates with protein G2R, a putative late transcription elongation factor. In contrast, studies of a dominant temperature-sensitive mutant of H5R by DeMasi and Traktman suggest a role in virion morphogenesis. A knowledge of the phosphorylation sites on the H5R protein is needed to test whether phosphorylation has a role in either of these processes, and we have made the first steps in this direction by identifying two threonine residues in the protein that are substrates for the B1R protein kinase.

## CONCLUSION

Conclusions Vaccinia virus protein kinase B1R phosphorylates the virus protein H5R in vitro at the threonine residues Thr-84 and Thr-85 within the region: EEYHQTTEKNSP. A synthetic peptide based on this sequence also acted as a substrate. We conclude that this sequence determines, at least in part, the substrate specificity of the vaccinia B1R protein kinase, although it is unclear which amino acid residues are the key determinants within this sequence. There are other phosphorylation sites for the kinase on protein H5R, but these remain to be determined.