

**VIRAL AND CELLULAR KINASES PLAY A CENTRAL ROLE IN VZV  
PATHOGENESIS AND ARE TARGETS FOR ANTIVIRAL KINASE  
INHIBITORS**

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Humans have co-evolved with herpesviruses, leading to finely tuned interactions between virus and cell proteins. Varicella zoster virus (VZV) encodes 70 genes; the function of some is to manipulate the cellular environment to favor virus replication at the expense of the host. During primary infection, VZV causes varicella, or chicken pox, and replicates in epithelial cells, T cell, neurons, and skin. Lifetime latency is established in ganglia and reactivation leads to zoster, a painful condition also known as shingles. VZV infects mainly non-dividing cells, thus the virus must induce the cellular DNA synthesis machinery it needs to replicate its own genome. Numerous viral and cellular enzymes, few of which have been identified, accomplish this. It is known that certain viral kinases are required for infection of T cells and skin, that phosphorylated viral glycoproteins are also essential for VZV growth *in vivo*, and that cyclin-dependent kinases are dysregulated in VZV-infected cells. A mouse model of VZV pathogenesis that consists of human thymus and skin tissues implanted in immunodeficient mice (SCID-hu) has been invaluable for studying the role of VZV kinases and glycoproteins. This model will also be important for testing new antiviral compounds that target viral and cellular kinases.

VZV encodes two serine-threonine protein kinases that are essential for replication *in vivo* but are not required for growth in cultured cells. ORF47 kinase is related to HSV-1 UL13 kinase and both viral proteins have kinase motifs with limited homology to casein kinase II. ORF47 has been cloned and characterized, and its viral substrates include two important transactivators and an essential glycoprotein, gE [1,2]. Cellular targets are not known but probably exist. Studies in SCID-hu mice show that VZV mutants that lacked ORF47 were unable to replicate in T cells or skin [3]. This is in contrast to ORF66 that was not required in skin but was important for T cell infectivity. ORF66 plays a key role in phosphorylating the major viral transactivator which determines its intracellular localization and packaging into the virion [4,5]. The closest cellular homologue of ORF66 is cyclin-dependent kinase 1 (cdk1 or cdc2).

Phosphorylation by cellular kinases is necessary for the function of two VZV glycoproteins, gE and gI, that form a heterodimer in infected cells. Interestingly, the cytoplasmic tail of gI is phosphorylated by cdk1 and this activity can be inhibited by roscovitine, a cdk inhibitor [6]. An unanswered question is whether ORF66 also phosphorylates gI. The cytoplasmic tail of gI is necessary for proper

envelopment of virus capsids by golgi-derived vesicles [7]. This may explain why VZV mutants lacking gI were unable to replicate in skin or T cells in the SCID-hu model [8]. VZV gI is necessary for normal trafficking of its partner, gE [9,10]. Movement of gE from the transgolgi to the plasma membrane, endocytosis, and return to the transgolgi are mediated by sites on the cytoplasmic tail. There is a consensus site for casein kinase II near the C-terminus of gE that is phosphorylated by both CKII and ORF47 [1]. In this assay, CKII was inhibited by DRB (5,6-dichloro-1- $\beta$ -D-ribofuranosyl-benzimidazole) while ORF47 was not; neither kinase was inhibited by roscovitine (personal communication). Studies are underway to learn whether deletion of the CKII consensus site in the gE tail will have an effect on gE trafficking and virus replication in the SCID-hu model.

When, for example, VZV infects fibroblast cells in the skin, it encounters cells that are in G1/0 phase of the cell cycle. In these quiescent cells, cyclin-dependent kinase 2 and its partner cyclin E are absent. Soon after infection with VZV, there is a dramatic induction of cdk2 and cyclin E. We will soon know if the increase in protein levels leads to a corresponding increase in kinase activity. Active Cdk2/cycE is required for the cell to cross the restriction point between G1/0 and S phase. It is during S phase that the cell has abundant nucleotides and enzymes used for DNA synthesis. A related herpesvirus, human cytomegalovirus, also induces cdk2/cycE activity, rendering the cell a fruitful place for viral DNA replication [11-13]. It will be important to determine how VZV alters the levels and activities of other cell cycle regulatory proteins, particularly cdk1. These proteins have great potential as new antiviral targets.

Two cdk inhibitors, roscovitine and purvalanol (rosco and purv), have potent antiviral activity against VZV. This supports the idea that cdk activity is important for VZV replication. Experiments in our laboratory indicate that the  $IC_{50}$  of rosco for VZV replication is approximately 12  $\mu$ M by plaque reduction assay. Using Real Time PCR, rosco inhibits VZV genomic DNA synthesis with an  $IC_{50}$  of 14  $\mu$ M. Rosco also causes downregulation of VZV mRNA, suggesting that rosco prevents viral DNA replication by shutting down viral gene expression. Confocal microscopy showed that rosco treatment restricts the expression of three major viral transactivators. The  $IC_{50}$  of purvalanol for VZV replication is 1-2  $\mu$ M. The effects of rosco and purv are reversible, the drugs are not cytotoxic, and they do not cause apoptosis at the levels tested. Numerous attempts to isolate VZV resistant to purv were unsuccessful, while mutants resistant to acyclovir were easily obtainable. In a recently developed human skin explant model, rosco inhibited the growth of VZV at the same levels used in cultured cells. Studies are planned to test the antiviral activity of rosco and purv in the SCID-hu model, using implanted mini diffusion pumps to continuously deliver the drugs. Cdk inhibitors show great promise for new treatments for VZV, especially as topical agents, and these compounds have already been useful tools to reveal the complex interactions between virus and cell proteins.

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