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ScienceDirect
Virology 353 (2006) 220–233
VIROLOGY
www.elsevier.com/locate/yviro

Vaccinia virus K1L protein supports viral replication in human and rabbit cells through a cell-type-specific set of its ankyrin repeat residues that are distinct from its binding site for ACAP2

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Received 9 April 2006; returned to author for revision 9 May 2006; accepted 26 May 2006
Available online 30 June 2006

Abstract

Vaccinia virus (VV) K1L is a host-range gene and encodes a protein comprised of six ankyrin repeats (ANKs). We showed here that a large portion of the K1L protein, except ankyrin repeat 1 (ANK1) and C-terminal halves of ANK2 and ANK3, can be deleted or substituted with an unrelated ANK with no adverse effect on VV replication in human HeLa cells. In contrast, only ANK4 and ANK6 can be mutated without impairing VV replication in rabbit RK13 cells. The growth rate of VV in HeLa cells was reduced differentially by substituting phenylalanine 82 or serine 83 of ANK2 and abolished completely by substituting both residues. These substitutions, however, did not affect K1L's ability to bind ACAP2, a GTPase-activating protein for ARF6. Our data support the hypothesis that surface residues of a few consecutive K1L ANKs mediate the host-range function by interacting with protein factors that are distinct from ACAP2.

Keywords: Poxvirus; Vaccinia virus; K1L; C7L; Host-range; ACAP2; Ankyrin repeat

Introduction

Vaccinia virus (VV) is the prototypical member of the poxvirus family and served as an effective vaccine for smallpox during its global eradication campaign (Moss, 2001). The origin and the natural host of VV are not entirely clear, but the wild-type VV strains are capable of replicating in a very broad range of cell lines of both avian and mammalian origins. VV strains with a more restricted host-range, notably the modified vaccinia virus Ankara (MVA) and NYVAC, have also been derived from the wild-type strains through empirical process or targeted gene deletions (Carroll and Moss, 1997; Tartaglia et al., 1992). MVA and NYVAC replicate efficiently in primary chicken embryo fibroblasts but abortively in most mammalian cell lines. They are highly attenuated in mammalian hosts and considered as a potential safe replacement for the currently licensed smallpox vaccine (Belyakov et al., 2003). They have also been used extensively as the vaccine vectors for other infectious diseases and cancer (Sutter and Staib, 2003). Their immunogenicities, however, are reduced compared to replication-competent VV strains, typically requiring booster doses to be administered to elicit optimal immune responses (Earl et al., 2004; Kaufmann and McMichael, 2005; Spearman, 2006).

For MVA, the genetic basis of its host-range restriction to avian cells is ill-defined (Wyatt et al., 1998), but, for NYVAC, the targeted deletion of two VV genes, K1L and C7L, is presumably the cause. VV mutants with deletion in both K1L and C7L genes replicate abortive in most human cell lines and the rabbit kidney RK13 cells (Drillien et al., 1981; Gillard et al., 1986; Perkus et al., 1990). The replication defect of the mutants in human cells can be complemented by either K1L or C7L or the cowpox virus CP77 gene, while the defect in RK13 cells can be complemented by either K1L or CP77 but not by C7L (Gillard et al., 1986; Perkus et al., 1990; Ramsey-Ewing and Moss, 1996). The host restriction of the mutants occurs at the translation of intermediate stage viral mRNA in human HeLa cells (Hsiao et al., 2004), while it occurs at the translation of early stage viral mRNA in RK13 cells (Ramsey-Ewing and Moss, 1996). However, the exact nature of the host restriction in

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doi:10.1016/j.virol.2006.05.032

nonpermissive cells and the mechanism by which CP77, K1L or C7L overcome the host restriction remain elusive today (McFadden, 2005).

Both CP77 and K1L contain multiple ankyrin repeats (ANKs), a common 33-residue protein motif that has been found in proteins as different as cytoskeletal organizers, cyclin-dependent kinases (CDK) inhibitors and signal transduction and transcriptional regulators (Mosavi et al., 2004; Sedgwick and Smerdon, 1999). ANK represents a structural scaffold for mediating protein–protein interactions (Sedgwick and Smerdon, 1999). The three-dimensional structures of the ANKs-containing proteins show very regular and conserved secondary and tertiary structure. Each repeat forms a structural unit, which consists of a β-hairpin, two antiparallel α-helices followed by a loop. The adjacent repeats are packed via hydrophobic interactions to form an elongated L shape structure with a large solvent-accessible surface. Despite this conserved structures, different ANKs-containing proteins interact specifically with their binding partners by containing variable surface residues in the repeats and by stacking different numbers of the repeats in one protein (Sedgwick and Smerdon, 1999).

Based on the studies of cellular ANKs-containing proteins, we hypothesized that the surface residues of a few consecutive ANKs of K1L mediate the host-range function by interacting with protein factors. To test our hypothesis and gain some insight into the molecular basis of the host-range gene function, we systematically examined all K1L ANKs for their contributions towards VV replication in both human HeLa cells and rabbit RK13 cells and towards the binding with ACAP2, the human homolog of a rabbit protein previously identified to bind K1L (Bradley and Terajima, 2005). Our data support the hypothesis that surface residues of a few consecutive K1L ANKs mediate the host-range function by interacting with protein factors that are distinct from ACAP2. It also demonstrates that the growth rate of VV vectors in human cells can be modulated by genetic engineering of the host-range genes.

Results

Construction of K1L mutant VVs

VV K1L and C7L provide an equivalent function for VV to replicate productively in many human cell lines. Therefore, to assess the effect of K1L mutations on VV replication in human cell lines as well as rabbit RK13 cells, we constructed a recombinant VV with neither K1L nor C7L genes (named vK1L$^-$C7L$^-$) as the parental virus for all the K1L mutants. As expected from previous studies of similar mutants (Perkus et al., 1990), vK1L$^-$C7L$^-$ replicated abortively in both RK13 and human HeLa cells but robustly in monkey VERO cells (data not shown). Restoring to vK1L$^-$C7L$^-$ the coding sequences for a K1L with a C-terminal V5 epitope tag and an adjacent green fluorescence protein (GFP) also restores the normal host-range of the virus (referred as vK1L-WT or WT hereafter). The epitope-tagged K1L protein (K1L-V5) was expressed from the natural K1L promoter by vK1L-WT and displayed the kinetics of synthesis of VV early gene (Fig. 1). The GFP was expressed from the widely used P11 promoter, which normally regulates

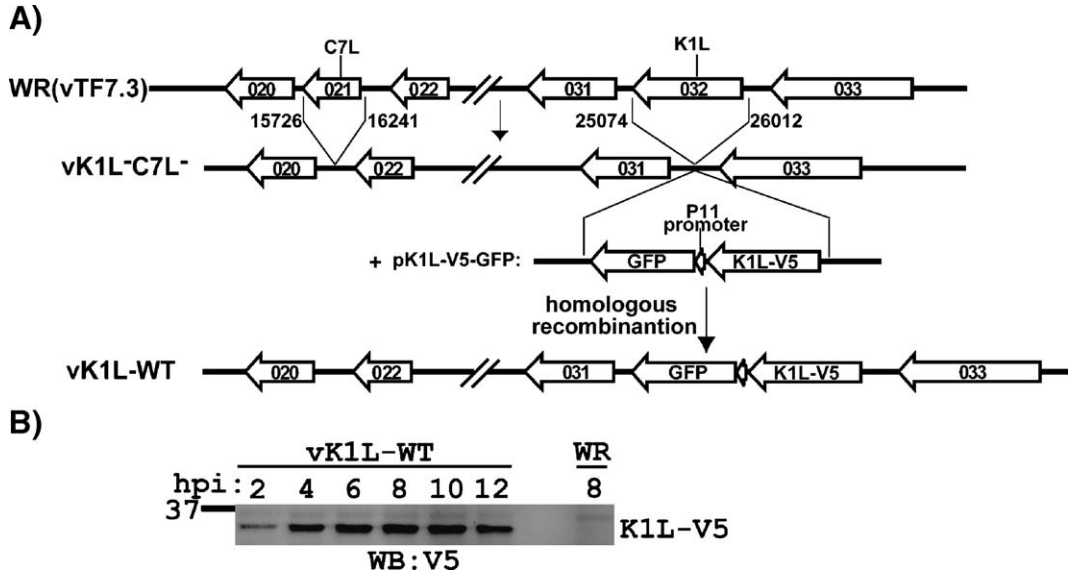

Fig. 1. Construction of recombinant VVs that depend on K1L's host-range function for replication in RK13 and human cell lines. (A) Schematic illustration of the construction and genomic organization of vK1L$^-$C7L$^-$ and vK1L-WT. vK1L$^-$C7L$^-$ was constructed by sequentially removing the K1L and C7L genes from VV strain vTF7.3 with the transient dominant selection method. The exact positions of the deletions are indicated by the corresponding nucleotide numbers of the complete WR genome sequence (accession number AY243312). The neighboring WR ORFs are indicated by their numerical nomenclature only. vK1L-WT was constructed by homologous recombination of vK1L$^-$C7L$^-$ and the plasmid pK1L-V5-GFP, which encodes the K1L with a C-terminal V5 epitope tag and the green fluorescence protein (GFP). P11 indicates the VV promoter that normally regulates late expression of the gene encoding the 11K protein (WR F18R). (B) The temporal expression of K1L proteins by vK1L-WT in RK13 cells. RK13 cells were infected with vK1L-WT or wild-type WR at a MOI of 10 PFU per cell. At the indicated time after infection (hpi), the level of K1L proteins was determined by Western blot with a monoclonal antibody against the V5 epitope tag. The same amount of total proteins as determined by Bradford protein assay was analyzed. The size of the molecular weight marker in kilodaltons is shown on the left.