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Barrier to autointegration factor (BAF) inhibits vaccinia virus intermediate transcription in the absence of the viral B1 kinase

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ABSTRACT

Barrier to autointegration factor (BAF/BANF1) is a cellular DNA-binding protein found in the nucleus and cytoplasm. Cytoplasmic BAF binds to foreign DNA and can act as a defense against vaccinia DNA replication. To evade BAF, vaccinia expresses the B1 kinase, which phosphorylates BAF and blocks its ability to bind DNA. Interestingly, B1 is also needed for viral intermediate gene expression via an unknown mechanism. Therefore, we evaluated the impact of B1-BAF signaling on vaccinia transcription. Strikingly, the decrease in vaccinia transcription caused by loss of B1 can be rescued by depletion of BAF. The repressive action of BAF is greatest on a viral promoter, and is more modest when non-vaccinia promoters are employed, which suggests BAF acts in a gene specific manner. These studies expand our understanding of the role of the B1 kinase during infection and provide the first evidence that BAF is a defense against viral gene expression.

Introduction

Vaccinia virus is the prototypical member of the large DNA virus family Poxviridae. Like all poxviruses, vaccinia exhibits significant autonomy from the host cell and carries out its entire lifecycle in the cytoplasm. The fact that vaccinia virus is capable of both DNA replication and transcription in this location is unique among DNA viruses, and is achieved by a large repertoire of virus-encoded proteins including multisubunit DNA and RNA polymerases and multiple accessory factors (Ahn et al., 1990; Amegadzie et al., 1991; Challberg and Englund, 1979; Condit et al., 1991; Earl et al., 1986; Klemperer et al., 2001; McDonald et al., 1997; Moss et al., 1991). Functional characterization of these proteins over the last three decades has provided broad mechanistic insights into genome replication and transcription, which are of interest to virology and cell biology alike. While poxviruses avoid obstacles such as chromatin which nuclear viruses face, cytoplasmic replication means that they are also challenged by host defenses specific to that environment. In turn, viruses employ countermeasures to inactivate the host defenses which are the most significant threat. Recent studies have revealed that the product of the vaccinia B1R gene is one such countermeasure.

Analysis of temperature sensitive mutant viruses, including the ts2 virus, which contain a point mutation within the B1 ORF (Condit et al., 1983; Rempel et al., 1990; Rempel and Traktman, 1992; Traktman et al., 1989) provided the first evidence of the essential role played by B1 during the vaccinia lifecycle (Banham and Smith, 1992; Lin et al., 1992; Rempel and Traktman, 1992). Subsequent genetic and biochemical analysis of B1 revealed it to be a Ser/Thr kinase essential for viral DNA replication as well as for optimal intermediate transcription (Banham and Smith, 1992; Kovacs et al., 2001; Lin et al., 1992), and also determined that the lesion found in the ts2 virus abrogates B1's kinase activity. The vaccinia B1 protein exhibits significant homology with a family of eukaryotic kinases now referred to as vaccinia-related kinases (VRKs) (Nezu et al., 1997; Nichols and Traktman, 2004). Importantly, Boyle and Traktman discovered that the cellular kinase VRK1, when expressed from the ts2 genome, rescued the DNA replication defect exhibited by this virus (Boyle and Traktman, 2004). This study suggested that B1 and VRK1 both target the same substrate needed to permit genome replication, and laid the foundation for the identification of the barrier to autointegration factor (BAF/BANF1) as a cellular substrate of B1 and VRK1 (Wiebe and Traktman, 2007).

BAF is a highly conserved 10-kDa DNA binding protein found in both the cytoplasm and the nucleus of many cell types, and has been found to be important for cell survival in multiple model systems (Cai et al., 1998; Cox et al., 2011; Furukawa et al., 2003; Lee and Craigie, 1998; Margalit et al., 2005, 2007; Puente et al.,

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2011; Zheng et al., 2000). Specifically, BAF is needed for survival and differentiation of both human and mouse embryonic stem cells (Cox et al., 2011). Additionally, attempts to deplete or knock out BAF in *Caenorhabditis elegans* and *Drosophila melanogaster* resulted in lethal phenotypes early in embryogenesis (Furukawa et al., 2003; Margalit et al., 2007). Recent data has also provided new insight of the importance of BAF in human disease. Specifically, a point mutation within the BAF coding region causes Nestor-Guillermo syndrome, a hereditary progeroid syndrome (Puente et al., 2011).

Extensive in vitro biochemical and structural studies of BAF have advanced our understanding of BAF's function and regulation (Bradley et al., 2005; Haraguchi et al., 2001; Harris and Engelman, 2000; Ibrahim et al., 2011; Margalit et al., 2005; Segura-Totten et al., 2002; Segura-Totten and Wilson, 2004; Umland et al., 2000). BAF binds double-stranded DNA independent of sequence (Lee and Craigie, 1998; Umland et al., 2000; Zheng et al., 2000), but does not bind ssDNA, ssRNA, or dsRNA (Ibrahim et al., 2011; Lee and Craigie, 1998). Through its ability to homodimerize, BAF can cross-bridge DNA to form higher order nucleoprotein complexes (Lee and Craigie, 1998; Zheng et al., 2000). Phosphorylation of BAF by either the viral B1 or cellular VRK1 proteins strongly inhibits BAF's ability to bind DNA, and regulates BAF in both the cytoplasm and nucleus (Nichols et al., 2006). In the nucleus, BAF interacts with LEM-domain proteins, histones, and transcriptional regulators (Dechat et al., 2004; Furukawa, 1999; Haraguchi et al., 2001; Lee et al., 2001; Margalit et al., 2005, Montes de Oca et al., 2005, 2009; Segura-Totten et al., 2002; Segura-Totten and Wilson, 2004; Shumaker et al., 2001). These partners allow BAF to play an important role during nuclear reassembly, and likely allow BAF to modulate gene expression as well. In the cytoplasm BAF is capable of inhibiting viral DNA replication (Wiebe and Traktman, 2007). This host defense activity of BAF depends on its DNA binding and bridging properties and is blocked through phosphorylation in the presence of active B1 kinase (Ibrahim et al., 2011).

In this manuscript, we further explore the importance of the B1-BAF signaling axis during poxviral infection. Specifically, we test the hypothesis that the action of BAF explains why B1 kinase is needed for intermediate transcription as well as DNA replication. We have determined that depletion of BAF from mouse L929 cells rescues the transcriptional defect of the ts2 B1-deficient virus. Interestingly, the impact of BAF is greatest on the viral G8 promoter as compared with other non-vaccinia promoters examined. Thus, our data suggests that while BAF can bind any dsDNA sequences, its ability to repress transcription is specific to the promoter BAF is acting on. Together, these data reveal a novel property of BAF as a transcriptional inhibitor and demonstrate that vaccinia is an excellent model system for future characterization of BAF's function as a regulator of gene expression.

Results

The regulation of viral intermediate gene expression requires the B1 kinase independently of its role in DNA replication

Temperature sensitive vaccinia viruses with lesions in the B1 kinase (ts2 and ts25) display a primary block at the stage of DNA replication at nonpermissive temperature (Condit et al., 1983). The requirement for B1 at this stage is linked to its ability to inactivate the cellular factor BAF via phosphorylation. If left unphosphorylated, BAF relocalizes to viral factories and impairs viral genome replication via its ability to bind and crossbridge DNA (Ibrahim et al., 2011; Nichols et al., 2006; Wiebe and Traktman, 2007). Interestingly, prior to the discovery of the B1-BAF signaling axis, Kovacs et al. have shown that B1 is also required for at least one post replicative stage in the viral life cycle. Specifically, using a plasmid transfection/infection approach to examine intermediate gene expression independent of DNA replication, it was discovered that intermediate promoter activity is impaired during infection with ts25 (Kovacs et al., 2001). However, the mechanism through which B1 contributes to gene expression has yet to be determined.

The goal of this present study was to further examine the role of B1 during vaccinia intermediate gene expression and test the hypothesis that without an active B1 kinase the cellular factor BAF is not only able to impede DNA replication, but viral transcription as well. To test this hypothesis, we first worked to confirm the role of B1 in intermediate gene expression as previously reported (Kovacs et al., 2001). L929 fibroblasts were transfected with plasmids expressing firefly luciferase under the intermediate promoters of the G8R, A2L, I1L genes or a synthetic intermediate promoter based on the consensus sequence described by Yang et al. (2012). These cells were then infected with WT or ts2 viruses at an MOI of 3 and incubated at 37 °C overnight before harvesting and measurement of luciferase activity. A temperature of 37 °C was chosen for our studies because we found that L929 cells are highly sensitive to heat induced stress at 39.7 °C. As we found that multiple temperature-sensitive viruses retain their ts phenotype at 37 °C in L929 cells (see Fig. 2 below), we chose to employ this temperature in our studies to minimize the impact of unknown stress responses on the interpretation of our data. To examine viral intermediate gene expression independent of DNA replication, the nucleoside analog cytosine arabinoside (AraC) was added during the infection, as previous studies have shown that the expression of vaccinia genes from a plasmid introduced into infected cells does not require viral DNA replication (Keck et al., 1990; Vos and Stunnenberg, 1988). As shown in Fig. 1A, expression from pG8-Luciferase (pG8-Luc) is inhibited in ts2-infected cells over 200-fold compared to WT-infected cells. This is in line with the previous observation by Kovacs et al. that the presence of a functional B1 kinase is necessary for the viral intermediate gene expression. Likewise, activation of each of the A2L, I1L, and consensus intermediate promoters was significantly decreased in the ts2 infected cells as compared to WT infected cells, demonstrating that B1 is important for expression of intermediate genes in general. As the pG8-Luc construct exhibited the greatest fold difference between WT and ts2 infections, we focused on it for the remainder of our study to allow for the greatest sensitivity in our assays.

Next, we tested whether the defect in intermediate gene expression of ts2 can be rescued by expression of B1 from the viral TK locus of the ts2 virus. Using recombinant ts2 viruses, Boyle and Traktman demonstrated that expression of B1 from the TK locus of the ts2 genome rescues the DNA replication defect of this virus (Boyle and Traktman, 2004). Using the same virus, we verified that in L929 cells the expression of B1 from the ts2 virus rescued viral yield compared to ts2 alone at either low or high MOI (Fig. 1C), as was previously observed in BSC40 cells (Boyle and Traktman, 2004). We also found that luciferase activity measured in ts2/B1 was 50-fold higher than that found in ts2-infected cells (Fig. 1B). These results support the role of the B1 kinase during viral intermediate gene expression, and demonstrate that expression of B1 from the ts2 genome can both rescue viral yield and enhance viral intermediate gene expression in L929 cells.

BAF affects viral intermediate gene expression in a B1 dependent manner

The above data indicate that the B1 kinase is required not only for DNA replication, but intermediate gene expression as well. To determine whether B1 is unique in functioning at both of these stages of the viral lifecycle, we examined whether intermediate gene expression was decreased during infection with other ts viruses displaying blocks in DNA replication. Specifically, two ts