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Recruitment of host translation initiation factor eIF4G by the Vaccinia Virus ssDNA-binding protein I3

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ABSTRACT

Poxviruses are large double-stranded DNA viruses that replicate exclusively in the cytoplasm of infected cells within discrete compartments termed viral factories. Recent work has shown that the prototypical poxvirus, Vaccinia Virus (VacV) sequesters components of the eukaryotic translation initiation complex eIF4F within viral factories while also stimulating formation of eIF4F complexes. However, the forces that govern these events remain unknown. Here, we show that maximal eIF4F formation requires viral DNA replication and the formation of viral factories, suggesting that sequestration functions to promote eIF4F assembly, and identify the ssDNA-binding protein, I3 as a viral factor that interacts and co-localizes with the eIF4F scaffold protein, eIF4G. Although it did not adversely affect host or viral protein synthesis, I3 specifically mediated the binding of eIF4G to ssDNA. Combined, our findings offer an explanation for the specific pattern and temporal process of eIF4G redistribution and eIF4F complex assembly within VacV-infected cells.

Introduction

Among the poxviruses that can infect humans, Variola Virus (VarV) is the causative agent of smallpox while Vaccinia Virus (VacV) was used as a vaccine in the eradication of smallpox and has become the laboratory prototype for the study of poxvirus infection (Moss, 2007). Both VarV and VacV belong to the *orthopoxvirus* genus, with close genetic, morphological and host range similarities. *Orthopoxviruses* are large, double-stranded DNA viruses whose genomes average 200 kbp, with VacV encoding approximately 200 genes. Poxviruses replicate exclusively in the cytoplasm of infected cells. After cell fusion and entry viral cores traverse the cytoplasm to specific sub-cellular sites that are the precursors of viral DNA synthesis. At early stages these cores rapidly produce and exude mRNAs due to the presence of a complete transcription system for early viral gene expression. As the infection proceeds the virus forms structures termed viral factories or replication compartments in the cytoplasm of the infected cell. Each incoming virus is capable of forming a unique replication compartment and these structures show a remarkable degree of self-sufficiency in terms of replicating viral DNA and transcription of the viral genome.

Despite this impressive compartmentalization and self-sufficiency poxviruses, like all viruses, remain dependent upon the host cell protein synthesis machinery to translate viral mRNAs into proteins (Walsh and Mohr, 2011). Similar to host messages, VacV mRNAs have a 7-Methyl GTP cap at their 5′ end (Boone and Moss, 1977; Martin and Moss, 1975; Venkatesan et al., 1980). Within the host cell a complex of eukaryotic translation initiation factors (eIFs), termed eIF4F plays a critical role in regulating the translation of capped messages ((Sonenberg and Hinnebusch, 2009) and Fig. 1A). eIF4F consists of eIF4E; a small cap-binding protein, eIF4A; an RNA helicase that facilitates ribosomal scanning and eIF4G; a large scaffolding protein on which the complex is built. In addition, polyA-binding protein (PABP) binds both the 3′ end polyA-tail of mRNAs and eIF4G, circularizing and stimulating the translation of mature messages ((Mangus et al., 2003; Sonenberg and Hinnebusch, 2009) and Fig. 1A). Finally, eIF4G also associates with the 40S ribosome through bridging interactions with eIF3 (Hinnebusch, 2006). eIF4F, therefore, plays a central role in recruiting the protein synthesis machinery to fully processed capped mRNAs to initiate their translation.

The assembly of eIF4F complexes is regulated, at least in part, by small eIF4E-binding proteins (4E-BPs). In their hypophosphorylated state, 4E-BPs act as translational repressors by competing with eIF4G for the same binding site on eIF4E (Fig. 1A). Signaling through the mTOR pathway results in 4E-BP phosphorylation, generating electrostatic repulsion that releases eIF4E to allow it to interact with eIF4G (Hara et al., 1997; Sonenberg and Hinnebusch, 2009; von Manteuffel et al., 1997). In contrast to many RNA viruses that inactivate eIF4F function, a growing number of DNA viruses are being found to stimulate the assembly of translation initiation complexes to maximize viral protein production (Arias et al., 2009; Castelló et al., 2009; Kudchodkar et al., 2004; McMahon et al., 2011; Moorman and

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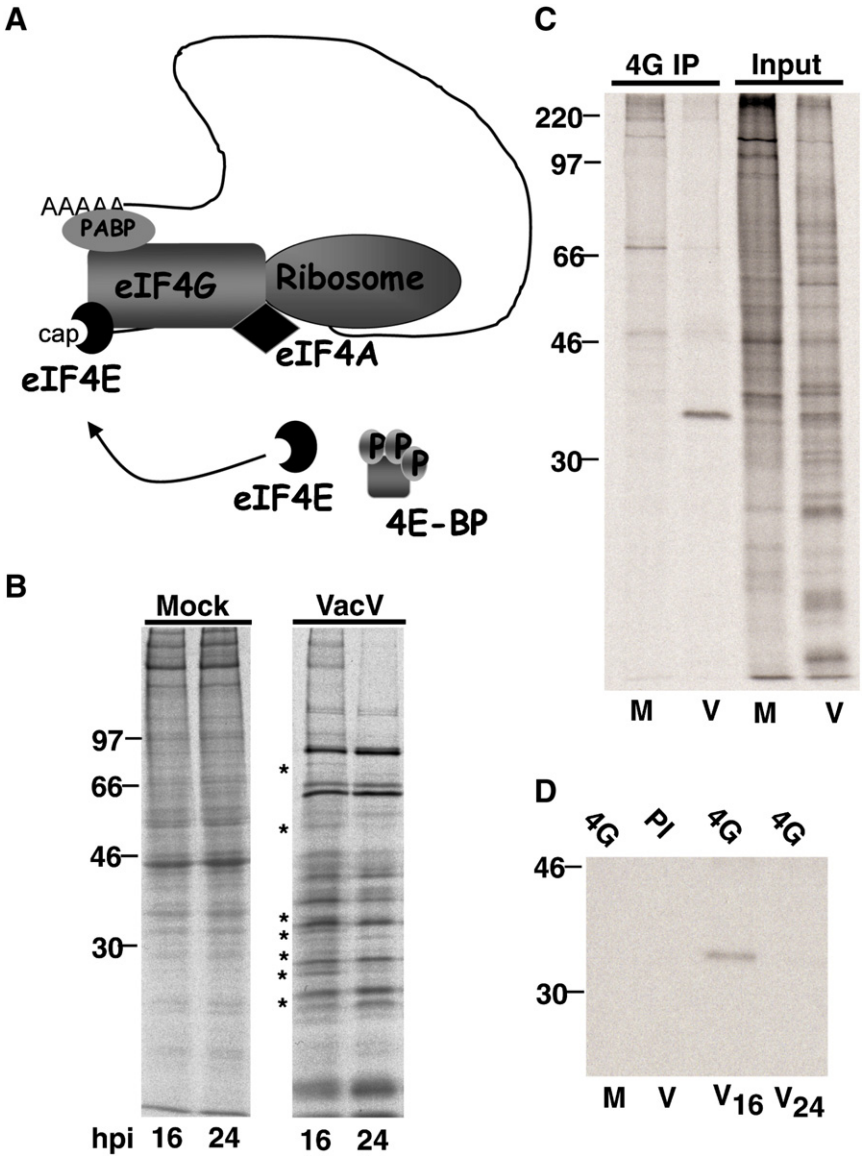

Fig. 1. A polypeptide synthesized in VacV-infected cells associates with eIF4G. (A) Recruitment of ribosomes to capped mRNAs by the translation initiation complex eIF4F. The 5′ 7-Methyl-GTP (cap) of mRNAs is bound by eIF4E, which in turn is bound by the scaffold protein, eIF4G. The RNA helicase, eIF4A also binds eIF4G to facilitate ribosome scanning. PolyA-binding protein (PABP) binds the n-terminus of eIF4G and the polyA-tail, bridging 5′ and 3′ ends of the mRNA. eIF4G indirectly recruits the 40S ribosome through interactions with eIF3. Formation of the eIF4F complex is repressed by small eIF4E-binding proteins (4E-BPs), which competitively inhibit eIF4E binding to eIF4G. In response to various stimuli mTOR signaling phosphorylates 4E-BPs, releasing eIF4E to join the eIF4F complex. (B) Serum starved NHDFs were mock-infected or infected with VacV at m.o.i. 10 for the indicated time in hours post infection (h.p.i.). 1 h prior to sample preparation cultures were metabolically labeled with ^{35}S-methionine/cysteine. Whole cell extracts were resolved by SDS-PAGE and fixed dried gels exposed to x-ray film. Migration of molecular weight markers (in kDa) is indicated to the left. Asterisks indicate proteins differentially-expressed at intermediate stage infection. (C) Serum starved NHDFs were mock-infected (M) or infected (V) at m.o.i. 5 for 15 h then metabolically labeled for 1 h. Soluble cell extracts were prepared and immunoprecipitated with anti-eIF4G antiserum. Immune-complex and input samples were resolved by SDS-PAGE and fixed, dried gels were exposed to x-ray film. Migration of molecular weight standards in kDa is indicated to the left. (D) Serum starved NHDFs were infected as described in C and metabolically labeled soluble cell extracts were prepared at 16 or 24 h.p.i. then immunoprecipitated with pre-immune serum (PI) or anti-eIF4G (4G) antiserum, as indicated. Immune complexes were resolved by SDS-PAGE and fixed, dried gels exposed to x-ray film. Migration of molecular weight markers in kDa is indicated to the left.

Shenk, 2010; O'Shea et al., 2005; Walsh, 2010; Walsh and Mohr, 2004; Walsh et al., 2005, 2008). Distinct from other mammalian DNA viruses, the *Poxviridae* and *Asfarviridae*, of which African Swine Fever Virus (ASFV) is the sole member, replicate exclusively in the cytosol of infected cells. Recent work has shown that in addition to stimulating eIF4F assembly both VacV and ASFV cause the redistribution of host translation factors to viral replication compartments (Castelló et al., 2009; Katsafanas and Moss, 2007; Walsh et al., 2008). How this rearrangement of the host translation system is accomplished remains unknown. Here, we show that eIF4F assembly is driven by events linked to viral DNA synthesis and factory formation and describe the identification of a VacV-encoded factor that associates with the eIF4F scaffold protein, eIF4G and recruits it to ssDNA.

Results

Identification of a VacV protein that binds eIF4G

Transformed or immortalized cell lines are frequently metabolically hyperactive, containing high basal levels of eIF4F and highly activated regulatory kinases that can mask the ability of some viruses to manipulate their activity. In contrast, the lowered metabolic state of serum-starved primary cells offers a means to study the ability of viruses to stimulate their host's translation system (Kudchodkar et al., 2004; Walsh and Mohr, 2004, 2006; Walsh et al., 2005), including both the redistribution and assembly of eIF4F complexes during VacV infection and the potential connections between these two