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Article Addendum

NSP-interacting GTPase

A cytosolic protein as cofactor for nuclear shuttle proteins

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Despite the significant progress in the identification of essential components of the nuclear transport machinery, some events of this process are still unclear. Particularly, functional information about the release of nuclear-exported macromolecules at the cytoplasmic side of the nuclear pore complex and their subsequent trans-cytoplasmic movement is lacking. Recently, we identified a cytoplasmic GTPase, designated NIG (NSP-interacting GTPase), which may play a relevant role in these processes. NIG interacts in vivo with the geminivirus NSP and promotes the translocation of the viral protein from the nucleus to the cytoplasm where it is redirected to the cell surface to interact with the viral movement protein, MP. Here we position the NIG function into the mechanistic model for the intracellular trafficking of viral DNA and discuss the putative role of NIG in general cellular nucleocytoplasmic transport of nucleic acid-protein complexes.

Introduction

In eukaryotic cells, bidirectional exchange of macromolecules between the nucleus and cytoplasm is mediated by receptors (exportin and importin) and occurs exclusively through nuclear pore complexes (NPCs). The regulated and selective communication between these organelles enables eukaryotic cells to separate transcription from translation as well as components of signaling pathways providing additional strategies to control gene expression and adaptive responses.[1,2] Despite the significant progress that has been made in identifying and characterizing the essential components of the nuclear transport machinery, some events of this process are still unclear. Particularly, our knowledge about the mechanisms for the release of nuclear-exported protein complexes at the cytoplasmic side of NPC and their subsequent trans-cytoplasmic movement is rudimentary. In mammalian cells, progress in the elucidation of these processes has been achieved by using viral nuclear shuttle proteins to target and isolate functional components of the nucleocytoplasmic transport machinery. Perhaps the best characterized RNA export protein is Rev from human immunodeficiency virus type 1 (HIV-1), which facilitates the nuclear export of unspliced and partially spliced viral RNAs.[3] In addition to identifying transport receptors and export/import nuclear signals, the Rev protein also revealed the human Rev-Interacting Protein (hRIP), which has been shown to be involved in the mechanism for the release of viral RNAs from the nuclear periphery to the cytoplasm.[4] Using a similar approach, we succeeded in isolating a novel transport GTPase, designated NIG (NSP-Interacting GTPase), as a functional target of the bipartite geminivirus nuclear shuttle protein (NSP).[5] NIG shares structural features with hRIP, such the presence of an ArfGap domain and a B-box zinc finger motif, but differs from the Rev cofactor in several other relevant aspects. These include an intrinsic GTPase activity exhibited by NIG, but not observed for hRIP, and the subcelullar localizations of the putative homologs. While the hRIP protein is found in the nucleus,[4] NIG localizes in the cytoplasm, as revealed by confocal microscopy of stably transformed root cells expressing a NIG-GFP fusion as well as of tobacco leaf epidermal cells transiently expressing YFP-NIG and NIG-GFP fused proteins.[5] A close examination of the fluorescence pattern emitted by the fused proteins in epidermal cells reveals an unusual distribution in the cytoplasm with a clear predominance of the fused protein around the nuclear envelope.[5] This is consistent with a role in assisting the release of exported proteins from the nuclear pore complexes at the cytoplasmic side. In fact, we demonstrated that NIG binds to the viral NSP in vivo and promotes its relocalization from the nucleus to the cytoplasm.

The redirection of NSP to the cytoplasm by overexpression of NIG in tobacco leaf cells is not an indirect effect of the NIG intrinsic GTPase activity because an unbalance in the GTP/GDP pools would be expected to promote NSP nuclear localization rather than its redistribution to the cytoplasm. One may predict that increased GTPase activity would cause GTP depletion and hence a block in the nuclear export of proteins by the consequent depletion of nuclear pools of RAN-GTP.[1,6] This indeed was not the case, as NSP was not confined to the nucleus by NIG action. Therefore, our recent results indicate that NIG plays a dynamic and direct role in the nuclear export and subsequent trans-cytoplasmic movement of the viral NSP. The NIG GTPase activity may be specifically involved in the regulation of its transport function rather than in the control of cellular GTP/GDP pools.

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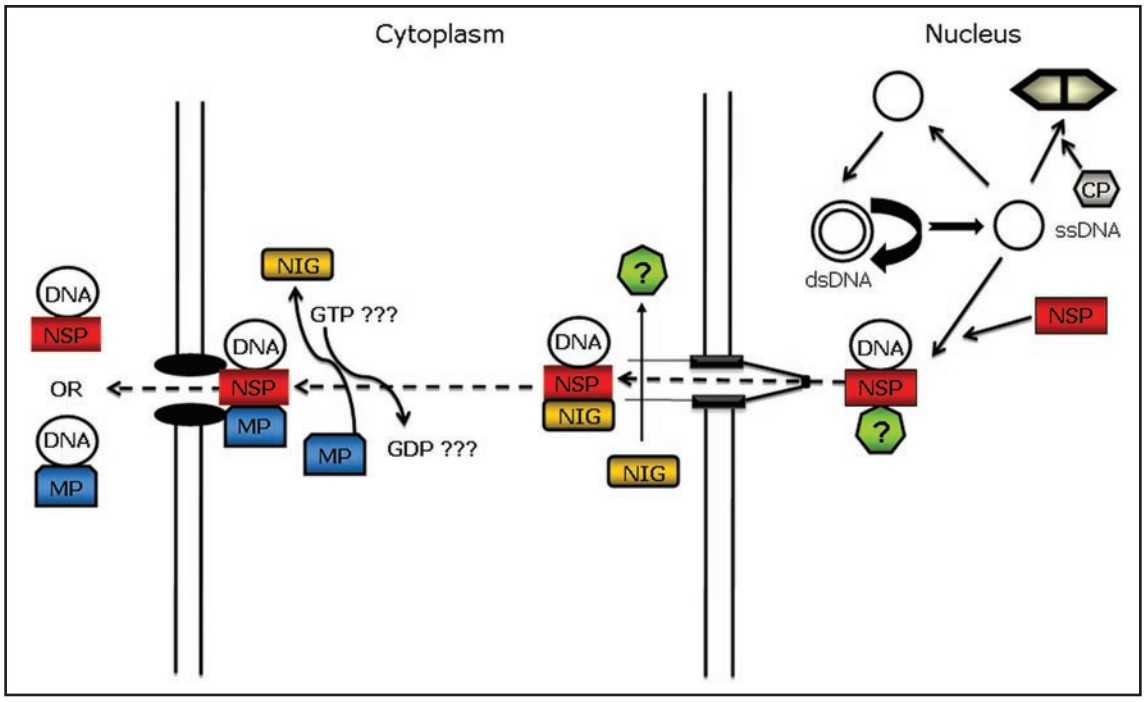

Figure 1. Proposed model for viral DNA intracellular trafficking. Geminiviruses replicate their circular, single-stranded DNA genomes via double-stranded DNA intermediates in nuclei of infected cells. During rolling-circle replication of viral DNA, the newly synthesized single-stranded DNA (ssDNA) may either re-enter the replication cycle, or be sequestered by the coat protein (CP) or NSP in the case of bipartite begomoviruses. Binding of NSP to viral DNA facilitates the intracellular movement of the viral genome from the nucleus to the cytoplasm via an exportin-like receptor (?). At the cytosolic side of the nuclear pore complex, NIG binds to NSP and redirects the viral DNA-NSP complex to the cell periphery where is replaced by MP. Thus, NIG may provide the directionality for the intracellular movement of the newly synthesized viral DNA. The NIG GTPase activity may regulate the assembly and/or disassembly of NIG-based complexes. MP either interacts directly with and transports the viral DNA or mediates the transport of the NSP-DNA complex to adjacent cells via plasmodesmata.

The Proposed Role of NIG in Viral DNA Intracellular Trafficking

The genome of species from the *Geminiviridae* family can be organized in either single or double configuration.[7] In bipartite geminiviruses from the *Begomovirus* genus, the virus-encoded replication, encapsidation and movement functions are segregated into two genomic components, designated DNA-A and DNA-B. The DNA-B component encodes two proteins, the nuclear shuttle protein (NSP) and the movement protein (MP), which act in a cooperative manner to mediate the intra- and intercellular trafficking of viral DNA. Our current findings together with previous results by others[8] support a proposed mechanistic model for viral DNA intracellular trafficking (Fig. 1). As geminiviruses replicate their genomes in nuclei of infected cells, they require a movement function to move the viral DNA from the nucleus to the cytoplasm. In the case of bipartite begomoviruses, this is accomplished by NSP that binds to viral DNA in the nucleus and utilizes the nuclear export machinery to move the viral DNA to the cytoplasm where it interacts with MP at the cell surface.[8] Evidence that nuclear export of NSP is mediated by exportin-like receptors comes from the observation that NSP contains a HIV Rev-like or TFIIA-like leucine-rich nuclear export signal (NES) that can be functionally replaced by TFIIIA NES in both nuclear export and infectivity.[9] Nevertheless, the putative export receptor for leucine-rich NESs that binds NSP and promotes its nuclear export in a Ran-GTP-regulated manner remains to be identified and is represented in Figure 1 by a question mark. Based on its properties, subcellular localization and capacity to move NSP-DNA complexes from the nucleus to the cytoplasm, we propose that NIG functions as cellular cofactor for NSP. At the cytoplasmic side of the nuclear pore complex, NIG would bind to NSP to facilitate the intracellular transport of viral DNA-NSP complexes from to the nuclear envelope to the cortical cytoplasm where it would be replaced by MP (Fig. 1). In support of this, we showed that NSP binds both NIG and MP in vivo, but NIG does not interact with MP and it is not found in NSP-MP complexes. Whether the GTPase activity of NIG regulates its transport function by controlling binding and release of substrates through cycles of GTP/GDP binding remains to be determined.

NIG as a Cellular Cofactor of Nuclear-Exported Complexes

A major perspective will be to determine the cellular function of NIG. Loss of NIG function does not appear to impair nuclear export of macromolecules as we could not associate any apparent phenotype in knockout lines grown under normal conditions. However, this result is not surprising because the Arabidopsis genome encodes two NIG homologs (At1g08680 and At4g32630), which might functionally replace NIG in protein trafficking. Because there is no advantage for plant cells to evolve a transport activity to facilitate virus intracellular movement, it is reasonable to predict that NIG acts at cellular protein complexes as well. Based on its biochemical properties and effects on NSP, NIG may function as a molecular switch for disassembly of transported complexes at the cytoplasmic side and/or a positive regulator of the subsequent movement of nuclear exported proteins to specific sites within the cytoplasm. The

identification of NIG cellular partners will allow us to test this model for NIG function.

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