

The role of cellular SH3 domain proteins in the replication cycle of influenza virus

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Introduction:

Influenza A and B viruses are the causative agents of seasonal flu epidemics. For efficient replication, the viruses need to recruit biosynthetic functions of their host cell, but in parallel they also have to subvert the potent innate defense reactions triggered after infection. The field has just started in recent years to define the specific host factors that contribute to influenza virus propagation and virulence.

We and others previously showed that the viral NS1 proteins are important pathogenicity factors that suppress the induction of antiviral type I IFN genes (1,2). Additional important NS1 activities do exist as evidenced by a strong apoptotic response and the attenuated growth of NS1 deletion viruses even in IFN-defective hosts (3). However, the molecular basis of this NS1 function is currently enigmatic. We have begun to close this gap by the identification of cellular binding partners for the NS1 proteins.

Previous work:

Our laboratory co-authored recent reports showing that the NS1 protein of influenza A virus (A/NS1) binds to and activates phospho-inositol 3-kinase (PI3K), a protein containing SH3 and SH2 domains, to trigger an anti-apoptotic reaction (4). SH3 is a prototypical protein interaction domain of about 60 amino acid that is found in many proteins of species from yeast to man, functioning in growth factor receptor signaling, cellular localization and regulation of GTPase activity (4). Interestingly, the interaction with PI3K is not conserved in the divergent NS1 protein of influenza B virus (B/NS1) (5). Nevertheless, we identified in the B/NS1 protein a class II binding motif for SH3 domains of the consensus $\Phi P x \Phi P x +$ (where x is any amino acid, and + is a basic residue). This motif is conserved among all sequenced influenza B viruses indicating that it mediates a vital interaction with an unknown cellular factor(s). In this proposal we will extend and scrutinize two key observations that suggest an important role of the conserved SH3 binding motif for virus propagation:

- A recombinant mutant influenza B/Lee/40 virus carrying alanine substitutions of the first ΦP dipeptide within the B/NS1-SH3 binding motif (SH3 mut) was generated by reverse genetics. The HA titer of the mutant virus in human lung epithelial cells was reduced by almost 90% in comparison to WT demonstrating that the SH3 binding motif is required for full level replication. Importantly, the mutant virus was a weak inducer of type IFNs and the kinase PKR in infected cells. This shows that the SH3 binding motif regulates a process that is different from the suppression of antiviral reactions that we analyzed before (3).
- By screening of 148 filter-immobilized human SH3 domains we identified three SH3 domain proteins (termed A, B and C) that strongly interacted with recombinant NS1 WT protein, but were highly reduced for binding to mutant NS1 (Kraemer & Wolff, unpublished). Protein A is a common adapter protein involved in cell signalling pathways that was also implicated in regulating apoptosis, protein B is a latent enzyme involved in the generation of second messenger molecules and protein C has a regulatory role for cytoskeletal organization. The three factors are therefore strong candidates for host cell factors that mediate the activities of the viral B/NS1 protein.

One recent study showed that the NS1 proteins of avian influenza A viruses (including H5N1) and the virus causing the 1918 pandemic interact with the SH3 domains of the Crk/CrkL proteins (6). It is currently unclear how the Crk/CrkL interaction precisely contributes to the pathogenicity of these viruses. However, this finding indicates that cellular SH3 domain proteins contribute to the propagation and spread of influenza A viruses.

Work hypothesis:

Host cell proteins carrying SH3 interaction domains are important regulators of the influenza virus replication cycle. Our goals are to identify the specific roles of the novel B/NS1 binding proteins A, B and C during virus replication *in vitro* and to assess their contribution to the pathogenicity of influenza viruses.

Proposed thesis topics:

- (1) Characterization of a novel host factor supporting replication and virulence of influenza B virus
- (2) The role of cellular SH3 domain proteins for influenza virus propagation

To approach our goals we will employ genetic, virological and biochemical methods that shall be conducted in the context of a PhD thesis project. This will involve the production of further recombinant mutant influenza viruses with altered host factor binding using established reverse genetic systems. These tools will allow us to study the kinetic dynamics of interaction of the three identified host factors with the B/NS1 protein by co-immunoprecipitation (IP) analysis. The contributions of the cellular proteins A, B and C to viral replication will be studied in siRNA-treated cells. According to the known functions of the proteins A, B and C, we will compare cells infected by wild-type or B/NS1-SH3 domain mutant virus for (i) induction of certain signalling pathways, their apoptotic response, enzymatic activity and cytoskeletal rearrangements. Virus-infected and/or transfected cells expressing the B/NS1 proteins and the host factors will be analyzed by confocal laser scanning microscopy to determine intracellular sites of colocalization. Finally, a mouse model of influenza is established in the laboratory to study the mutant viruses *in vivo*.

Interlinkage: remains to be determined. We offer long-term expertise in the analysis of pathogen interactions with interferons and other innate immune reactions for GraKo projects.

References

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