PI3K signalling during influenza A virus infections

B.G. Hale¹ and R.E. Randall
Centre for Biomolecular Sciences, University of St. Andrews, St. Andrews, Fife KY16 9ST, U.K.

Abstract
Recent work has demonstrated that the PI3K (phosphoinositide 3-kinase) signalling pathway is important for efficient influenza A virus replication. Activation of PI3K in virus-infected cells is mediated by the viral NS1 protein, which binds directly to the p85β regulatory subunit of PI3K and causes the PI3K-dependent phosphorylation of Akt (protein kinase B). Given that recombinant influenza A viruses unable to activate PI3K signalling are attenuated in tissue culture, the PI3K pathway could be a novel target for the development of future anti-influenza drugs.

Influenza A virus
Both seasonal and pandemic outbreaks of influenza A virus pose a serious threat to global public health [1]. Three major human pandemics occurred in the 20th Century. The first, in 1918, known as ‘Spanish flu’, was the most severe and is estimated to have caused over 40 million deaths worldwide [1]. Two further pandemics in 1957 (‘Asian flu’) and 1968 (‘Hong Kong flu’) had much lower, yet still significant, mortality rates [1]. Recent human infections caused by highly pathogenic avian influenza A viruses (including H5N1 subtypes) have led many to believe that another global pandemic is inevitable.

Influenza A virus is a member of the Orthomyxoviridae family of enveloped viruses. The virus genome consists of eight single-stranded, negative-sense RNA segments, which encode up to 11 viral proteins [2,3]. Segment 8 encodes two polypeptides: NS1 and NEP [2]. NS1 is not a component of the virus particle, but is expressed early in infected cells [2]. As a multifunctional virulence factor, NS1 contributes significantly to disease pathogenesis by modulating virus replication, cell death and host immunity [3,4]. In particular, a major role for NS1 during infection is to counter the host’s innate ability to respond to virus insult. NS1 achieves this by suppressing both antiviral signalling and the production or action of specific antiviral effector proteins [3,4].

NS1 binds p85 and activates PI3K signalling
Recent studies have demonstrated that the PI3K signalling pathway is activated during influenza A virus infection [5–7]. Although a weak and transient stimulation of PI3K may regulate a very early step in virus entry [5], a greater and more sustained activation of PI3K is readily detectable approx. 6 h after infection [5,6]. This latter PI3K activity is caused by the viral NS1 protein, which appears to be both necessary and sufficient to stimulate PI3K signalling during infection [5,6]. For example, unlike wild-type influenza A virus, a mutant virus lacking NS1 was unable to induce the phosphorylation of Akt (protein kinase B) at Ser-473 [5]. Additionally, stable expression of NS1 caused the constitutive phosphorylation of Akt, which was sensitive to the general PI3K inhibitor, LY294002 [6].

NS1 activates PI3K signalling by interacting with the p85 regulatory subunit of PI3K [6]. In vitro pull-down assays using purified recombinant proteins demonstrated that NS1 is able to bind directly and efficiently to the p85β subunit of PI3K, but not to the related p85α isoform [6]. The relative affinity of NS1 for p85α and p85β in vivo requires further examination, as NS1 has been reported to co-precipitate with p85α from virus-infected and plasmid-transfected cells [7]. However, it is not clear if the interaction with p85β is direct, and the in vitro data clearly indicate that NS1 is extremely selective in predominantly targeting p85β [6]. Isoform discrimination would be intriguing, as distinct physiological roles for p85α and p85β have yet to be identified. Thus NS1 may be an invaluable biotechnological tool for delineating p85β-specific signalling pathways.

Determinants of the NS1–p85 interaction
Both p85 isoforms consist of an N-terminal SH3 (Src homology 3) domain, a Bcr (B-cell receptor) homology domain, and two SH2 (Src homology 2) domains, which flank the p110-binding (inter-SH2) domain [8]. Recent mapping studies by Shin et al. [7] suggest that NS1 can independently co-precipitate with both the SH3 domain and the C-terminal SH2 domain of p85. NS1 has a strain-specific length of 230–237 amino acids [2] and contains two functional domains: an N-terminal RBD (RNA-binding domain) and a C-terminal effector domain [4]. The RBD is not essential for the interaction of NS1 with p85β, as a recombinant NS1 protein lacking the first 72 amino acids (i.e. the RBD) is still sufficient to interact with full-length p85β in vitro (B.G. Hale, unpublished work). The effector domain of NS1 contains a putative YXXM-like motif (amino acids 89–93: Tyr-Leu-Thr-Asp-Met) [6,7]. Tyrosine-phosphorylated YXXM motifs

Key words: Akt, antiviral drug, influenza A virus, NS1 protein, phosphoinositide 3-kinase (PI3K), p85β

Abbreviations used: PI3K, phosphoinositide 3-kinase; RBD, RNA-binding domain; SH, Src homology

¹To whom correspondence should be addressed (email bg1@st-andrews.ac.uk).
Thus it is not clear whether a true pYX₇₅ of cellular PI3K-recruiting proteins are responsible for binding to p85 SH2 domains [9]. Mutational studies reveal that both Tyr-89 and Met-93 are essential for the interaction of NS1 with p85β [6]. However, structural analysis of the homodimeric NS1 effector domain indicates that Tyr-89 is exposed in a cleft formed between the two monomers, whereas Met-93 is mostly buried within the molecule [10]. Thus it is not clear whether a true pYX₇₅-like motif mediates NS1–p85 binding, as mutation of Met-93 (or even Tyr-89) could potentially destabilize the NS1 dimer. There is also no evidence yet to suggest that Tyr-89 can be phosphorylated during infection. Shin et al. [7] have recently proposed that two polyproline motifs in NS1 (amino acids 164–167, Pro-Xaa-Pro, and amino acids 213–216, Pro-Pro-Xaa-Pro) may be involved in interacting with the p85 SH3 domain [7], but individual mutational analyses have yet to be performed. Further work is clearly required to determine the basis of the NS1–p85 interaction.

**PI3K activation is important for efficient influenza A virus replication**

The single amino acid substitution of phenylalanine for Tyr-89 (Y₈⁹F) prevents NS1 from binding p85β and subsequently activating PI3K signalling [6]. A recombinant influenza A virus (A/Udorn/72 strain) that expresses NS1 with the Y₈⁹F mutation does not induce Akt phosphorylation at Ser-473, and is attenuated under tissue-culture conditions [6]. In MDCK (Madin–Darby canine kidney) cells, the mutant virus forms smaller plaques, and grows to titres approx. 10-fold lower than wild-type virus [6]. A similar phenotype has recently been reported for a recombinant influenza A virus (A/Puerto Rico/8/34 strain) expressing NS1 with a total of six mutations, including Y₈⁹F and five substitutions of alanine for proline [7]. Like the single Y₈⁹F mutant, this virus does not activate PI3K [7], but the contribution of mutating each proline residue to its attenuated phenotype was not characterized.

**PI3K signalling as a potential anti-influenza drug target**

Antivirals may provide an important initial defence against rapidly emerging and antigenically novel pandemic strains of influenza A virus [11]. Targeting virus-activated signalling cascades (such as PI3K) could be an attractive strategy for anti-influenza drugs [11]. Chemical inhibition of PI3K (using LY294002 or wortmannin) has already been shown to greatly reduce the yield of influenza A virus under tissue-culture conditions [5,6]. As various PI3K inhibitors are already under investigation as potential long-term therapies for cancer and chronic inflammatory disorders, it may be possible to develop similar compounds for use as short-term antivirals. The advantage of targeting a host-cell pathway is that treatments may be less susceptible to virus mutation and drug resistance. However, unknown toxic side effects on uninfected tissues could be cause for concern. An alternative antiviral strategy is to specifically inhibit the activation of PI3K by NS1. However, as this is a virus-specific target, there is potential for the development of drug resistance. Nevertheless, this approach is very attractive as Tyr-89 of NS1 appears wholly conserved among human and avian influenza A viruses, thus compounds targeting this virus–host interaction may be effective against all influenza A strains.

**Concluding remarks**

Many viruses hijack PI3K signalling in order to regulate host activities such as cell survival, gene transcription and protein synthesis [12]. The biological reasons for influenza A virus activating PI3K remain unclear, from both a virus and a host perspective. Further studies on the mechanism and consequences of NS1-mediated PI3K activation have the potential to provide novel insights into the biology of both influenza A virus infections and cellular PI3K regulation.

**References**


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