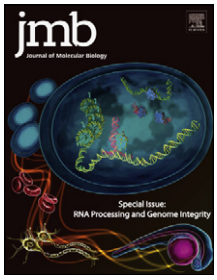

The NS1 Protein from Influenza Virus Stimulates Translation Initiation by Enhancing Ribosome Recruitment to mRNAs

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Abstract

The non-structural protein NS1 of influenza A viruses exerts pleiotropic functions during infection. Among these functions, NS1 was shown to be involved in the control of both viral and cellular translation; however, the mechanism by which this occurs remains to be determined. Thus, we have revisited the role of NS1 in translation by using a combination of influenza infection, mRNA reporter transfection, and *in vitro* functional and biochemical assays. Our data show that the NS1 protein is able to enhance the translation of virtually all tested mRNAs with the exception of constructs bearing the Dicistroviruses Internal ribosome entry segment (IRESes) (DCV and CrPV), suggesting a role at the level of translation initiation. The domain of NS1 required for translation stimulation was mapped to the RNA binding amino-terminal motif of the protein with residues R38 and K41 being critical for activity. Although we show that NS1 can bind directly to mRNAs, it does not correlate with its ability to stimulate translation. This activity rather relies on the property of NS1 to associate with ribosomes and to recruit them to target mRNAs.

Introduction

Influenza A viruses are major human pathogens with pandemic outbreaks such as in 1918 that had caused over 20 million deaths worldwide [1,2]. Influenza is an enveloped virus that belongs to the Orthomyxoviridae family and possesses 8 negative single-stranded RNA genomes that encode up to 17 different proteins [3]. The viral life cycle commences by virus entry into the cell where incoming vRNPs are transported toward the nucleus to undergo transcription and replication; these are performed by the viral RNA-dependent RNA polymerase with the support of the host RNA polymerase II and transcriptosome machinery [3]. The viral mRNAs harbor a 5′ methylated cap that is stolen from cellular transcripts in a process called “cap-snatching” [3]. This occurs by a subtle subversion of the cellular transcriptional machinery whereby the RNA-dependent RNA polymerase binds to the m^7GTP cap of cellular transcripts to cleave it about 9 to 17 nt downstream to their 5′ end. This cellular fragment of RNA is then used as a primer for viral transcription [4–6], resulting in the synthesis of properly capped mature viral mRNAs and their CRM1-dependent export to the cytoplasm, where they are translated by the host cell machinery into proteins [7]. Such hijacking is accompanied by the degradation of the cleaved cellular transcripts together with the impairment of nucleocytoplasmic export and contributes to the shut-off of cellular gene expression [8,9].

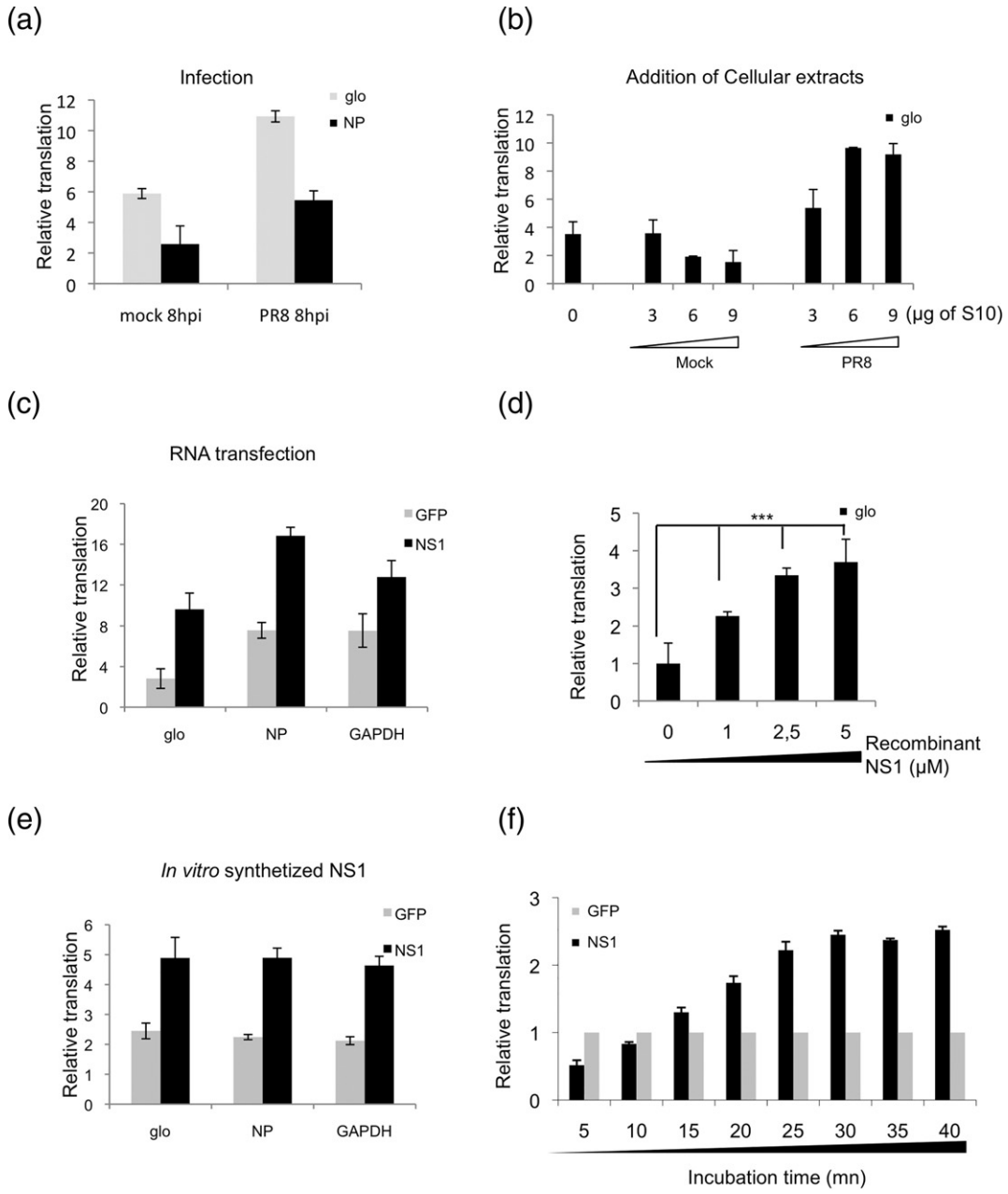

Fig. 1. Viral and cellular translation is enhanced in influenza-infected cells. (A) Capped and polyadenylated luciferase reporter mRNAs driven by the 5′UTRs derived from the β-globin (glo) or the NP (NP) segment of PR8 influenza strain were electroporated in A549 cells that were infected, or not (mock), with the influenza PR8 strain at a multiplicity of infection of 6. Then, 8 h post-infection, luciferase activity was determined. (B) Capped and polyadenylated luciferase reporter mRNAs driven by the β-globin 5′UTR were added to the RRL supplemented with increasing concentrations of cytoplasmic extracts (from 3 µg to 9 µg) prepared from infected or mock-infected A549 cells with the PR8 influenza strain. Luciferase activity was determined after 30 min of incubation at 30 °C. (C) Capped and polyadenylated luciferase reporter mRNAs driven by the 5′UTRs derived from β-globin (glo), GAPDH, or the NP genes were electroporated in A549 cells that have been previously transfected with RNAs coding for the GFP or the NS1 protein as indicated. Luciferase activity was determined for each construct 1 h post-electroporation. (D) Capped and polyadenylated luciferase reporter mRNAs driven by the 5′UTRs derived from β-globin was translated in the RRL in the presence of increasing amounts of recombinant NS1 protein as indicated. After 30 min of incubation, luciferase activity was determined. (E) Capped and polyadenylated luciferase reporter mRNAs driven by the 5′ UTRs derived from β-globin (glo), GAPDH, or the NP segment were translated in the RRL in the presence of *in vitro* synthesized GFP or NS1 proteins as indicated. (F) Capped and polyadenylated luciferase reporter mRNAs driven by the 5′ UTRs derived from β-globin were translated in the RRL in the presence of *in vitro* synthesized GFP or NS1 proteins. Luciferase activity was determined at different times as indicated on the figure (from 5 to 40 min of incubation). All results are expressed as mean +/- SD of three independent experiments.