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How a rotavirus hijacks the human protein synthesis machinery

Gabriele Varani and Frédéric H.-T. Allain

The NSP3 protein from rotaviruses recognizes a unique sequence at the 3' end of the rotaviral mRNA. By doing so, it promotes translation of viral proteins while repressing host protein synthesis. The structure of the NSP3 protein bound to a viral 3' end sequence reveals how this occurs and suggests how it might be possible to design a new class of antiviral drugs.

Rotaviruses are a class of double-stranded RNA viruses responsible for pediatric diarrhea, which causes the death of approximately one million children worldwide each year¹. The viral genome is composed of 11 segments of double-stranded RNA that encode six structural proteins forming the viral capsids (named VP for viral proteins) and six nonstructural proteins (NSPs)². The 36 kDa nonstructural protein 3 (NSP3) is essential for promoting the synthesis of viral proteins. Its N-terminal domain binds the 3' end of viral mRNA and its C-terminal domain interacts with translation initiation factor eIF4G. These interactions are functionally equivalent to the interactions between human poly(A) binding protein (PABP), and both the poly(A) tail at the 3' end of eukaryotic mRNAs and eIF4G. Through these interactions, the rotavirus achieves circularization of its mRNA and selectively boosts the efficiency with which the host translational machinery synthesizes viral proteins³ (Fig. 1).

In a recent issue of *Cell*, Burley and colleagues⁴ report the high resolution structure of the N-terminal domain of an NSP3 protein (from group A simian agent rotavirus) bound to a consensus sequence found at the 3' end of the viral mRNA. The structure reveals how the virus hijacks the human translation machinery to enhance synthesis of viral proteins and simultaneously shut down production of host proteins. It also reveals a new and surprising structure for RNA recognition and

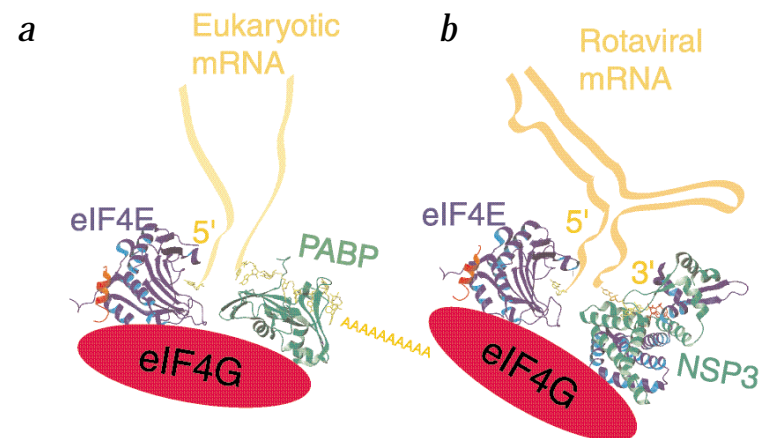


Fig. 1 The 5' and 3' ends of viral and eukaryotic mRNA synergistically stimulate initiation of protein synthesis. **a**, In host mRNAs, poly(A) binding protein (PABP) binds the 3' poly(A) tail and eIF4E binds the 5' cap; together, they interact with eIF4G, which delivers the mRNA to the ribosome. **b**, Rotaviral mRNAs have no poly(A) tail, but the viral NSP3 protein binds the consensus 3' sequence 5'-GACC-3', which also interacts with eIF4G, similar to the mechanism of action of human PABP. The atomic resolution structure of the eIF4E–eIF4G peptide–7methyl GDP ternary complex²² (1EJF), of the PABP–A_n complex²¹ (1CVJ) and of the recently determined NSP3 dimer–5'-UGACC-3' complex⁴ (PDB accession code 1KNZ) were used to prepare this schematic figure. Unfortunately, we do not yet know exactly how the pieces are assembled to form the translation apparatus. Figs 1 and 2 were generated using MOLMOL²⁵.

explains the sequence-specific recognition of the 3' end of the viral mRNA by NSP3. The deep burial of RNA bases within a tunnel formed by the interaction of two NSP3 monomers suggests a possible means for designing new antivirals that selectively target NSP3 and inhibit its activity.

Translating rotaviral mRNA

Rotaviruses are icosahedral viruses with three capsids enclosing a double-stranded

RNA genome. The innermost capsid consists of two structural proteins VP1 and VP3. VP1 is the RNA-dependent RNA polymerase (RdRP) and VP3 is the mRNA capping enzyme. These proteins allow viral RNA replication and 5' capping to occur within the virus, but synthesis of viral proteins is dependent upon the host translational apparatus. The rotaviral mRNAs are capped at their 5' ends but their 3' ends are not polyadenylated;

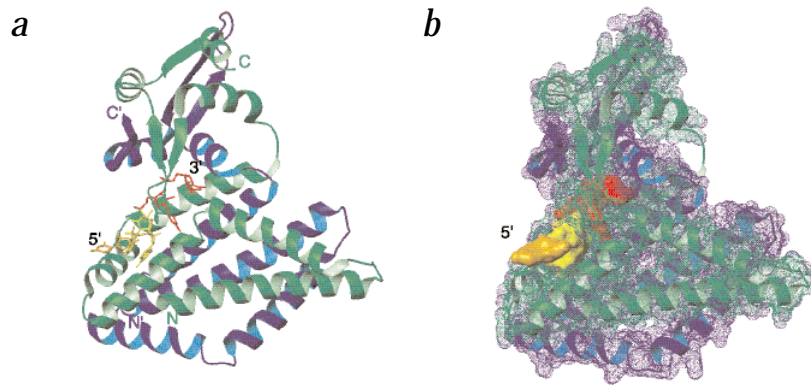


Fig. 2 NSP3 homodimer binds the viral mRNA 3' ends and buries these nucleotides within a deep tunnel. **a**, Ribbon representation of the NSP3 dimer-5'-UGACC-3' structure⁴. Each NSP3 monomer is shown as a ribbon in a different color. The two 3' cytosines (C4 and C5) are red, G2 and A3 are yellow, and U1 is orange. **b**, Same view of the complex as in (a) but with the solvent accessible surface added to the protein dimer and the RNA. This image shows how deeply buried the two terminal cytosines are.

rather, they terminate with tetranucleotide sequences that are conserved among viral groups: 5'-GACC-3' in Group A rotaviruses, 5'-(C/A)(C/A)CC-3' in Group B and 5'-GGCU-3' in Group C.

Because viral mRNAs do not have poly(A) tails, they are not recognized by the PABP of eukaryotic cells. This should, in theory, make viral protein synthesis less efficient than host synthesis because in the latter, the 5' cap and 3' poly(A) tail synergistically enhance translation⁵. The cap binding protein eIF4E and the poly(A) binding protein PABP both bind initiation factor eIF4G⁶, a multipurpose adaptor protein that is responsible for delivering capped and polyadenylated mRNAs to the ribosome⁷ (Fig. 1a). *In vivo*, translation initiation factors are limiting and mRNAs compete for translation, thereby enhancing the synergy observed *in vitro*. If capped-only mRNAs are translated inefficiently, how do rotaviruses overcome the absence of a poly(A) tail to allow translation of their mRNAs?

Viruses have evolved exquisite mechanisms to exploit the cellular protein synthesis machinery and gain an evolutionary advantage during infection. Flaviviruses (such as hepatitis C) and picornaviruses (such as the virus that causes foot-and-mouth disease) utilize a cap-independent mechanism of translation initiation that relies on an RNA structure at the 5' end of the viral mRNA that directly interacts with the ribosome and initiation factor eIF3 (refs 8,9). Rotaviruses use a different strategy: they have a set of unique sequences at the 3' ends of their viral RNAs, and they encode NSP3, which is functionally equivalent to the poly(A) binding protein in that it binds simultaneously the unique viral mRNA 3' end and host eIF4G (Fig. 1b). Because the binding sites for PABP and NSP3 on eIF4G overlap, and because the affinity of eIF4G for NSP3 is stronger than for PABP, increased translational activity of

the viral mRNA occurs while translation of host mRNAs is rendered less efficient^{10,11}.

NSP3 proteins from each viral group bind specifically to 3' terminal sequences that are unique to each group. These proteins have functional roles analogous to those of eukaryotic PABP. First, they protect the viral mRNA 3' end from cellular nucleases; second, they recruit eIF4G by interacting with it¹¹. The structure of the N-terminal domain (residues 4–164) of NSP3 from Group A (NSP3A) in complex with a hexanucleotide containing the Group A-specific 3' terminal sequence (5'-GUGACC-3') reported by Burley and coworkers⁴ reveals how recognition of the viral RNA is achieved. It is remarkable and unique in many respects.

Structural analysis of NSP3

The NSP3A structure reveals an intertwined homodimer with an unusually large protein-protein interface (7,800 Å²) and with a fold that has no similarity to any known protein structure (Fig. 2). Most remarkably, the dimer is asymmetric, although it is composed of identical subunits adopting similar secondary structures. The asymmetric homodimer binds a single RNA molecule, and it is very likely that RNA recognition is responsible for breaking the symmetry: completely different sets of amino acid side chains from each subunit are used to contact the RNA. In addition, the protein structure is unstable in the absence of the RNA ligand. Although there are other examples of asymmetric homodimers, this is the first example of a protein homodimer binding RNA asymmetrically.

In the complex, the RNA adopts an extended structure and is located in a highly basic deep cavity or tunnel that lies within the dimer and creates a 'dead end' for the 3' terminal nucleotides (Fig. 2). The four nucleotides of the consensus

group A terminal sequence (5'-GACC-3') are specifically recognized by a combination of van der Waals, stacking, salt bridge and hydrogen bond interactions with protein side chains of both subunits, and some of the contacts are water-mediated. Another surprising observation is that NSP3A utilizes an α -helical surface to bind single-stranded RNA. Although not unprecedented, this mode of recognition is distinctively different from the paradigm exemplified by the RNA recognition motif¹² (RRM) and by anticodon-binding domains of tRNA synthetases, where single stranded RNA bases are recognized on the surface of β -sheets.

The structure rationalizes the ability of NSP3 protein to protect the viral mRNA from degradation *in vivo*. The 3' terminal cytosine is completely buried within the protein (Fig. 2b), making it almost inaccessible to solvent, and the three nucleotides that follow are also deeply buried. The cytosine 2' and 3' hydroxyl groups, a feature unique to the 3' end of the mRNA, are both recognized by the protein, thereby preventing recognition of internal tetranucleotide sequences.

The structure as well as thermodynamic data provided by Burley and colleagues suggest that the free NSP3A may consist of a metastable arrangement of secondary structure elements, which envelop the RNA to form a highly stable ternary complex⁴. Induced fit has been reported as a nearly ubiquitous feature of RNA-protein recognition, but the present structure reveals new heights, in that the protein structure itself is very significantly stabilized in the presence of the RNA. Although folding of domains or local secondary structure motifs is common and coupled with specific binding in protein-DNA¹³ and protein-RNA recognition^{14–16}, the stabilization of a new protein fold upon RNA binding is a very surprising observation.

The structure not only explains how the viral mRNA 3' end is recognized by the viral protein and how proteins that belong to different viral groups are specific for their cognate mRNA sequences, but it also rationalizes the role of the protein in promoting viral mRNA translation through its functional homology with PABP. By interacting simultaneously with eIF4E (which binds the cap) and NSP3 (which binds the viral mRNA 3' end), the 5' and 3' ends of the viral RNA are brought into close spatial proximity, presumably allowing the circularization of the mRNA and synergistic enhancement of translation from both 5' and 3' ends, in close analogy to the mechanism used by the host poly(A) binding protein (Fig. 1)¹⁷.

Still to come

The NSP3–RNA complex⁴ is the first atomic resolution structure providing information on how a virus uses the cellular machinery to activate the translation of its own genes. In addition to providing fundamental insight into how this occurs, it also suggests how it might be possible to interfere selectively with viral protein synthesis. The RNA is buried deeply within a highly basic tunnel, and four nucleotides are sufficient to provide the entire binding energy^{4,18}. Thus, it may be possible to find small molecules that, by binding selectively to this crevice, prevent the association of NSP3 with the viral mRNA. Because there is no known cellular counterpart to NSP3, and its fold appears to be unique, it is possible that this strategy would achieve a high level of selectivity. In the absence of a genetic system that could be used to screen for such compounds,

structure-based approaches could be particularly useful to bias library searches toward the discovery of inhibitors. The results presented by Burley and colleagues⁴ make this approach feasible.

While the structure of the NSP3–RNA complex provides insights into how the rotavirus may hijack the host's translation machinery to synthesize its own proteins, several questions remain. Among these questions is how replication of the viral RNA occurs, because this process is known to start at the 3' end of the mRNA. The two cytosines at the 3' end of the viral mRNA must be accessible to ensure binding of the replicase complex for priming and replication by the RNA-dependant RNA polymerase (VP1). These residues are so deeply buried in NSP3, and so tightly bound (Fig. 2), that it appears unlikely that they could be exposed passively, suggesting that an unknown activity may be responsible for removing NSP3 from the viral mRNA 3' end. Another unanswered question is how the pieces of the puzzle that comprise the machinery responsible for initiating protein synthesis are put together. We know how the cap is recognized by eIF4E^{19,20}, how PABP recognizes the poly(A) tail²¹, and now how NSP3 recognizes the viral mRNA 3' end⁴. We even know the structure of fragments of eIF4G²² and PABP^{23,24} involved in protein–protein interactions during initiation of protein synthesis (Fig. 1). But how do these pieces fit together to function? There is still much to learn about this fascinating process.

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