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used to induce two-dimensional crystallization of proteins and viruses (Steven et al., 1978).

The P3 shell of RDV and its analogs in reo, BTV, and L-A virus is a highly conserved feature. In each case, the shell formed by 60 dimers displays a quaternary structure in which subunits are staggered around 5-fold symmetry axes with one, designated A, making contact with 5-fold related subunits near the 5-fold axis and the second (B), making close contact with A subunits on each side, but not contacting its 5-fold related equivalents. There is extensive symmetry mismatch in this shell, with equivalent parts of each subunit making different interactions with neighbors. It is likely that an A/B dimer is the stable form in solution prior to assembly and that they form pentamers of dimers that assemble into the inner shell.

RDV contains the largest genome of any dsRNA virus studied by crystallography. The 25.7 kilo base pair (kbp) dsRNA exists in 12 segments compared with 23.5 kbp in 10 segments for reovirus and 19.2 kbp and 10 segments for BTV. The RNA is inside the P3 protein shell along with gene products P1, the RNA directed RNA polymerase, P5, a guanylyltransferase and P7, a nonspecific nucleic acid binding protein. Some of the density for P7 is visible in the X-ray structure, as it interacts with the P3 and has icosahedral symmetry in one region. No density was assigned to the other internal proteins. By analogy with other dsRNA viruses, each pentamer of the P3 shell is likely to contain an RNA replication complex, and it is probable that each segment of RNA is associated with a specific pentamer and polymerase for its replication. Unlike RDV and BTV, the orthoreovirus core particle has well ordered pentamers of the protein λ2 (analogous to P5 of RDV) that adds the 7-methyl-G cap to the 5′ end of the nascent RNA, allowing the domains and active sites for three distinct reactions required for this process to be identified. The polymerase exists as one copy per pentamer in RDV, BTV, and orthoreovirus and has not been seen in any of these particles due to the lack of icosahedral symmetry. The structure of the orthoreovirus polymerase was, however, determined as an expressed, independent protein (Tao et al., 2002).

It is remarkable that in five years the *Reoviridae* family has become one of the best structurally characterized virus groups. The results have been exceptionally interesting and informative as the structures determined characterize viruses infecting mammals, insects, and now plants, with common themes obvious in their structures, but novel features required for the individual niche that each occupy. An interesting challenge remaining in the study of RDV is a structure-based mechanism for the insect-induced "activation" of the virus for infection of plants. This will probably require the structure determination of P2.

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Structure, Vol. 11, October, 2003, ©2003 Elsevier Science Ltd. All rights reserved. DOI 10.1016/j.str.2003.09.011

Unraveling the Replication Machine from Negative-Stranded RNA Viruses

The atomic structure of the Borna-disease virus nucleocapsid protein represents the first detailed structural information for such essential element in the negative-stranded RNA virus replication machine.

The negative-stranded RNA viruses (NSVs) include very important human and animal pathogens, like the rabies, measles, mumps, influenza viruses as well as many viruses causing severe hemorrhagic fevers, for instance, the Ebola-like viruses, the Hantaan-like viruses, and the South American hemorrhagic fever viruses. In contrast to their positive-polarity RNA counterparts, the NSVs cannot directly express their genetic information upon entering into an infected cell. Instead, their genome must first be transcribed. As such, all of the protein factors required for transcription, including a specific RNA-dependent RNA polymerase are transferred to the infected cell together with the viral RNA. Thus, the functional unit for virus RNA transcription, and for virus RNA replication later on in the infectious cycle, is a ribonucleoprotein complex or RNP, in which the negative-stranded RNA template is associated with the polymerase. Some of the NSVs such as the Mononegavirales group contain a single RNA genome while others, includ-

ing the influenza, bunyaviruses, and arenaviruses, contain a specific number of RNA segments. These RNA molecules form separate RNPs and are transcribed and replicated independently.

The most abundant protein in the RNPs is an RNA binding protein present in multiple copies called the nucleocapsid protein (N) or nucleoprotein (NP) in the various NSVs. Typically, the N(NP) is a medium-size protein of about 50 kDa that associates regularly all along the virus genome and protects the RNA molecule from nuclease attack to a variable degree in the different virus species. Thus, the N(NP) serves as a structural protein and determines the general morphology of the RNP but is by no means a passive element in the transcription and replication processes. First, the N(NP) is essential for the RNA polymerase to copy the template since no significant productive synthesis can be achieved on a naked viral RNA. In other words, the real template for a NSV polymerase is the N(NP)-RNA complex. Chemical probing has shown that the sugar phosphate backbone in the RNA is protected by N(NP) binding, while the Watson-Crick positions of the bases are reactive (Baudin et al., 1994; Iseni et al., 2000). This suggests that the RNA polymerase can read the template RNA without the need to dissociate it from the bound N(NP). Second, many mutations in the N(NP) show defective virus RNA synthesis, some of which do not alter RNA binding. Altogether, the present evidence indicates that the N(NP) nucleates a long series of interactions with virus and cellular factors necessary for RNA transcription and replication, intracellular RNP localization and eventually encapsidation.

In spite of the central role of the N(NP) in NSV multiplication and the possibility to express many of these proteins to high level, structural information has been scanty. Now the atomic structure of Borna-disease virus (BDV) N protein is reported by Rudolph et al. (2003) in this issue of *Structure*, establishing a baseline reference in our understanding of NSV RNP structure and function.

Borna-disease is a rare fatal encephalitis, originally detected in Germany, which affects horses and other farm animals. It is produced by BDV, a member of the Mononegavirales that, contrary to most other viruses of this group, transcribes and replicates its RNP in the nucleus of the infected cell (de la Torre, 2002). In spite of normally producing persistent infections and being a very difficult virus to work with, the BDV N protein is the first among the NSV N(NP)s whose structure has been solved at atomic resolution. The structure reported is essentially α-helical and represents a novel fold. In the crystal, the N protein forms tetramers in which each monomer interacts by its N- and C-terminal regions with the adjacent ones, in a head-to-tail fashion. A large fraction of the surface area of each monomer is buried in these interactions that include hydrogen bonds, salt bridges and van der Waals contacts. This suggests that the interactions among monomers in the crystal most probably are biologically relevant for the virus RNP structure and function. In addition, the atomic structure reported provides insights into the possible areas of N protein involved in RNA binding. Two alternatives are proposed by the authors, namely the positively charged central channel in the 4-fold axis of symmetry and a large cleft that runs diagonally along the tetramer. The structure also allowed the identification of residues probably involved in the interaction of N and P (p23) proteins, which is essential for RNA replication in all Mononegavirales.

The structural evidence suggests that the tetramer could be a biologically relevant entity in BDV RNA replication and such an idea would fit with the tetrameric structure of Sendai virus P protein (Tarbouriech et al., 2000) . Similarly, a role for dimeric NP has been proposed for influenza virus RNA replication (Ortega et al., 2000). However, there is no evidence for a dimeric or tetrameric repetitive unit in the RNP morphology of NSVs. Three-dimensional models for the N(NP) from influenza virus and several Mononegavirales have been derived by electron microscopy (Bhella et al., 2002; Martín-Benito et al., 2001; Schoehn et al., 2001). They all have a banana-like shape and are able to multimerize to form rings or helicoids, even in the absence of RNA, due to the presence of two contact sites per monomer. These interactions among N(NP) monomers are quite flexible, as various ring sizes and/or helix pitches can be obtained (Bhella et al., 2002; Schoehn et al., 2001). Moreover, fully replicating, recombinant influenza virus RNPs can be produced in which the number of NP monomers in the circular structure varies from 8 to 14, depending on the length of the template RNA (Martín-Benito et al., 2001; Ortega et al., 2000). It is possible that the tetrameric BDV N protein, if present as a biological entity in the infected cell, would represent an intermediate in the biosynthesis of progenie RNPs. The interactions among consecutive monomers in the helicoidal structure would be quasiequivalent to those in the tetramer. Moreover, the two alternative RNA binding sites proposed by Rudolph et al. (2003) in the tetramer would constitute a single RNA binding path along the N protein multimer, as the positively charged channel seen in the tetramer would not be present in the helicoidal structure. Determination of the N-RNA stoichiometry and the general structure of native BDV RNPs will be required to put the atomic structure of N into the context of the virus replication machine. Likewise, docking this atomic structure into the known three-dimensional models of the N(NP) of other NSVs and modeling their protein sequences into the BDV N atomic structure will tell us about its generality for the N(NP)s from other NSVs.

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Exploring Actin Five Steps at a Time

A global mutagenesis survey of actin structure and function shows that many mutants retain the ability to polymerize despite its very high sequence conservation.

One always feels compelled to begin any article on the protein actin by writing, "Actin is a very highly conserved protein found in all eukaryotic cells, where it forms a major component of the cytoskeleton and, in combination with myosin, a major molecular motor system." One has only to add that it is a major structural protein of the myofibril, the structural organelle that produces the contractile characteristic of muscles, again due to the actomyosin motor system, to inform the reader why the study of actin has been a major research activity for many years. There can be few proteins that have as many and diverse protein binding partners. Estimates of how many actin binding proteins (ABPs) there are vary between organisms. The *Drosophila* genome, for instance, contains at least 80 actin binding proteins (Goldstein and Gunawardena, 2000), probably more, and these can be grouped into a smaller number of ABP families.

Actin exists in two forms, the soluble monomeric G-actin and the filamentous F-actin. It is in its filamentous, polymeric F-actin form that actin has its biological functions. Where and how much F-actin forms by polymerization, the structures it forms, and its polarity all affect cell shape and cell movements. Additionally, it provides tracks for the movement of, and movement within, cells driven by myosins. All these functions are regulated by interactions with ABPs. It is therefore crucial that we understand the relationships between the actin sequence, its structural dynamics as a monomer and as a polymer, and its interactions with the myriad ABPs.

Progress toward these goals at the structure/function levels has been slower than with many other proteins for two reasons. The great propensity of actin to polymerize at high concentrations rather than form crystals has, until recently, prevented structural determinations of actin, except in combination with monomer binding proteins (DNase I, profilin, a gelsolin fragment and vitamin D binding protein) that prevent formation of F-actin. Cocrystals of actin with myosins or with F-actin binding proteins have not been achieved. Successful structural studies with this latter group of ABPs have been achieved only by EM reconstructions of F-actin with α-actinin, myosin, tropomyosin, etc., but, as yet, these provide limited resolution. Molecular genetic approaches to actin structure and function have a long history (Hennessey et al., 1993), but there have been difficulties (largely overcome, especially in yeast) in recovering actin mutants and expressing the mutant proteins in sufficient quantities for biochemical investigation of actin structure and function. Actin requires a chaperone to fold into its native state and cannot be expressed in bacteria.

Previous investigators have studied the effects of single amino acid substitutions; few multiple residue mutations have been made. Now, in this issue of *Structure*, Rommalaere et al. (2003) report on their studies in which they have scanned the complete actin polypeptide chain using a variant of alanine-scanning mutagenesis. They replaced serial, nonoverlapping five-residue segments with alanines throughout the length of the polypeptide chain and then assayed the mutant proteins for retention of structure, polymerization, interactions with specific ABPs, and finally for in vivo effects by transfection of cultured mammalian cells. This heroic effort was only possible because they used in vitro transcription/translation to express the mutant actins and developed techniques to measure the biochemical activities of very small amounts of protein. This approach has been very informative, and they have integrated their data with reference to the actin monomer structure and information of known binding sites from earlier studies. Five-residue substitutions are not subtle interventions and a major surprise is that so many of these mutations formed actin capable of polymerization, at least as measured by copolymerization with an excess of normal actin. One may question whether this is true polymerization, but, clearly, sufficient structure remains in the mutant to permit binding to other actin monomers. Overall, their results confirm the actin-actin binding sites proposed from F-actin atomic models and monomer ABP binding sites already known. These are G- rather than F-actin binding proteins, as the small amounts of mutant protein expressed prevent F-actin binding studies. The effects of the mutants on cell morphology and the localization of the mutant actins in the cells are interesting and show some correlation with the authors' interpretations from in vitro data. Mutants affecting polymerization in vitro did not colocalize with F-actin structure in vivo; other mutants provided a range of cellular phenotypes. These