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Nucleoproteins and nucleocapsids of negative-strand RNA viruses

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A hallmark of negative-strand RNA viruses (NSVs) is that their genomes never exist as free RNA, but instead are always assembled with many copies of a single nucleoprotein (N) to form highly stable nucleocapsids. Moreover, viral genomes are the only RNAs in infected cells that are assembled with N. The mechanism by which this specific association occurs, for both the segmented (s) and non-segmented (ns) viruses, has recently become clearer due to our expanding knowledge of N protein and nucleocapsid structures.

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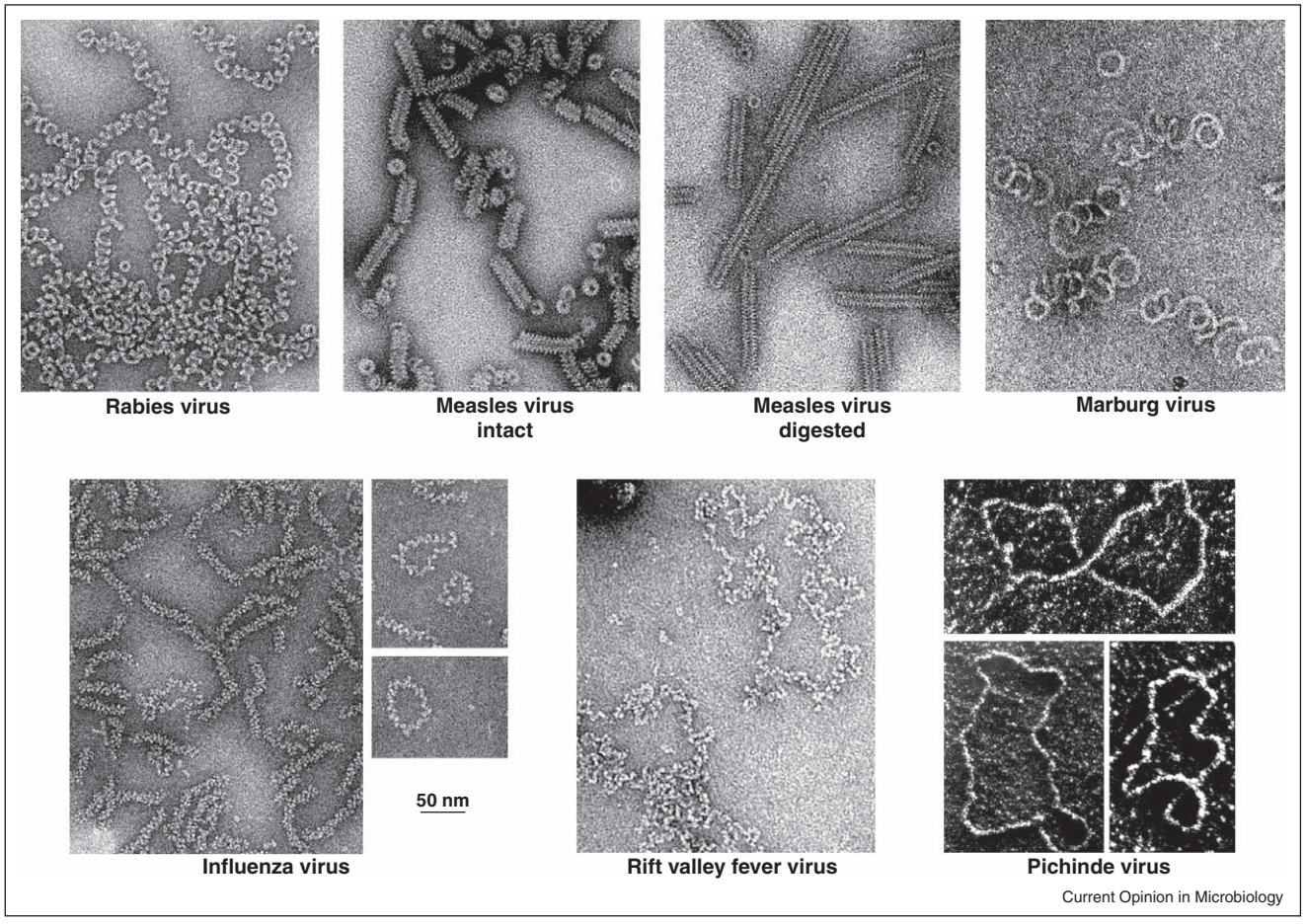
Negative-strand RNA viruses (NSVs) are divided into two orders, depending on whether their viral genomes are non-segmented (ns) or segmented (s). The order *Mononegavirales* (nsNSV) contains four families; *Rhabdoviridae* (e.g. rabies virus and vesicular stomatitis virus (VSV)); *Paramyxoviridae*, with two subfamilies, *Paramyxovirinae* (e.g. measles and Sendai viruses) and *Pneumovirinae* (e.g. respiratory syncytial virus (RSV)); *Bornaviridae* (e.g. Borna disease virus (BDV)), and *Filoviridae* (e.g. Marburg virus). These viruses have a single, long RNA genome coding for at least five proteins, always in the order 3′–Nucleoprotein (N)–Phosphoprotein (P)–Matrix–Glycoprotein–Large protein (L)–5′; L being the RNA-dependent RNA polymerase. The order *Multinegavirales* (sNSV) contains three families; *Arenaviridae* with two segments (e.g. Lassa fever virus); *Bunyaviridae* with three segments (e.g. Rift Valley fever virus (RVFV), and *Orthomyxoviridae* with six to eight genome segments (e.g. influenza virus).

N-RNAs of the mononegavirales form long, flexible and helical nucleocapsids (Figure 1, top row). Together with their P–L polymerase, these nucleocapsids are active in mRNA synthesis and genome replication. Contact between their N-RNAs and polymerases is made through P, the polymerase cofactor. In the absence of N, the polymerase can initiate synthesis on the naked viral RNA, but its processivity is reduced [1] (S Whelan, unpublished data). In infected cells, N binds only to genome RNAs and not to cellular RNA, nor viral mRNAs. However, when expressed in the absence of the other viral proteins, recombinant nucleoproteins bind cellular RNAs and form complexes almost indistinguishable from the viral structures. All N-RNAs in the top row of Figure 1 form relaxed structures, except that of measles virus after treatment with trypsin. Trypsin removes the C-terminal 125 amino acids (N-tail) and converts the structure into a tight helical rod. When rhabdovirus and filovirus nucleocapsids are integrated into virus particles, they form ordered, tightly packed and stiff helices with a defined diameter that determines the shape of the virus [2•,3•]. For the sNSV, the viral polymerase binds directly to the complementary ends of the genome RNA without the help of a phosphoprotein, forming a panhandle structure [4–6] and resulting in circular nucleocapsids that have a more wavy structure (Figure 1, bottom). These nucleocapsids remain relaxed for bunyaviruses and arenaviruses, but form flexible supercoils for influenza viruses. Circular influenza N-RNAs are visualized only under low salt conditions [5].

The atomic structures of four nsNSV nucleoproteins are now available: BDV N that crystallised as a tetramer in the absence of RNA [7], and those of rabies virus, VSV, and RSV, which crystallised in the form of recombinant N-RNA rings containing 10 or 11 N-protomers [8,9,10••]. These four proteins are homologous, with helical N-terminal (N-ter) and C-terminal (C-ter) domains and extensions (Figure 2). The protomers in the three N-RNA rings make extensive contacts between their C-ter domains, with N-ter extensions reaching to the back of the neighbouring N to make an additional domain exchange contact. For the rhabdoviruses, the C-ter extension also goes to the back of the N-protomer at the other side for additional contacts beyond those made by N-ter extensions (cf. figure 3 in reference [6]). The net result is an exceedingly stable N-RNA structure. The C-ter extension of RSV N is very flexible and partly invisible in the atomic structure, and the homologous domains of measles and Sendai virus are intrinsically disordered and bind to P [11,12]. For the three N-RNA ring structures, the RNA binds in a positively charged (blue) cleft between the N-ter and C-ter domains (Figure 2, bottom row). This channel, from which the RNA has been

Figure 1



Electron micrographs of NSV nucleocapsids. All samples were negatively stained except for the nucleocapsids of Pichinde virus that were prepared by metal shadowing. The nucleocapsids of rabies, influenza, Pichinde and Rift valley fever virus were isolated from virus, whereas the measles and Marburg nucleocapsids were produced in recombinant form. The second panel of measles virus shows the RNP after trypsin digestion. The influenza virus nucleocapsids in the large panel were prepared for EM in 150 mM NaCl whereas the circular complexes were prepared in 15 mM salt. All micrographs have the same magnification indicated by the bar underneath the circular complexes of influenza virus. Picture of RVFV nucleocapsids courtesy of Raymond and Smith [18••], picture of Pichinde virus nucleocapsids courtesy of Young and Howard [28] and pictures of measles virus N-RNA courtesy of Desfosses and Gutsche [14•].

removed for clarity, appears as a hole in the nucleoprotein. For BDV, residues mutated in the cleft between the two domains prevent RNA binding, suggesting the same binding site [13].

A remarkable difference between the rhabdovirus and RSV N-RNA rings is that the RNA binds at the inside of the rings for the rhabdoviruses, and the outside of the ring for RSV. The internal position of the VSV RNA suggested by the crystal structure was confirmed in the virus particle [2•], where the helical turns with the smallest diameter (at the tip of the bullet) have a very similar structure to those of the recombinant N-RNA rings. The external position of the RNA at the outside of the RSV N-RNA rings was supported by docking the RSV N-RNA crystal structure in a helical reconstruction of measles N-RNA [14•]. This reconstruction also showed that the C-ter domain of recombinant N points towards the inside of the helical coil. As the inside of this helix is much too narrow to accommodate the 13 N-tails per helical turn of the nucleocapsid, this explains why the intact nucleocapsid forms a loose coil; presumably the natively unfolded N-tails [11] must escape from the interior of the helix between the helical turns [15••]. Removal of N-tail with trypsin allows the helical turns to touch, resulting in a tight coil (Figure 1).

Figure 3 shows the atomic structures of influenza, RVFV and Lassa nucleoproteins, all crystallised without RNA. In contrast to the nsNSV, these three nucleoproteins are all different and no structural homology can be found. The influenza virus nucleoprotein is a helical globular protein