**Virus structure**

**First comparison of two animal viruses in three dimensions**

from Stephen C. Harrison

The full three-dimensional structures of two small, positive-stranded RNA animal viruses, reported recently in *Nature* and *Science*, are dramatic crystallographic achievements as well as major contributions to structural virology. The technical significance of the human rhinovirus (HRV14) structure described by Rossman and colleagues has already been detailed in these columns. With the publication of the poliovirus structure by Hogle, Chow and Filmen, the structure of these two related viruses can now be compared. It is clear that they have many remarkable details in common, a number of which they also share with the RNA plant viruses. Moreover, the structures have important implications for assembly, antigenicity, receptor binding and uncoating.

The so-called picornaviruses, of which poliovirus and rhinovirus are examples, are built from 60 copies of each of four viral proteins, known as VP1–4, and a single genomic RNA with a small protein (VPg) at its 5′ terminus. All the viral proteins are synthesized as part of a polyprotein precursor cleaved as shown in Fig. 1a. The cleavages generating VP0 (= VP4 + VP2), VP3, and VP1 are carried out by a virus-encoded protease. Usually copies of VP0, VP1, and VP3 also form an RNA-free shell, known as a procapsid and believed to be a precursor to the virus particle. The cleavage of VP0 to VP4 + VP2, required for RNA incorporation, may be autocatalytic or it may be facilitated by some other part of the capsid structure. The mature virion appears to contain one or two uncleaved VP0 chains.

How do these proteins fold and how do the proteins form a virus particle? The poliovirus and rhinovirus structures show that the folded polypeptide chains of VP1, 2 and 3 all have very similar core structures—β-rolls that are strikingly similar to the S domain of tomato bushy stunt virus (TBSV) and southern bean mosaic virus (see Fig. 1b). These compactly folded cores have long N-terminal extensions, shorter C-terminal extensions, and significant insertions at the positions of one or more loops between β-strands. VP4 is, in effect, part of the N-terminal extension of VP2. The proteins are packed in the icosahedrally symmetrical particle, as shown in Fig. 1c, with the N-terminal extensions (including VP4) toward the inside of the particle. In poliovirus and HRV14, the character and length of the extensions and insertions are very similar, but comparison with other picornaviruses suggests some variability in these elaborations, probably significant for antigenicity.

No features attributable to RNA are seen in either of the electron density maps. The crystallographic methods used would only have seen RNA, segments bound in identical ways to all 60 proteins. Thus, just as in the plant viruses, there is no fixed way in which RNA interacts with ordered parts of the protein.

The disposition of N-terminal arms in these structures is remarkable. The arm of VP1 folds into a large loop lying against the inner surface of VP3. The arm of VP3 extends out of VP1 from its N-terminus near the 5-fold axis of the particle, where it interacts with four other symmetrically related structures in a five-stranded β-structure that Hogle et al. describe as a twisted tube. The N-terminus of VP4 lies near the twisted tube, so that five VP4 segments contribute to the 5-fold structure.

The remaining part of VP4 extends toward the particle’s 3-fold symmetry axis, and its C-terminus lies near the N-terminus of VP2, from which it is cleaved. This description shown in the diagram in Fig. 1c implies that the arms can fold only as the subunits associate—that is, the important tertiary structure of the N-terminal extensions cannot be realized without the quaternary organization of the capsid.

**What are the implications of this pattern of organization for viral assembly?**

Steps thought to occur in the virus formation pathway include...
processing cleavages to form VP0, VP1 and VP3. Association of these units to form a 6S protomer, and association of 6S species into pentameric 14S structures. The VP0/VP1/VP3 assembly unit in these viruses is clearly defined by the interactions of the N-terminal arms just described, as well as by the dispositions of C-terminal extensions from VP1 and VP3 (see Fig. 1c). The significance of the 14S species (a pentamer of these protomeric assembly units) is also clear from the convergence of five VP3 N-termini in the twisted tube and their association with segments of VP4 (Fig. 1c). The phenomenon of regulating assembly by interaction of extended arms was first discovered in the plant viruses.14. The assembling units are not just rigid, preformed 'bricks'; they have extensions that reach across adjacent units to form second- and third-nearest neighbour interactions.

Another conclusion about assembly that can be drawn from the HRV14 and poliovirus structures is pointed out in both papers. cleavage of VP0, VP1 and VP3 from each other is probably an early event, preceding formation of the 6S protomer, since the C- and N-termini generated by the cleavages are not at all near each other in the virion. Indeed, N-termini are on the inside of the particle and C-termini are on the outside.

By contrast, the proximity of the C-terminus of VP4 and the N-terminus of VP2 is consistent with the later occurrence of the corresponding cleavage. The position and character of this junction gives no obvious clue, however, to the observed linkage between VP0 cleavage and RNA incorporation. Polio- and rhinovirus proteins, unlike many plant-virus coat proteins and unlike the core subunits of alphaviruses, do not have positively charged 'R-domains' at the extremities of their N-terminal extensions. The mechanism of RNA condensation in these structures is therefore not yet evident. It is probably appropriate to consider again whether RNA is inserted into a preformed capsid, as suggested by previous in vivo kinetic data on poliovirus, or whether RNA is condensed by association with 14S pentamers as they assemble into a shell.

The implications of these structures for studies of viral antigenicity are no less striking. Viral epitopes can be assigned by
The question of whether major extinctions in the history of life have a clocklike spacing has been hotly debated. Some good syntheses of palaeontological data have resulted, and the debate has produced a general search for other periodic signals in the Earth's history. One possibility is periodicity in the geological record of the reversals of the Earth's magnetic field — though the consensus has been that the fine structure of the magnetic record is purely stochastic. A danger with all such analyses is that they tend to yield 'an answer', which may hide real processes behind the statistical methods used, as is well illustrated by the recent history of this subject.

In 1983, Negri and Tewari argued for a 30-Myr stationary periodicity in the magnetic field and Marzouk et al. claimed a 15-Myr periodicity. Earlier this year, from my own analyses, I recognized a 30-Myr signal in phase with, and possibly a harmonic of, the 15-Myr signal. The 15-Myr analysis was devoted in Nature and now, on page 409 of this issue, Lutz challenges my case for a 30-Myr periodicity. He has shown by an elegant argument that the 30-Myr signal is probably sensitive to the length of the time series: when the record is truncated by progressively eliminating the most recent events, the spectrum changes, showing that the 30-Myr peak is an accident of record length. Lutz is correct. And I apologize to the readers of my earlier paper.

While the implications of Lutz's study for the other claims of periodicity in magnetic reversals are not yet clear, since those studies used different statistical techniques, they will now have to be examined very carefully. The new result also has implications for the periodicities in biological extinction and impact cratering — that have been claimed. Two of the three studies of extinction used essentially the same statistical techniques that I used with the magnetic data, but as I pointed out, the extinction and magnetic data are different. I am happy to report...