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*[1] and in *Science*[2], are dramatic crystallographic achievements as well as major contributions to structural virology. The technical significance of the human rhinovirus (HRV14) structure described by Rossmann and colleagues[1] has already been detailed in these columns[3]. With the publication of the poliovirus structure by Hogle, Chow and Filmen[2], 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 from a single genomic RNA with a small protein (VPg) at its 5′ terminus[4]. All the viral proteins are synthesized as part of a polyprotein precursor cleaved as shown in Fig. 1*a*. The cleavages generating VP0 (= VP4 + VP2), VP3, and VP1 are carried out by a virus-specific protease. Sixty copies of VP0, VP1 and VP3 can form an RNA-free shell, known as a procapsid and believed to be a precursor to the virus particle[4]. 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 they 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 domains of tomato bushy stunt virus (TBSV) and southern bean mosaic virus[5–7] (see Fig. 1*b*). 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. 1*c*, 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 new electron density maps. The crystallographic methods used would only have seen RNA segments bound in identical ways to all 60 protomers. Thus, just as in the plant viruses[5–7], 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 beneath 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.*[2] 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. 1*c*, 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 virion formation pathway include the initial

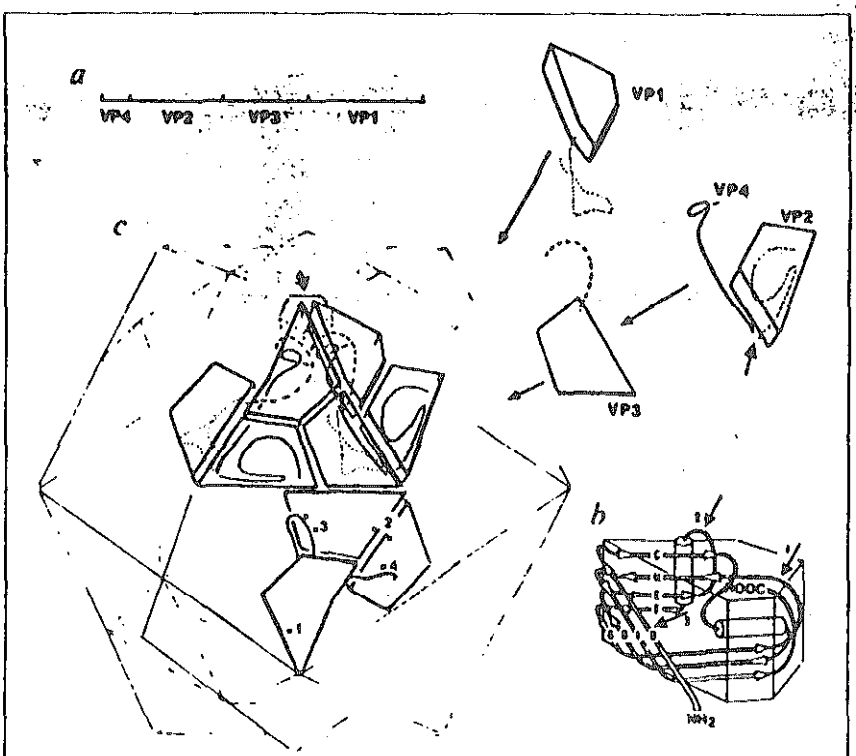

Fig. 1. *a*, Order and approximate relative sizes of VP1 - 4 in the polyprotein from which they are cleaved. *b*, Folded structure of the polypeptide chain in the 'core' of VP1, VP2 and VP3 (adapted from Hogle *et al.*, ref. 2). Such a domain, based on two β sheets, is sometimes termed a 'swiss roll' or 'jelly roll'. The arrows labelled 1, 2 and 3 indicate the positions of significant insertions in VP1, VP2 and VP3 respectively, both in poliovirus and HRV14. Since the upper part of the diagram corresponds to the outside of the virion, all the insertions, as well as the C-terminus, can be seen to project outwards. *c*, Packing of VP1 - 4 in the capsids of HRV14 and poliovirus. Three protomers (1 copy each of VP1 - 4) are shown, with the elements of the upper right-hand protomer exploded from the icosahedral particle. The full capsid contains 60 protomers, symmetrically covering the icosahedral shell (see Fig. 2*b* in ref. 1). Here special attention is given to the N-terminal arms of VP1 - 3 and to VP4 (drawn as for poliovirus[2]; HRV14 seems essentially similar[1]). The arm of VP1 is shown dotted, that of VP3 dashed and the arm of VP2 and all of VP4 solid. Note the way the arm of VP3 loops under VP1 and interacts with four other VP3 arms in a twisted tube around the 5-fold axis (indicated by heavy curved arrow and outlines of a pentagonal prism). A loop near the N-terminus of VP4 also 'backs up' this twisted tube structure. The point at which VP4 is cleaved from VP2 is shown by an arrow on the exploded VP2 subunit. Clearly VP4 is part of the N-terminal extension of VP2. In the protomer in the lower half of the figure, positions are indicated of four clusters of residues (numerals 1 - 4), identified by Hogle *et al.*[2] as principal neutralization sites in poliovirus. Similar sites have been identified in HRV14 (refs 1, 13). The insertion and C-terminal extension of VP1 are also shown, as residues in them contribute to clusters 3 and 4 respectively. Note how they enhance the overall contribution of VP1 to the viral surface.

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[1,2]: 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

various methods but probably the most specific involves isolation and characterization of 'monoclonal release mutants' — viral mutants selected for resistance to a neutralizing monoclonal antibody. Correlation of such mutations with three-dimensional structure of a viral protein was shown to be useful by Wiley *et al.*[17], who found that in epitopes thus identified in influenza virus, haemagglutinin clustered on the surface of the subunit, often in protruding loops. Hogle *et al.*[2] comment that sites located in this way in poliovirus[11,12] appear to cluster into three or four patches on the surface (see Fig. 1c) and a similar but not identical set of patches in HRV14 are described by Rossmann *et al.*[1] from the work of Sherry and Rueckert[11]. All the mutation sites occur in exposed loops, which are fully accessible to antibody binding. These loops include all the protruding insertions in the three principal poliovirus subunits and two of the three in HRV14. The polio VP1 C-terminus, draped over VP3, is also implicated. Residues in such loops can probably be more variable in sequence than residues in the core.

The way in which the insertions and C-terminal extensions decorate much of the viral exterior suggests a mechanism of antigenic disguise, in which potentially variable structures cover much of the accessible antigenic surface. Similar suggestions have been made for influenza virus haemagglutinin, where the conserved residues essential for receptor (sialic acid) binding are in a deep pocket, inaccessible to antibodies[18], and where acquisition of a glycosylation site in one strain has led to the covering of a protein epitope by the new oligosaccharide[19].

The unanticipated similarity of the cores of VP1, VP2 and VP3 to each other and to the S-domains of plant-virus coat proteins emphasizes how alike small positive-stranded RNA viruses are in both plant and animal hosts. Such a relationship was already evident from the homology of viral RNA-dependent RNA polymerases, pointed out by several groups[16-18]. The implications for our understanding of viral evolution cannot yet be adequately assessed. A possibility, with important consequences for virology, is that animal and plant viruses share a fairly recent history, perhaps through the mediation of insect hosts.

An important factor for tissues tropism and pathogenesis of a virus is its binding to receptors. Picornavirus receptors have been partly characterized but not yet purified, and different members of the family appear to have different receptors[4]. The shape and packing of VP1 gives rise to a depression on the surfaces of HRV14 and of poliovirus, which Rossman *et al.*[1] suggest may be the site of receptor interation. VP1 has been implicated in receptor binding in foot-and-mouth disease virus[20].

One consequence of binding to a cell is that these viruses undergo a conformational change that leads to release of VP4 (ref. 4). Since VP4 is an internal protein, and since the core domains are rather tightly packed, the shell must expand or breathe in some manner for VP4 to escape. The expansion of plant viruses such as TBSV provides a plausible model for such a process[20]. This transformation is a cooperative rearrangement of subunit packing in the shell, conserving many of the interactions in the native particle while a few others are altered or ruptured.

It has been suggested[21] that neutralization of poliovirus involves cross-linking of a few subunits by divalent antibody molecules, which would indeed inhibit a cooperative structural transition. Studying altered forms of the picornavirus particles may thus provide significant insight into initial events in uncoating. Understanding of subsequent events will require information not yet available from cell biological experiments on the form in which RNA enters the cytoplasm. Is it still encapsidated (for example, by VP1–3), or is uncoating coupled to membrane traversal? Either possibility leads to structural puzzles.

It should be evident from this summary that X-ray crystallography has just begun to contribute to our understanding of virus assembly and viral pathogenesis. The two structures just completed show that the technology and methodology — developed in work on plant virus structures[6-8,22] and first applied to problems of human viral infection in work on influenza virus haemagglutinin[23] — are firmly in place. Indeed, the HRV14 and polio virus studies make an interesting contrast in this respect, for Rossmann's group has used synchrotron data collection and a supercomputer, whereas Hogle's group has relied on a laboratory X-ray generator and a small VAX computer. Methods aside, what is evident from the parallel features of these two structures is that elucidation of the regulated assembly, of the complex antigenicity, and of the incompletely characterized binding and uncoating of these viruses can now proceed from clear three-dimensional pictures. □

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Geology

Rise and fall of periodicity

from David M. Raup

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 (and perhaps still is) 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 problems with the data set and statistical methods used, as is well illustrated by the recent history of this subject.

In 1983, Negi and Tiwari[1] argued for a 32-Myr stationary periodicity in the magnetic field and Mazaud *et al.* claimed a 15-Myr periodicity. Earlier this year, from my own analysis[2], I recognized a 30-Myr signal in phase with, and possibly a harmonic of, the 15-Myr signal. The 15-Myr analysis was debated in *Nature*[3], and now, on page 409 of this issue[4], Lutz challenges my case for a 30-Myr periodicity. He has shown by an elegant experiment that the 30-Myr signal is predictably 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[5] that have been claimed. Two of the three studies of extinction used essentially the same statistical techniques that I used with the magnetic data[6], but, as Lutz points out, the extinction and magnetic data are different. I am happy to report