

The dsRNA *Viridae* and their catalytic capsids

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dsRNA viruses share similar replicative strategies and the same unusual inner capsid architecture. The fold of the inner capsid protein is not conserved, however.

The structures of viral capsids, the protein coats that protect viral DNA or RNA, have long been studied as paradigms for how proteins interact with each other. While icosahedral virus capsids represent some of the largest complexes for which structures have been determined to date, they are also some of the simplest, often consisting of just one polypeptide building block used multiple times in constructing the capsid shell. Frequently the capsid functions as a vehicle to deliver the viral genome to its destination in the host cell. In this case, the component proteins serve a structural role and have no enzymatic activities. The double-stranded RNA (dsRNA) viruses, however, differ from other groups of viruses in that they never release their genome from its capsid. Therefore their capsid must function not only as protective armor but also as a machine that synthesizes and modifies mRNA. On page 725 of this issue, Naitow *et al.*¹ present the atomic resolution structure of one such enzyme complex, the capsid of L-A virus, a member of the Totivirus family. They find a capsid architecture typical only to the dsRNA viruses and yet a capsid protein with a novel fold unlike that of other dsRNA virus proteins studied. In their description of the capsid protein, they also detail the first structure to date of an mRNA decapping enzyme.

Although dsRNA viruses are a diverse group, they share a common replicative strategy that is reflected in their capsid architecture (reviewed in ref. 2). In addition to the *Totiviridae*, such as the L-A virus, the *Reoviridae*^{3–5}, the *Cystoviridae*⁶, and the *Birnaviridae*⁷ have also been structurally characterized. Members of these families all have a segmented dsRNA genome that is surrounded by 2–3 concentric protein shells. These form the complete virion capsid. The outermost capsid shells function in viral attachment and host cell penetration, while the innermost ‘core’ enters the host cell cytoplasm, where it serves as a miniature nucleus. It thus acts as a compartment specialized to

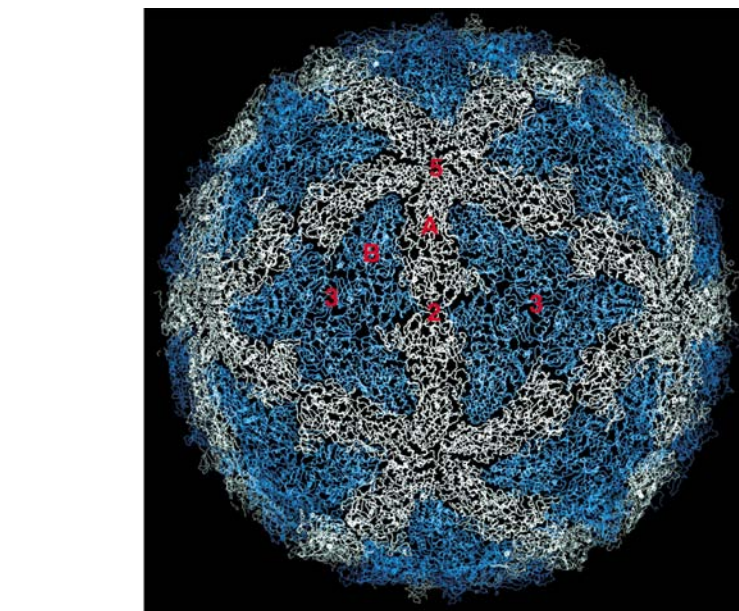


Fig. 1 The inner capsid architecture of the *Reoviridae*^{14,15} is indistinguishable from that of L-A virus (compare with Fig. 1 of Naitow *et al.*¹). An icosahedral five-fold axis is labeled (number 5). Other numbers refer to two- and three-fold symmetry (number 2 and 3), respectively. Protein subunits forming sets A and B (letters A and B, see text for discussion) make different, non-equivalent sets of specific contacts with their neighbors. While such a non-equivalent arrangement is unusual for viral capsids, it is typical for dsRNA viruses.

transcribe the dsRNA genome into functional mRNA, which is passed into the host cell cytoplasm for translation. The protein capsid always contains an RNA-dependent RNA polymerase — frequently part of a multi-component transcription complex — that functions as a replicase during viral assembly and a transcriptase thereafter. In the *Reoviridae*, the transcription complex appears to be tethered beneath the five-fold icosahedral axis of the viral capsid, which contains an aperture to allow passage of the newly synthesized mRNA into the cytoplasm^{8–10}. The inner capsid in dsRNA viruses consists of 1–3 protein species. With the exception of the *Birnaviridae*⁷, the innermost layer consists of 120 copies of one polypeptide species. This copy number is unusual and ‘forbidden’ according to the principles of quasi-equivalence (explained below) by

which most icosahedral viral capsids are constructed.

The L-A virus is among the simplest dsRNA viruses known (reviewed in ref. 11). Because it is transmitted intracytoplasmically and never leaves its *Saccharomyces cerevisiae* host, it has not had to devise a strategy for entry into a host cell and therefore has no outer capsid shell. It consists of just two proteins: 120 copies of the capsid protein Gag and, packaged with the RNA genome, ~2 copies of the polymerase pol. Cryo-EM reconstruction of L-A¹² has shown an arrangement for Gag that does not conform to the tenets of quasi-equivalence described by Caspar and Klug in 1962¹³. Icosahedral viruses have strict 60-fold symmetry in their capsid structure, such that all 60 subunits interact identically with each other. That is, in the simplest

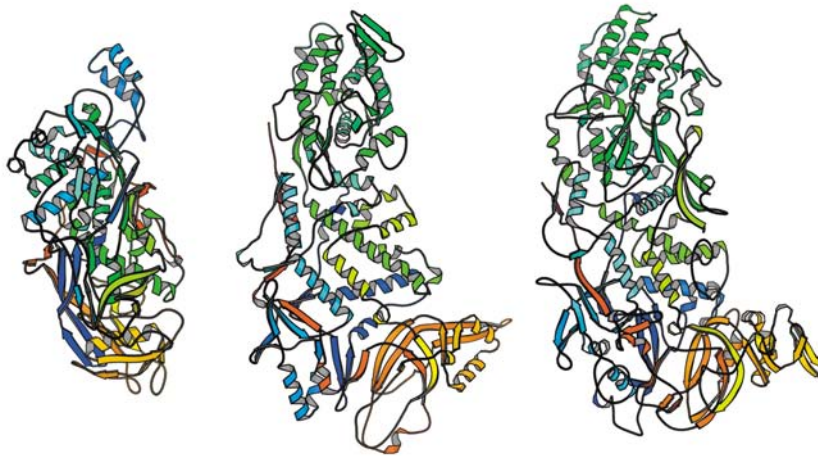


Fig. 2 The inner capsid protein of L-A virus (left) has a different fold from the inner capsid protein of bluetongue virus VP3 (middle) and orthoreovirus λ 1 (right).

viruses, each of 60 polypeptides has precisely the same interface with its neighbors. Nevertheless, for more complex viruses, 60 copies of the capsid protein cannot form a shell large enough to accommodate the viral genome, and an icosahedral subunit typically consists of protein multimers. In such larger capsids all monomeric subunits are no longer related by strict icosahedral symmetry; however, they are often arranged quasi-equivalently. Their local environments remain similar and the interactions between adjoining subunits are mostly conserved. Thus, a given protein has had to evolve only one set of specific interactions with its neighbors.

What is so striking about the architecture of the L-A virus inner capsid reported by Naitow *et al.*¹ is that the capsid protein can make two completely different sets of specific interactions. Thus, in L-A, there are two sets of Gag, A and B. Five monomers of Gag in set A directly surround the icosahedral five-fold axis and form a pore through which mRNA may exit the capsid shell. Five copies of Gag in set B are interdigitated between the A subunits to form a decamer^{1,12} (Fig. 1; see also Fig. 1 of Naitow *et al.*¹). Except at a very small interface between Gag A and Gag B from adjacent decamers, the interface between A and its neighbors differs from the contacts between B and its neighbors almost entirely^{1,12}. In comparison, VP3 and λ 1, the inner capsid proteins of two members of the *Reovirus* family (bluetongue and orthoreovirus, respectively), have the same non-equivalent arrangement as Gag^{14,15} (Fig. 1). However, both VP3 and λ 1 are higher molecular weight proteins, almost twice as large as Gag, and are plate-like: they form a protein shell that is about

half as thick as the L-A capsid (20 *versus* 46 Å) with a surface area roughly three times as extensive. Their function appears to be mostly structural — to organize the outer capsid shells, to protect the dsRNA genome, and to help organize the capsid interior. Gag, on the other hand, in addition to its structural role, also functions as an enzyme. It is globular with an active site trench. To form whole capsids, copies A and B of the VP3 and λ 1 gene products adopt different conformations *via* domain/subdomain reorientations. They also differ in the conformations of loops at their peripheries. In contrast, Gag A and B differ only with respect to the conformations of their peripheral loops. Finally, the fold of Gag bears only a very faint resemblance to either VP3 or λ 1: all three proteins are predominantly α -helical, they have an α -helical bundle nearest the icosahedral five-fold axes and β -sheets near the two- and three-fold axes. Closer inspection shows that Gag's tertiary structure is different from that of VP3 or λ 1.

Based on the observations that the dsRNA virus inner capsid shell seems always to consist of 120 subunits arranged in the same non-equivalent fashion, it is tempting to speculate that all dsRNA viruses share the same inner capsid architecture and have, in fact, evolved from the same ancestor. The atomic resolution structures of the bluetongue and reovirus cores show that VP3 and λ 1 have recognizably similar folds and support the notion that the *Reoviridae* share a common lineage. The structural dissimilarity between Gag and either VP3 or λ 1 suggests that either the *Reoviridae* and the *Totiviridae* independently evolved their unusual inner capsid architecture or that their inner capsid proteins have diverged

dramatically — a view favored by Naitow, *et al.*¹.

The dsRNA viruses share a similar replicative strategy and the same inner capsid architecture, but their capsid proteins may have different structures. They also adopt different strategies for protecting their mRNA once it is extruded into the cytoplasm. Since eukaryotic mRNA is protected from degradation at its 5' end by a 7-Me-GpppG capping moiety¹⁶, *Reoviridae*² and *Birnaviridae*¹⁷ have developed elaborate apparatuses to cap newly synthesized viral mRNA on its way out of the viral capsid. Rather than stabilizing its own mRNA, the L-A virus deprotects the host cell mRNA by decapping it, thus turning it into a decoy that will divert the host cell Ski1p/Xrn1p exoribonuclease system away from the viral mRNA^{18,19}. Encoding only Gag, which functions simultaneously as a capsid protein and as a decapping enzyme, rather than encoding the four enzymatic activities required for capping RNAs, considerably reduces the size of the viral genome inside the capsid.

Biochemical studies have shown that His 154 of Gag forms a covalent adduct with 7-methyl-GMP (m⁷GMP), an inhibitor of the decapping reaction²⁰. The position of His 154 locates the Gag active site on the surface of the capsid, where it has ready access to cellular mRNA¹. Four loops, including residues 144–163, surround a trench that likely binds cellular mRNA. Because the side chain of His 154 extends into the solvent in the absence of mRNA substrate and the active site loops appear flexible, Johnson and co-workers¹ suggest that loop movement during catalysis may enclose the mRNA in the trench. Efforts are currently underway to determine a co-crystal structure of the L-A capsid bound to m⁷GMP.

The present structure only hints at the organization of the capsid interior, where the dsRNA is loosely packed close to the Gag shell. As in all viruses, the interior does not have icosahedral symmetry, and the transcription complex is not visible in the crystal structure¹. By analogy with the *Reoviridae* and in order to place pol close to the presumed site of mRNA extrusion, it has been postulated that pol may lie directly beneath Gag near the five-fold icosahedral axis^{1,12}. The specifics of how pol works there — first as a replicase and subsequently as a conservative transcriptase²¹ remain a mystery. In fact, the mechanism of how replication and transcription take place in dsRNA viruses is only beginning to emerge now with the publication of the first dsRNA polymerase structure²².

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Where chaperones and nascent polypeptides meet

Véronique Albanèse and Judith Frydman

Two recent studies provide evidence for a direct interaction between the ribosomal protein L23 at the exit tunnel of the ribosome and the bacterial chaperone trigger factor, and between the eukaryotic L23 homolog and the signal recognition particle. These findings indicate that the exit site of the ribosome may physically link translation to the cytosolic components that guide nascent polypeptides to their correct fate.

Proteins are synthesized on the ribosome, an organelle-sized ribonucleoprotein complex that transforms the linear genetic code from nucleic acid into polypeptide chain. After synthesis, each polypeptide chain adopts a specific three-dimensional structure and performs a specific function in the cell. The crystal structure of the ribosome¹ has opened the door to understanding the mechanism of this complex machinery at high resolution and how it interacts with other cellular components.

As newly translated polypeptides emerge from the ribosome, they face a formidable task in the crowded environment of the cell — finding the right location in the cell and folding into their correct structure for proper function. While some newly synthesized polypeptide chains fold in the cytosol, others are targeted to intracellular compartments prior to folding. For instance, proteins destined for the secretory apparatus carry an N-terminal signal sequence, which is recognized cotranslationally by the signal recognition particle (SRP) and targets the protein to the endoplasmic reticulum (ER)². Translation is temporarily halted upon engagement of SRP and resumes when the ribosome nascent chain complex docks onto the Sec61 translocon².

Folding of cytosolic proteins is complicated by the vectorial nature and the rela-

tively slow elongation rate of the translation process. The nascent chain is initially constrained in the 100 Å long ribosomal exit tunnel, which is too narrow (~15 Å) to allow either folding or aggregation to occur^{1,3}. However, after emerging from the ribosome, the partially synthesized, aggregation-prone polypeptide is exposed to the crowded cellular milieu^{4–6}. These nascent polypeptides interact cotranslationally with several cellular proteins called molecular chaperones, which recognize and bind exposed hydrophobic sequences, thereby preventing aggregation and facilitating folding to the native state^{4–6}. Molecular chaperones also bind and stabilize stress-denatured proteins, thus raising the question of how these chaperones are recruited to the ribosome-bound polypeptide. In the simplest model, chaperones could recognize the unfolded polypeptide in a stochastic manner, just as they would recognize any other unfolded protein in the cytosol. Alternatively, folding and translation could be coordinated by the specific recruitment of chaperones to the site of protein synthesis. Indeed, studies in eukaryotic cells provide evidence for the existence of mechanisms that couple translation and chaperone-mediated folding^{7,8}. In a recent issue of *Nature*, Kramer *et al.*⁹ provide evidence that, in a bacterial system, molecular

chaperones are not only functionally, but also structurally coupled to translation.

The role of chaperones in *de novo* folding is best characterized in *Escherichia coli*. Three bacterial chaperones are thought to participate in the folding of newly translated polypeptides in the cytosol: trigger factor (TF), the Hsp70 DnaK and the chaperonin GroEL (Fig. 1b). TF appears to be the first player in the folding of nascent chains, recognizing relatively short hydrophobic stretches and protecting them from aggregation¹⁰. DnaK can then bind longer chains and allow larger polypeptides to fold^{11,12}. TF and DnaK seem to have partially overlapping functions, and mutants that are defective in the chaperone functions of both proteins exhibit a synthetic lethal phenotype^{11,12}. Finally, GroEL functions post-translationally to assist folding of a subset of cytosolic proteins¹³.

What is the mechanism that brings chaperones to bind nascent chains? Because of its early association with translating polypeptides and the presence of a ribosome-targeting domain, TF appeared to be a good candidate to gain insight into the interaction of chaperones with ribosomes. Kramer *et al.*⁹ demonstrate that binding of TF to nascent chains is mediated by a direct and specific interaction of this chaperone with the ribosomal protein L23.

