Those magnificent molecular machines: logistics in dsRNA virus transcription

Double-stranded (ds) RNA viruses face two major challenges when infecting a cell: the cellular replicative machinery does not operate on dsRNA genomes, and dsRNA provokes a strong apoptotic response. To overcome these problems, dsRNA viruses conceal their genomes from the host cell in an enclosed icosahedral viral core, but then need to carry with them all the necessary enzymatic requirements for replication and transcription. dsRNA virus genomes are often segmented (up to 12 segments). Whereas the transcription and capping reactions have been well documented (see, for example, Luongo et al., 2000; Reinisch et al., 2000), the mechanism used for genome packaging, and the site for minus strand synthesis, have not been well defined. A paper by Diprose et al. (2001) now sheds new light on the trafficking of the precursors and transcription products of an active dsRNA virus core. These cores are symmetrical (icosahedral) particles with 2-, 3- and 5-fold axes of symmetry (Figure 1; Grimes et al., 1998).

As there are no data to suggest the mechanisms for the RNA replication cycle in Reoviridae, I will use an analogous model to convey the complexities of dsRNA virus genome duplication. Insight into the steps involved has been provided by studies of the bacterial dsRNA virus phi6 (Figure 2; Butcher et al., 1997; Mindich, 1999; Poranen and Bamford, 1999). As the packaging density is close to that of crystalline nucleic acid and the transcription and minus strand synthesis velocities are 30 and 120 nucleotides per second, respectively (Makeyev and Bamford, 2000a,b), a severe logistical challenge is apparent. Simultaneous transcription of all the segments utilizes ~300 nucleotides per second, releasing pyrophosphates and making nascent mRNA molecules ~30 bases in length. Intuitively, this trafficking had been appreciated, but there had been little understanding of how this might occur.

The X-ray structures of the cores of other dsRNA viruses, the bluetongue virus (Grimes et al., 1998; Gouet et al., 1999) and reovirus (Reinisch et al., 2000), have been published over the last few years. These viruses are >50 MDa particles with a diameter of some 700 Å. Although the non-icosahedral portions of the structures (including the polymerase subunit) have not been resolved, a wealth of data on the capsid structure and genome organization has been provided by these structures.

The report by Diprose et al. (2001) provides a number of crystallographic snapshots of the bluetongue virus core, including oligonucleotide, nucleotide, pyrophosphate and a number of ions associated with the protein shell. Although these are static structures, they illustrate marvellously how the precursors and products may be trafficking during transcription. When the core particle enters the cell, it senses the changed ionic environment, activates transcription and obviously must have maximal access to the cellular NTP pool. Diprose et al. (2001) observed that physiological magnesium concentration (9 mM) triggers conformational changes in the core. The particle expands slightly, particularly around its icosahedral 5-fold axes, enlarging the pores positioned at these locations enough to accommodate single-stranded (ss) RNA. These pores bind nucleotides with their bases stacked along the icosahedral 5-fold axis of the virus particle and the phosphate moieties facing the pore walls. Oligonucleotide incubation revealed an even stronger difference in electron density within these pores, accounting for ~15 bases. These observations confirm that the pores serve as the portals for mRNA exit (X in Figure 2), in accordance with previous lower-power electron-microscopy-based results (see, for example, Lawton et al., 1997).

In a search for NTP signals, it was observed that between two of the inner core T2 proteins, which are juxtaposed to one another in different conformations (N in Figure 2), there is an

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**Fig. 1.** A schematic illustration of a dsRNA virus genome replication cycle. The infection process brings into the cell the core containing the dsRNA genome segments (a). Upon uncoating, the particle is activated to synthesize single-stranded (ss) RNA copies from each genome segment. These exit through machinery occupying the icosahedral particle vertices (b). The ssRNA segments are of positive polarity and are utilized as mRNAs (c), leading to the synthesis of proteins that assemble into new polymerase complex particles (d). The transcripts from (b) are also utilized as genomic precursors, which are packaged into the procapsids, also through the machinery located at the icosahedral vertices (e and f). This process requires energy in the form of NTPs. The packaged ssRNA genomic precursors are used as templates from which the polymerase subunits associated with the particle produce progeny genomic dsRNA segments (i). The packaged particles mature into infectious virions, which are released from the cells. Provided by Dr Eugene Makeyev.
The exit of the pyrophosphate may take place through a pore on the VP7 layer, ensuring the virus its share of the cellular NTP pool. This arrangement obviously enriches NTPs in the proximity of the adjacent VP7 trimers (C sites, but drawn only on the P trimer for clarity). The right panel shows binding sites on the inner VP2 shell. The icosahedrally unique molecules A and B are in green and red, respectively. The difference electron density for the three binding sites is drawn: mRNA leaves the particle at site X, NTPs are thought to traverse the particle via the pore marked N and density due to a phosphate is marked F. Figure taken from Diprose et al. (2001).


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