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

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 (f). The packaged particles mature into infectious virions, which are released from the cells. Provided by Dr Eugene Makeyev.

Fig. 2. The left panel shows key features in the bluetongue virus VP7 layer. In the close-up, 260 VP7 proteins forming the core surface are depicted, with the VP3 layer beneath. The VP7 trimers that form the icosahedral asymmetric unit are marked P, Q, R, S and T and are shown in different colors. Difference electron density is drawn in white, with black shadows. NTPs bind at the inter-trimer site (I). Cations bind along all of the 3-fold axes of the 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).

Fig. 3. Cartoon showing the movement of the NTP raw material into the bluetongue virus core particle and the site of egress of the product mRNA transcript and by-products PPI, Pi and NDPs. Figure taken from Diprose *et al.* (2001).

additional pore to which NTPs/NDPs bind. The exterior side of this pore opens like a funnel through the outer protein VP7 layer, giving access to the bulk solvent. In addition to this pore, several positions on the outer shell (between some, but not all, of the adjacent VP7 trimers; I in Figure 2) were found to bind NTP. This arrangement obviously enriches NTPs in the proximity of the NTP pore, ensuring the virus its share of the cellular NTP pool. The exit of the pyrophosphate may take place through a pore on

the 2-fold icosahedral axis, as phosphate was found bound at this location.

It is amazing how serial structural snapshots are able to generate a framework of the dynamic events that must be taking place when this molecular machine is going at full steam (Figure 3). It is equally amazing that it was possible to collect this information in spite of the transient nature of the interactions studied. I would not be surprised if Diprose and co-workers are currently hectically collecting further data on transcribing cores, cursed with exiting mRNA blowing the crystals up. They seem to be enjoying projects that have been considered impossible to do.

References

- Butcher, S.J., Dokland, T., Ojala, P.M., Bamford, D.H. and Fuller, S.D. (1997) Intermediates in the assembly pathway of the double-stranded RNA virus $\phi 6$. *EMBO J.*, **16**, 4477–4487.
- Diprose, J.M. *et al.* (2001) Translocation portals for the substrates and products of a viral transcription complex: the bluetongue virus core. *EMBO J.*, **20**, 7229–7239.
- Gouet, P., Diprose, J.M., Grimes, J.M., Malby, R., Burroughs, J.N., Zientara, S., Stuart, D.I. and Mertens, P.P.C. (1999) The highly ordered double-stranded RNA genome of bluetongue virus revealed by crystallography. *Cell*, **97**, 481–490.
- Grimes, J.M., Burroughs, J.N., Gouet, P., Diprose, J.M., Malby, R., Zientara, S., Mertens, P.P.C. and Stuart, D.I. (1998) The atomic structure of the bluetongue virus core. *Nature*, **395**, 470–478.
- Lawton, J.A., Estes, M.K. and Prasad, B.V.V. (1997) Three-dimensional visualization of mRNA release from actively transcribing rotavirus particle. *Nature Struct. Biol.*, **4**, 118–121.
- Luongo, C.L., Reinisch, K.M., Harrison, S.C. and Nibert, M.L. (2000) Identification of the guanylyltransferase region and active site in reovirus mRNA capping protein $\lambda 2$. *J. Biol. Chem.*, **275**, 2804–2810.
- Makeyev, E. and Bamford, D.H. (2000a) Replicase activity of purified recombinant protein P2 of double-stranded RNA bacteriophage $\phi 6$. *EMBO J.*, **19**, 124–133.
- Makeyev, E. and Bamford, D.H. (2000b) The polymerase subunit of a dsRNA virus plays a central role in regulation of viral RNA metabolism. *EMBO J.*, **19**, 6275–6284.
- Mindich, L. (1999) Precise packaging of the three genomic segments of the double-stranded-RNA bacteriophage $\phi 6$. *Microbiol. Mol. Biol. Rev.*, **63**, 149–160.
- Poranen, M.M. and Bamford, D.H. (1999) Packaging and replication regulation revealed by chimeric genome segments of double-stranded bacteriophage $\phi 6$. *RNA*, **5**, 446–454.
- Reinisch, K.M., Nibert, M.L. and Harrison, S.C. (2000) Structure of the reovirus core at 3.6 Å resolution. *Nature*, **404**, 960–967.

Dennis H. Bamford

Department of Biosciences and Institute of Biotechnology, PO Box 56 (Viikinkaari 5), FIN-00014 University of Helsinki, Finland

Tel: +358 9 191 59100; Fax: +358 9 191 59098;

E-mail: dennis.bamford@helsinki.fi

Received January 21, 2002; revised February 8, 2002; accepted February 15, 2002

DOI: 10.1093/embo-reports/kvf074