Structural Studies on a dsRNA Virus

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Viruses are complex assemblies of macromolecules, and the simplest of parasites. They are not simple boxes of genetic information which use the equipment of the host cell for replication. Rather, they are complex bioloigal systems which achieve a variety of functions with considerable economy and elegance. We have studied the structure of Bluetongue virus (BTV). BTV is the prototype virus of the genus *Orbivirus,* and belongs to the family Reoviridae. This is the largest family of double-standed RNA viruses, and as such, these viruses face certain characteristic problems in their life-cycle. They enter the cell, usually shedding an outer capsid layer in the process to leave a smaller particle, termed the core in the case of BTV. This core remains intact in the cytoplasm of the infected cell where it is activated by the presence of nucleotide triphosphate substrates and commences to act as a factory, producing capped mRNA. The genome consists of 10 segments of RNA, which are transcibed independently by transcription complexes which appear to consist of three proteins, thought to possess helicase, polymerase, and capping activities. These are contained within an icosahedrally symmetric shell made up of 780 copies of VP7(T13) and 120 copies of VP3(T2). The structure is, therefore, an intriguing mixture of symmetries, some icosahedral and some not. Since the core contains nearly 1000 protein subunits, its analysis, by single-crystal x-ray diffraction, was a major undertaking. We were fortunate in being able to make use of beamline ID2 at the ESRF, which enabled the complex, crowded, and weak diffraction patterns to be resolved. We have determined the structure of the core of BTV-1 (SA) at approaching 3.5-Å resolution and that of BTV-10 (USA) at about 6.5-Å resolution. The higher resolution structure has revealed the fold and detailed interactions of the proteins which obey icosahedral symmetry whilst the comparison of the two structures at lower resolution reveals something of the positioning of the other proteins (the enzymatic complex) and, surprisingly, a substantial portion of the RNA genome of the virus. We are able to suggest a number of stages in the assembly of the virus and feel that the structure we observe throws light on the way the core acts as a transcriptional machine.