Virus Research 182 (2014) 5–20

Contents lists available at ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres

The molecular biology of Bluetongue virus replication

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ARTICLE INFO

Article history:
Available online 25 December 2013

Keywords:
Virus structure
Replication
Molecular biology
Host factors

ABSTRACT

The members of *Orbivirus* genus within the *Reoviridae* family are arthropod-borne viruses which are responsible for high morbidity and mortality in ruminants. Bluetongue virus (BTV) which causes disease in livestock (sheep, goat, cattle) has been in the forefront of molecular studies for the last three decades and now represents the best understood orbivirus at a molecular and structural level. The complex nature of the virion structure has been well characterised at high resolution along with the definition of the virus encoded enzymes required for RNA replication; the ordered assembly of the capsid shell as well as the protein and genome sequestration required for it; and the role of host proteins in virus entry and virus release. More recent developments of Reverse Genetics and Cell-Free Assembly systems have allowed integration of the accumulated structural and molecular knowledge to be tested at meticulous level, yielding higher insight into basic molecular virology, from which the rational design of safe efficacious vaccines has been possible. This article is centred on the molecular dissection of BTV with a view to understanding the role of each protein in the virus replication cycle. These areas are important in themselves for BTV replication but they also indicate the pathways that related viruses, which includes viruses that are pathogenic to man and animals, might also use providing an informed starting point for intervention or prevention.

1. Introduction

Bluetongue virus (BTV) is a complex, non-enveloped, double stranded RNA (dsRNA) virus and is the archetypal member of the genus *Orbivirus* of the family *Reoviridae*. The family *Reoviridae* comprises of segmented dsRNA viruses, possessing icosahedral non-enveloped capsids with a double layered architecture. This large group of dsRNA viruses represent the causative agents of several economically and medically significant diseases and as such present a great challenge in virological understanding.

BTV, the prototype of the *Orbivirus* genus is a pathogen of livestock and is common throughout the world including Europe, causing serious periodic outbreaks. Consequently it has been studied extensively as a model system for related viruses and substantial progress has been achieved in sequence, biochemical and structural studies of BTV. Indeed, BTV was the first virus of this genus to be characterised at a genetic level and in its structure and replication.

Through the testing study of this virus many innovations have been achieved which have now become the standard tools of molecular virology. BTV provided the first whole genome sequence of a dsRNA virus and the first virus-like particle (VLP), a game changing discovery, now in widespread use for many viruses both for vaccine development and as a tool for basic research. Indeed VLP based assay system has facilitated to dissect the structure of BTV and the virus assembly pathway. Simultaneously, the structure of an integral virus core protein was solved, which became a paradigm in the field. The structure was the first for a large RNA virus and was later merged with cryo-electron microscopy (cryo-EM) data to give a unique high resolution 3D image of the viral core. This structure constituted the first complex viral capsid and biological assembly to be solved at atomic resolution. Latterly the virus replication cycle, notably virus entry, replication and egress have been examined. Recent significant developments include the first helper virus free, *in vitro* T7 reverse genetics system for dsRNA viruses, which will allow major studies of how the virus causes disease (and generation of new vaccines), and the development of an *in vitro* cell-free assembly system which gives rise to infectious virus particles in the absence of cellular factors. Further the first 3D image of the whole virus has been obtained, illustrating how BTV may enter into the host cells.

These breakthroughs have provided the initial model system from which the understanding of the *Orbivirus* genus as a whole and to some extend the other members of the family has been possible. This review is focused on the molecular biology of the current understanding of BTV replication, the role of each viral protein in virus replication, the basic virological study of which has allowed rational designing of highly attenuated efficacious vaccines against BTV infection. This article summarises the current

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http://dx.doi.org/10.1016/j.virusres.2013.12.017

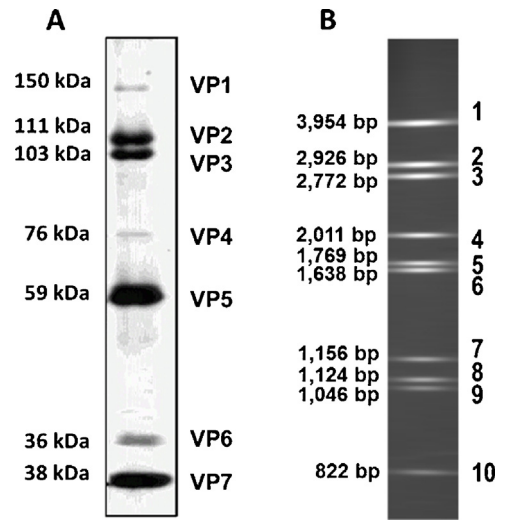

Fig. 1. The virion constituents. (A) SDS-PAGE of purified virions. The minor core proteins VP1, VP4 and VP6 are present at a relatively lower concentration to that of the remaining structural proteins. (Sizes are calculated based on molecular weights for BTV serotype 10). (B) The ten segments of the dsRNA profile of purified core particles. (Sizes for BTV serotype 10).

understanding of BTV replication at molecular and structural levels.

2. The virion structure

The structure and function paradigm of biological assemblies dictates that in order to comprehensively understand the biology of the virus, it is informative to know the structure and organisation of the virus. To this effect, significant progress has been made to identify the organisation of BTV at an atomic level. Initial transmission electron microscopy (TEM) of purified virus particles and density migration of chemically treated virions indicated an icosahedral double shelled structure of the virus particle, existing as both whole particles and cores from which the outer layer proteins were removed (Huismans et al., 1987b; Martin and Zweerink, 1972; Van Dijk and Huismans, 1980). Indeed, from the expression of individual subsets of structural proteins, using a recombinant baculovirus system, it was possible to reconstitute particles displaying the biophysical characteristics of particles obtained from virus preparations (French and Roy, 1990; French et al., 1990; Loudon and Roy, 1991). From this and protein profiling of purified particles and cores, the order and organisation of the virion has been established.(Matsuo and Roy, 2013).

The particle consists of seven structural proteins three of which (VP1, VP4 and VP6) are minor components having low relative concentrations in the particle in relation to the major components of VP2, VP3, VP5 and VP7 (Fig. 1A). These minor components are not necessary to reconstitute virus-like and core-like particles (CLP) by recombinant baculovirus expression, indicating that they are not components of the protein capsid and serve as encapsidated cargo.

The core particle itself comprises of VP3 and VP7 which encapsidate the ten genomic dsRNA segments (Fig. 1B) along with minor protein components. The core is then decorated with a further protein capsid layer to form mature virus. This constitutes VP2 and VP5 which act as outer capsid proteins and are surface exposed to a varying extent (Fig. 2A and B). Higher resolution cryo-EM (Hewat et al., 1994; Nason et al., 2004; Prasad et al., 1992) and X-ray crystal structure (Grimes et al., 1998) of the core particle revealed the finer

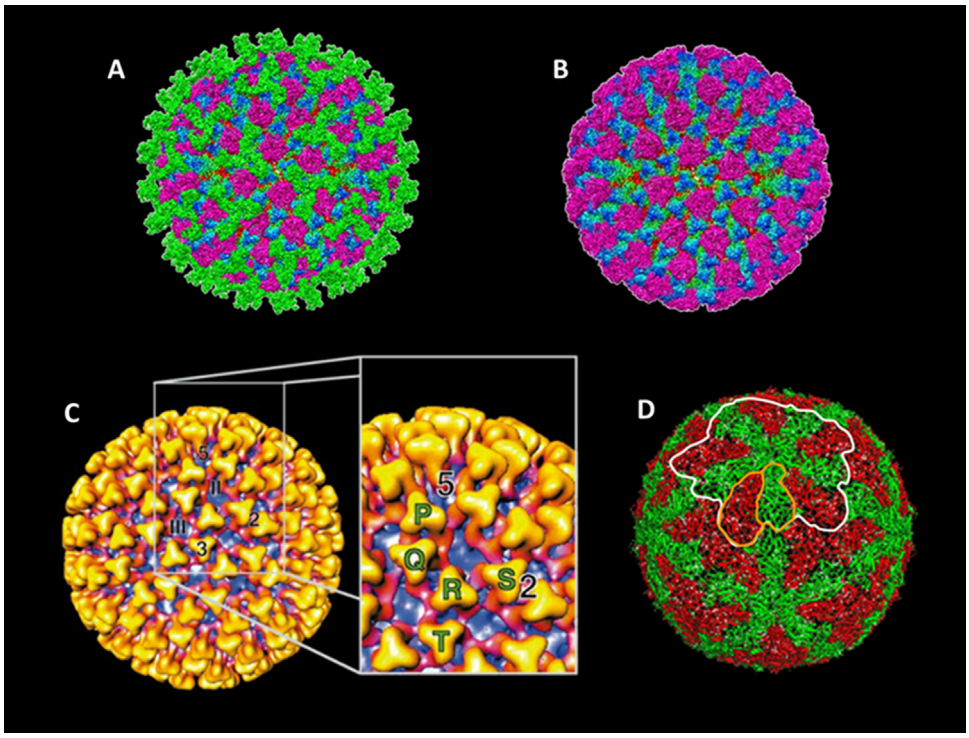

Fig. 2. Structure of the particle and inner capsid layers. (A) The 7 Å Cryo-EM structure of the complete particle. Triskelion shaped trimers of VP2 (green) are exposed on the outer surface. VP5 trimers (purple) sit under VP2 trimers between the triskelion arms. Regions of the underlying VP7 (blue to green gradient) and VP3 (red) can be seen. (Adopted from Zhang et al., 2010.) (B) The structure of the mature particle lacking the VP2 trimer layer (colouring as in A). (Adopted from Zhang et al., 2010 (C)). The 23 Å Cryo-EM structure of the core particle. VP7 trimers (gold to red gradient) are surface exposed with regions of the VP3 sub-core layer (purple) visible below. 2, 3 and 5 denote the icosahedral 2-fold, 3-fold and 5-fold symmetry axis respectively. II and III denote the position of class II and class III channels. The cut away annotates the P, Q, R, S and T crystallographic positions. (Adapted from Grimes et al., 1997 (D)). The 3.5 Å X-ray crystal structure of the sub-core particle. Two isoforms of VP3 are present, A (green) and B (red). These form a dimer (outlined in orange) which associate to form a decamer (outlined in white), twelve of which compose the sub-core layer. (Adapted from Grimes et al., 1998).