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Molecular Biology of Varicella–Zoster Virus

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Morphology of the Virion and Genome Organisation

Varicella–zoster virus (VZV), also known as human herpesvirus 3 (HHV3) belongs to the herpesvirus family (Herpesviridae). This classification is based on the morphological characteristics of the virus and its physical and chemical properties. The Herpesvirus Study Group of the International Committee on the Taxonomy of Viruses (ICTV) divided the members of this family into three subfamilies: Alphaherpesvirinae, Betaherpesvirinea and Gammaherpesvirinea. Based on its host spectrum, the length of the replicative cycle, the cytopathic effect in vitro and the particularities in the establishment of latency, VZV together with herpes simplex virus type 1 (HSV1; HHV1) and type 2 (HSV2; HHV2) were grouped into the subfamily of Alphaherpesvirinae. Moreover, by its genome organisation, VZV was classified into the genus varicellovirus, whereas HSV was classified into the genus simplexvirus, for overview see [1].

Though symptoms of an infection with one of these herpesviruses differ strongly from each other, the morphology of the particles and the biological properties are very similar. VZV is characterised by a strongly limited spectrum of infectable host cells, which are, in fact, exclusive cells of human or simian origin.

An important characteristic of herpesviruses is the architecture of the virion. Its size varies between 120 and 300 nm and is described to have a polygonal or round shape with a clearly visible central dot [2, 3]. Until now, it is not exactly known, how many polypeptides are involved in the assembly of the virion, but an average of 30–35 is reported. The virion is structured by four distinct components: envelope, tegument, capsid and core with the genome (fig. 1a).

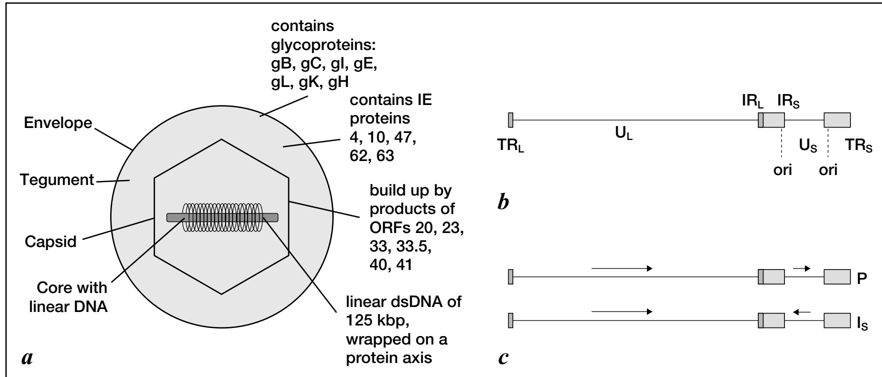

Fig. 1. Schematic drawing of the VZV. ***a*** Overview of its morphology: the important elements – envelope, tegument, capsid and core – are indicated on the left-hand side, important components of each element are given on the right-hand side. ***b*** General structure of the VZV genome. The genome (124,884 bp) can be divided into the unique long (U_L) and unique short (U_S) region, which are flanked by terminal repeats long and short (TR_L, TR_S) and internal repeat long and short (IR_L, IR_S). The origins of DNA replication are located in IR_S and TR_S. ***c*** Isomeric forms of VZV DNA: The P and I_S isomers make up more than 95% of the packaged VZV DNA.

The outer covering envelope has a typical trilaminar appearance [4]. It consists of different membranous elements captured during the transport of the new particles through the nuclear membrane network, Golgi apparatus, rough endoplasmic reticulum, cytoplasmic vesicles and cell surface elements [4–7]. The envelope is interspersed by spikes made up of viral glycoproteins. The VZV genome encodes glycoproteins gB, gC, gE, gH, gI, gK and gL as well as the putative glycoproteins gM and gN [8–10]. The enveloped particles have a final diameter of 180–200 nm and a pleomorphic to spherical shape.

The next inside layer, located directly underneath the envelope, is the tegument [11]. It does not have any distinct properties, but its thickness seems to be variable: virions located in cytoplasmic vacuoles obviously have a thicker tegument than those located in the perinuclear space [12]. The proteins encoded by the open reading frames (ORF) 4, 10, 47, 62 and 63 are found inside the tegument [13, 14]. The tegument surrounds the nucleocapsid.

The nucleocapsid has an icosahedric shape of 100–110 nm in diameter. It is composed of exactly 162 capsomers. Due to the morphology of this capsid structure, it is not possible to distinguish between members of the Herpesviridae. All capsomers occur in a 5:3:2 axial symmetry in which pentameric proteins form the vertices of an 80–120 nm icosahedron. The facets are comprised by hexameric

elements [15]. The capsid is built up by the proteins encoded by ORFs 20, 23, 33, 33.5, 40 and 41 (fig. 1a) [9].

The VZV genome is located inside the nucleocapsid. The DNA is coiled upon a protein axis. This combination of linear DNA and proteins is called core [16]. The genome is a linear double stranded DNA molecule of approximately 125 kbp in length and an average G–C content of 46%. This is the smallest genome known in the family of herpesviruses. During transition of the DNA from the capsid into the nucleus of an infected cell, it changes from a linear state into a circular one. It contains at least 69 unique ORFs and three duplicated genes (ORFs 62–71, 63–70 and 64–69, for overview see [10]). The VZV genome consists of two covalently linked segments, U_L and U_S (long, L and short, S), which are composed of unique sequences. Both of these unique segments are flanked by inverted repeat sequences: U_L by IR_L (internal repeat long) and TR_L (terminal repeat long), U_S by IR_S (internal repeat short) and TR_S (terminal repeat short) (fig. 1b). In the genome of the VZV strain Dumas, which is completely sequenced [17], the U_L element has a length of 104,836 bp flanked by 88 bp inverted repeats and the U_S region, which is 5,232 bp in length, is surrounded by inverted repeats of 7,319 bp.

VZV DNA isolated from purified virions can be found in two predominant isomeric forms designated as P (prototype) and I_S (showing an inverted U_S region) (fig. 1c) [18–22]. Other isomeric forms can only be found at very low levels representing 2–5% of the virion DNA. DNA purified from VZV nucleocapsids is infectious as it was first demonstrated by Dumas et al. [23].

The VZV genome contains two origins of replication (ori) [24, 25]. These elements, consisting of a 46 bp palindromic sequence which centres are composed of 16 TA dinucleotide repeats, are located within the inverted repeats flanking the U_S region (fig. 1b). Three internal elements inside these ori-sequences, designated A, B and C, are recognised by the viral origin binding protein encoded by ORF 51 [26].

The Replication Cycle of VZV

The replication cycle of VZV is divided into three different phases: (i) virus adsorption and entry, uncoating, transportation of the capsid to the nucleus and release of the viral DNA into it, (ii) viral gene transcription and translation as well as synthesis of viral DNA and (iii) assembly of new virions, enveloping and egress.

The replication cycle begins when the virus adsorbs to its specific receptors on the surface of the target cell. The adsorption is mediated by viral glycoproteins, the receptors have not yet been precisely identified. However, recent

data indicate that the mannose 6-phosphate receptor plays a major role during attachment since at least four VZV envelope glycoproteins contain mannose 6-phosphate [27, and references there in]. After fusion of the viral envelope and the cellular membrane, capsid and tegument proteins are released into the cytoplasm. The capsid is transported to the nuclear pores and releases its nucleic acids by an unknown mechanism. With regard to this process, it is noteworthy that the cytoskeletal architecture of the host cell was found to be altered after infection. Microfilaments and microtubules were subject to reorganisation, while intermediate filaments remained unaffected. These data support the thesis that cellular filament systems play an important role in the transport of virions or nucleocapsids as it is known from HSV [28].

The following expression of viral genes runs according to a very precise cascade. Immediate-early genes (IE; ORFs 4, 61, 62, 63; [29–38]) are transcribed first within a few hours of infection in the absence of de novo protein synthesis. The IE proteins have regulatory functions on the subsequent gene transcription.

Next to the virus-encoded transactivator proteins, cellular transcription factors are also involved in the regulation of VZV gene expression. Most VZV promoters contain *cis*-acting elements which are recognised by ubiquitously expressed cellular factors. The bi-directional promoter of the ORFs 28 and 29 is activated by cooperation of cellular upstream stimulatory factor and the major transactivator protein encoded by ORF 62 (IE62) [39–41]. Other cellular factors of importance are Sp1 and Ap1. Sp1 is one essential factor for the trans-regulation of the activating upstream sequence-element inside the viral glycoprotein I promoter [42] as well as it is implicated in the regulation of the viral glycoprotein E expression by substituting the TATA-box binding protein to initiate transcription [43, 44]. The expression and activation of Ap1 increased significantly after infection of cells with VZV and a knockout of this factor leads to a significant decrease of virus replication [45]. To achieve AP-1 activation, VZV takes advantage of pre-existing cellular signalling pathways such as the MAPK cascades [46]. The ORF61 protein has been demonstrated to be involved in the regulation of this pathway [47].

The induction of transcription of a secondary class of genes, named early-(E) genes, which can be translated into early proteins before the onset of viral DNA replication is dependent on the cooperation of viral IE proteins and cellular transcription factors. Almost all E genes encode proteins with enzymatic properties involved in the replication of viral DNA, like the DNA polymerase (ORF 28), the polymerase processivity factor (ORF 16), the helicase (ORF 55), the primase (ORF 6), the helicase/primase accessory factor (ORF 52), the single-strand DNA binding factor (ORF 29) and origin binding protein (ORF 51).

The VZV DNA replication process itself can be divided into different steps [48]. At first, the linear viral DNA circularises followed by the start of the

replication process, which involves the rolling circle-mechanism leading to the formation of head-to-tail concatemers [24]. Isomerisation may occur by homologous recombination between the inverted repeats. Finally, the concatemers are cleaved to generate linear DNA which is packaged into virions.

After DNA replication has begun, late (L) genes are transcribed. Proteins belonging into the group of L products are the glycoproteins as well as those proteins that build up the virus particles.

Due to the aim to achieve a strict and efficient expression of all classes of genes and to repress an up-come of host defence mechanisms, VZV mediates a process known as host shut-off which results in the degradation of cellular mRNA. In contrast to HSV-1, the VZV mediated shut-off is not an immediate-early process but a delayed one. The ORF17 protein, which is the homologue to the HSV virion host shut-off (*vhs*) factor U_L41, is not the main actor to gain the shut-off [49, 50]. Due to its transrepressing properties, recent reports indicate a role of IE63 in putting on the shut-off effect [51, 52].

The degradation of mRNA includes also transcripts of VZV IE genes what is thought to be a part of the switching process from the IE to the E and L gene transcription during the replication cascade [53]. Viral E and L transcripts are also degraded as a consequence of the shut-off [53, 54]. However, evidence is increasing that a broad range of cellular genes are not influenced by the shut-off.

In addition to the host shut-off as a mechanism against host defence, VZV is also capable to prevent the induction of interferon-stimulated anti-viral systems such as PKR and RNase L [55, 56].

After the expression of all three classes of genes has occurred, the newly replicated genomes are wrapped on the protein core, packed inside the newly synthesised capsids and transported outside the host cell. It is still not definitely clear how and in which form the nucleocapsids are transported out of the nucleus and towards the egress. Different hypotheses are still proposed. A widely accepted model is that the capsids get a temporary envelope gained from inner nuclear membranes while entering the perinuclear space. These newly formed particles reach the lumen of the rough endoplasmatic reticulum (rER). The envelope fuses with the rER membrane. The processes resulting in this temporary enveloping at the inner nuclear membrane and the fusion with the rER membrane are not understood yet. Further hypotheses give unknown functions of some glycoproteins in these events. Following this dis-envelopment, naked particles bud into large cytoplasmic vesicles. The viral glycoproteins are released from the trans-Golgi network in additional vesicles that fuse with the cytoplasmic vesicles prior to virion formation. The assembly of fully enveloped virions with functional glycoproteins occurs in these vesicles while they are forwarded to the cell surface. The viral particles are released by exocytosis [8, 57–59]. According to another scenario, cytosolic capsids are wrapped by cisternae of the

trans-Golgi network which already contain the glycoproteins. The tegument is thought to bind to those glycoproteins [60].

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