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Analysis of minimal sequences on JC virus VP1 required for capsid assembly

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Human JC virus (JCV) belongs to the family of Polyomaviridae. The viral capsid is composed of 72 capsomeres. Five VP1 molecules make up a capsomere structure. To investigate the minimal sequences on JCV VP1 polypeptide required for capsid assembly, the first 12 (ΔN12) and 19 (ΔN19) amino acids at the N-terminus and the last 16 (ΔC16), 17 (ΔC17), and 31 (ΔC31) amino acids at the C-terminus of VP1 were truncated and expressed in *E. coli*. The VP1 proteins of ΔN12 and ΔC16 were able to self-assemble into a virus-like particle similar to that of wild-type (WT) VP1. However, the mutant proteins of ΔN19, ΔC17, and ΔC31 formed a pentameric capsomere structure as demonstrated by a 10–50% sucrose gradient centrifugation and electron microscopy. These results suggest that the 12 amino-terminal and 16 carboxy-terminal amino acids of VP1 are dispensable for the formation of virus-like particles, and further truncation at either end of VP1 leads to the loss of this property. *Journal of NeuroVirology* (2001) **7,** 298–301.

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JC virus (JCV), a human polyomavirus, is an etiologic agent for progressive multifocal leukoencephalopathy (PML) (Padgett *et al*, 1971; Major *et al*, 1992). Human oligodendrocytes are permissive for JCV infection (Kenny *et al*, 1984; Major *et al*, 1992). During lytic infection, the structure proteins, VP1, VP2, and VP3, of polyomavirus are transported into the nucleus (Chang *et al*, 1992a; Chang *et al*, 1992b) to interact with viral genome (Chang *et al*, 1993) for progeny formation. The major shell protein, VP1, makes up about 75% of JCV capsid (Tooze, 1981; Frisque *et al*, 1984). Therefore, the VP1 protein is responsible for maintaining the structural integrity of the virus. It is also essential in the virus attachment protein (VAP) sites for infection of host cells (Liu *et al*, 1998), hemagglutinins for agglutination of erythrocytes (Liu *et al*, 1998) and is essential in DNA packaging (Chang *et al*, 1993; Ou *et al*, 2001) during virus assembly.

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The major capsid protein VP1 of JCV is able to self-assemble into virus-like particles (VLP) in the absence of the minor capsid proteins, VP2 and VP3, when expressed in either eukaryotic (Chang *et al*, 1997; Goldmann *et al*, 1999) or prokaryotic cells (Ou *et al*, 1999). In this study, the minimal sequences on VP1 polypeptide for VLP assembly were further investigated. The three-dimensional structure of JCV VP1 (Figure 1) was constructed by the SWISS-MODEL protein modeling program (Peitsch, 1996), referring to X-ray crystallography of SV40 VP1 (Liddington *et al*, 1991). Five truncations on VP1 polypeptide (Figure 1) were generated by deleting the 5′- and 3′-end of the VP1 gene. The JCV VP1 expressing plasmid, ΔpF JCV1 (Ou *et al*, 1999), was used as a template for generating mutant VP1 genes by polymerase chain reaction (PCR). To amplify the first 12 and 19 amino acids truncated VP1, ΔN12, and ΔN19, the sense primers, 5′-ACATGTGGATCCATGCCCGTGCAAGTTCCAAAA-3′ and 5′-ACATGTGGATCCATGCTTATAAGAGGAG GAGTA-3′, were used with an antisense primer, 5′-ACATGTGGATCCTTACAGCATTTTTGTCTGCAA-3′. The antisense primers, 5′-ACATGTGGATCCTTAG

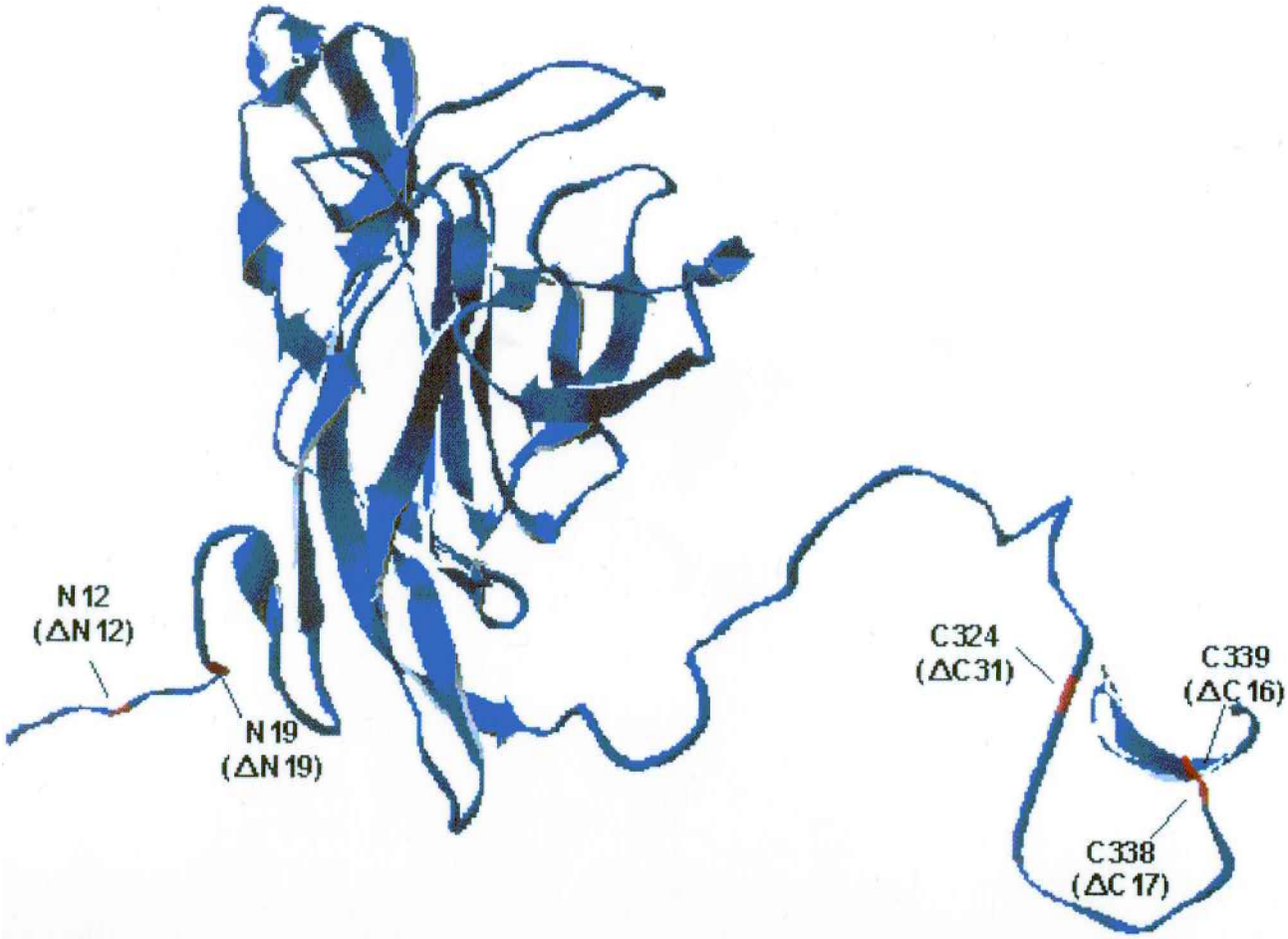

Figure 1 Stereo drawing of JCV VP1. The structure of JCV VP1 was constructed by the Swiss-Model Modeling program referring to the X-ray crystallography of SV40 VP1 (Liddington *et al*, 1991). The truncated amino acids of VP1 are colored in red.

TCTGGGTCCCCTGGGAG-3′, 5′-ACATGTGGATCCT TATGGGTCCCCTGGGAGTTC-3′, and 5′- ACATGTG GATCCTTACTCCTCTATTTGAGC-3′ were used with a sense primer, 5′-CACTTGGGATCCATGGCCCCAA CAAAA-3′, to generate the last 16, ΔC16, 17, ΔC17, and 31, ΔC31, amino acids truncated VP1, respectively. The amplified DNA fragments were cloned into a prokaryotic expression vector, ΔpFlag (Chang *et al*, 1993), at the *Bg*/II restriction site. The constructs were transformed into *E. coli* JM109 cells and the mutant proteins were expressed by isopropyl-B-D-thiogalactopyranoside. The cells bearing VP1 proteins were lysed by sonication (Chang *et al*, 1996). The mutated VP1 proteins in *E. coli* lysates were identified by Western blot using rabbit anti-JCV VP1 serum (Chang *et al*, 1996). The results showed that all VP1 mutants were expressed in *E. coli* and recognized by the monospecific antibody (Figure 2). The VP1 proteins were further analyzed by a 10–50% sucrose gradient centrifugation at 45,000 rpm for 1.5 h using a Beckman SW55 Ti rotor (Ou *et al*, 2001). Fractions were collected and analyzed by Western blot for determination of VP1 distribution. The maximum amount of ΔN12 and ΔC16 was located at fraction 7, similar to that of WT VP1 (Figure 3). The sedimentation coefficient of ΔN12 and ΔC16 was approximately 200S when compared with murine polyoma virus particle (data not shown). However, the maximum distribution of ΔN19, ΔC17, and ΔC31 were found at fraction 25 (Figure 3). The sedimentation coefficient of these proteins was approximately 12S when compared with catalase in a 5–20% sucrose gradient centrifugation (date not shown). The morphologies of ΔN12 and ΔC16 were observed under an electron microscope after a 10–50% sucrose gradient centrifugation. ΔN12 formed a virus-like particle (VLP) with a homogenous diameter of 42 nm similar to that of WT VP1 (Figure 3). ΔC16 also self-assembled into a VLP with heterogeneous sizes measuring from 30 to 55 nm (Figure 3). However, both VLPs of ΔN12 and ΔC16 showed a strong hemagglutination activity (Figure 3). VP1 proteins of ΔN19, ΔC17, and ΔC31 mutants were further purified by an immunoaffinity chromatography (Cai *et al*, 1994) after a 5–20% sucrose gradient centrifugation. These VP1 mutants formed a capsomere-like structure without hemagglutination activity (Figure 3).

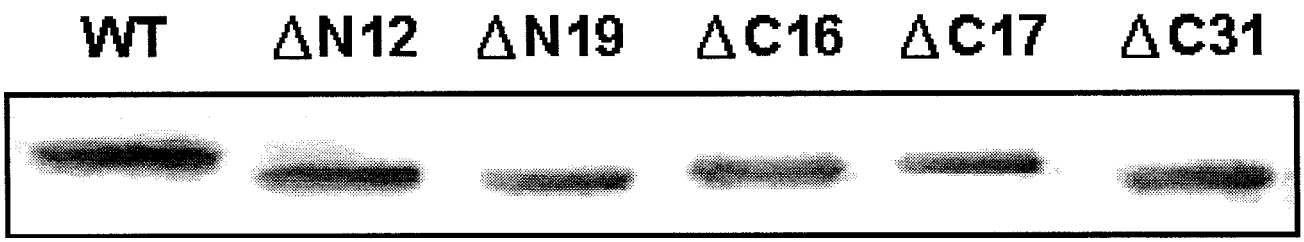

Figure 2 Western blot of various JCV VP1 proteins. The *E. coli* cell lysates containing various VP1 proteins, WT, ΔN12, ΔN19, ΔC16, ΔC17, and ΔC31 were resolved by SDS-PAGE and then identified by Western blotting using a rabbit anti-JCV VP1 serum.

The major capsid protein VP1 of human JCV was able to self-assemble into a VLP when expressed in insect cells (Chang *et al*, 1997; Goldmann *et al*, 1999) and *E. coli* (Ou *et al*, 1999). In this study, we further identified the minimal sequences on VP1 polypeptide for VLP assembly. Our results indicate that the regions of the first 12 amino acids at the N-terminus and the last 16 amino acids at the C-terminus of VP1 are dispensable for VLP assembly.

X-ray crystallography of SV40 illustrated the interactions between VP1 molecules in capsid structure

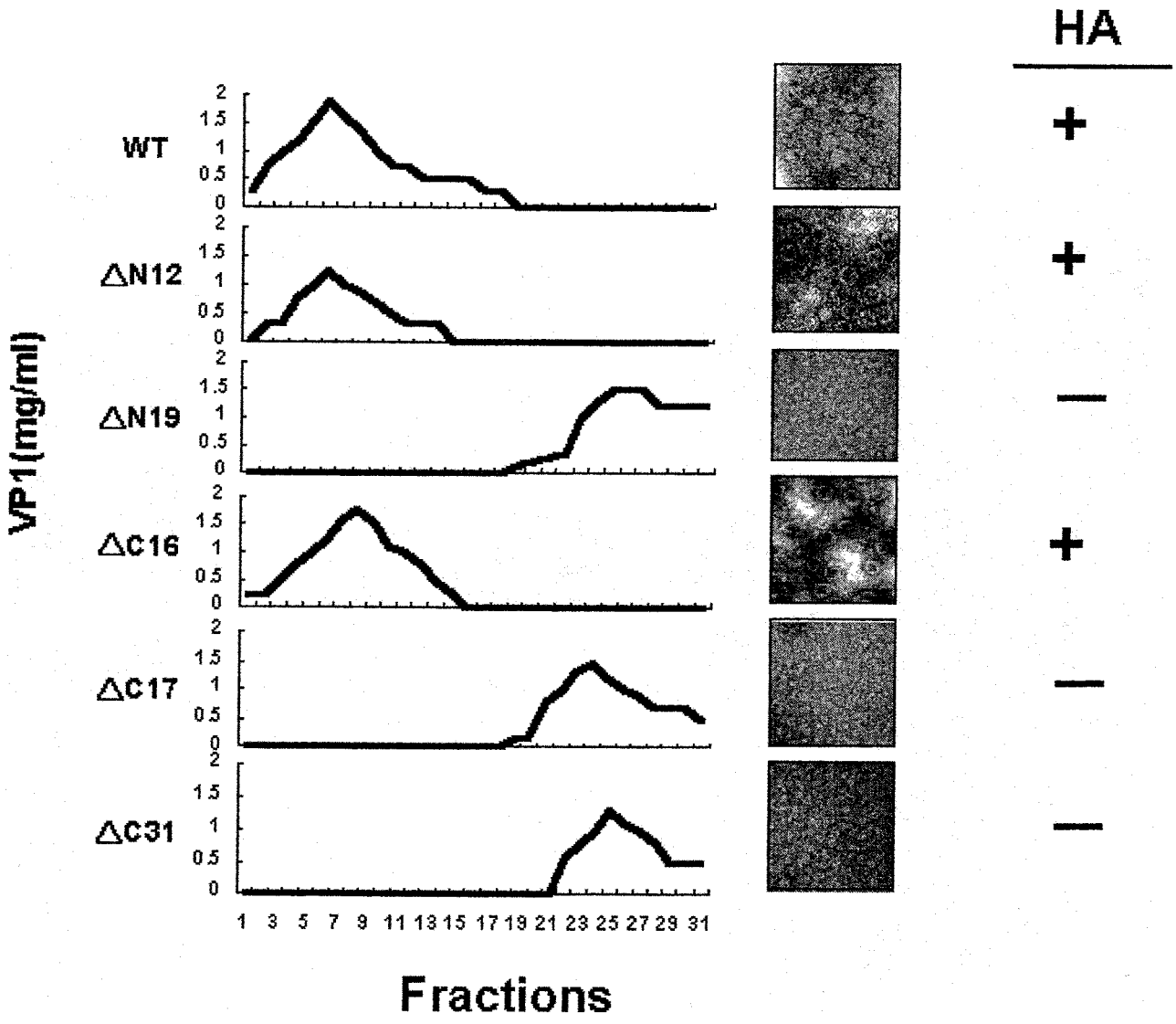

Figure 3 Analysis of JCV VP1 mutant proteins by sucrose gradient centrifugation, electron microscopy and hemagglutination assay. The *E. coli* cell lysates expressing various VP1 proteins were analysed by a 10–50% sucrose gradient centrifugation. The purified VP1 proteins were observed under electron microscope and assayed for hemagglutination activity.

(Stehle *et al*, 1996). The last 49 amino acids, 313–361, at the C-terminus of SV40 VP1 invade another pentamer to interact with the N-terminal region of a VP1 molecule. The C-terminal amino acids 330–336 form strand *J* and clamp the N-terminal strand A, amino acids 1–32, of a VP1 molecule in the different pentamer to form the six-stranded A*J*BIDG2 sheet. The region of the first 24 amino acids of JCV VP1, corresponding to the first 32 amino acids of SV40 VP1, may be involved in clamping with the region of the last 31 amino acids including strand *J*. Our study showed that truncation at either strand A (the first 19 amino acids) or strand *J* (the last 31 amino acids) results in incapability of capsid assembly. Therefore, these findings are in agreement with the structural configuration.

The last 17th amino acid, Asp338, of JCV VP1 corresponding to Asp345 of SV40 VP1, was predicted to be involved in calcium ion binding (Stehle *et al*, 1996). Therefore, when the last 17 residues were deleted, _C17 was not able to assemble into a capsid-like structure. Instead, truncation of the last 16 amino acids (_C16) retained capsid structural integrity. The VLPs of _C16 were not as homogeneous and stable as those of WT VP1 presumably due to the instability of calcium binding in the capsid structure of _C16. However, it needs to be further verified whether Asp338 of JCV VP1 is involved in calcium ion binding for capsid formation. The calcium-binding sites on VP1 polypeptide for JCV capsid assembly are currently being investigated in our laboratory.

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