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Analysis of DNA-binding activity of the JC virus minor capsid protein VP2

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To investigate the DNA binding activity of the JC virus minor capsid protein, VP2, both wild-type and mutant VP2 were cloned and expressed in *Escherichia coli.* Southwestern blotting was employed for the DNA-binding assay. The results showed that VP2 was able to bind to DNA, except when either the last 13 or the last 29 amino acids were truncated. The results indicate that the DNA-binding domain of VP2 is located within the last 13 amino acids. Furthermore, we also demonstrated that Lys^{332} and Lys^{336} within the DNA-binding domain are crucial for DNA binding. The findings may provide further information for understanding the mechanism of virion assembly of the JC virus. *Journal of NeuroVirology* (2003) **9(suppl. 1),** 21–24.

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The human JC virus (JCV) is a neurotropic virus. When JCV infects oligodendrocytes, myelin-producing cells, in the human brain, viral progenies are generated, and the infected cells are subsequently lysed (Sweet *et al*, 2002). Destruction of oligodendrocytes results in a fatal disease, progressive multifocal leukoencephalopathy (PML) (Padgett *et al*, 1971; Major *et al*, 1992). During the late phase of the lytic infection, the three structural proteins, VP1, VP2, and VP3, are synthesized and assembled with viral genomic DNA for viral progeny formation in infected cells (Tooze, 1981; Frisque *et al*, 1984).

The molecular mechanism of capsid assembly of the JCV is not well documented. The major capsid protein, VP1, of the JCV is able to self-assemble into a capsid-like structure when expressed in insect cells (Chang *et al*, 1997), yeast (Chen *et al*, 2001), and *Escherichia coli* (Ou *et al*, 1999). The capsid-like structure can be dissociated in the presence of a chelator (ethylene diaminetetraacetic acid [EDTA]) and a reducing agent dithiothreitol [DTT] (Chang *et al*, 1997). The findings indicate that disulfide bonds and calcium ions may be involved in maintaining the integrity of the viral capsid structure (Chang *et al*, 1997). The capsid-like structure was further demonstrated to contain disulfide bonds that cause dimeric and trimeric VP1 linkages (Chen *et al*, 2001). Disulfide bonds were demonstrated to play an important role in maintaining the integrity of the JCV capsid-like structure by protecting calcium ions from chelation (Chen *et al*, 2001).

The viral capsid is composed of 72 capsomeres (Tooze, 1981). Five VP1 molecules make up a capsomere structure. Protein-protein interactions between pentameric capsomeres appear to be indispensable for capsid formation. The interaction domains on JCV VP1 for linking capsomeres were investigated (Ou *et al*, 2001a). When the first 12 amino acids of the N-terminus were truncated or the last 16 amino acids of the C-terminus were truncated, VP1 was able to self-assemble itself into capsid-like particles similar to those of wild-type VP1. VP1 mutants, which were further truncated by deleting the first 19 or the last 17 amino acids, formed a pentameric capsomere structure and failed to assemble into a capsid-like structure. These results indicate that the first 19 amino acids at the N-terminus and the last 17 amino acids at the C-terminus of VP1 are required for interactions between capsomeres during capsid assembly (Ou *et al*, 2001a).

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The virion contains the major capsid protein, VP1, the minor capsid proteins, VP2 and VP3, and viral genomic DNA (Tooze, 1981). The mechanism of DNA encapsidation for viral progeny formation is not fully understood. Recently, the DNA encapsidation domain of JCV VP1 for pseudovirion formation was identified (Ou *et al*, 2001b). The N-terminal region of VP1, containing the positively charged amino acids, Met^{1}- Ala- Pro- Thr- Lys- Arg- Lys- Gly- Glu- Arg- Lys- Asp^{12}, was demonstrated to be the DNA encapsidation sequence. The sequence also showed DNA-binding activity. Taken together, the results suggest that the first 12 amino acids of the N-terminus of JCV VP1 may be involved in viral genome encapsidation during viral progeny maturation.

The minor capsid proteins, VP2 and VP3, and the viral genome are packaged inside the capsid particle (Tooze 1981; Liddington *et al*, 1991; Griffith *et al*, 1992). Therefore, DNA-protein interactions may occur between the viral genome and the minor capsid proteins. VP2 contains two positively charged stretches, Lys^{319}- Lys- Lys- Arg- Arg- Lys^{324} and Lys^{332}- Thr- Ser- Tyr- Lys- Arg- Arg- Ser- Arg- Ser- Ser- Arg- Ser^{344}, at the C-terminus. It is proposed that these two positive stretches interact with DNA molecules. To analyze DNA-binding activities of the minor capsid proteins, the VP2 gene of the JCV was amplified by Polymerase chain reaction (PCR) using the sense primer, 5′-CACTTGGGATCCATGGGTGCCGC ACTTGCACTTTTG-3′, and antisense primer, 5′-CACTTGGGATCCTTAACTTCTA GAACTTCTACTCCT-3′. The last 13 amino acids (ΔC13), from amino acids 332 to 344, and the last 29 amino acids (ΔC29), from amino acids 316 to 344, of VP2 were truncated using PCR to generate the mutant genes. The antisense primers, 5′-CACTTGGGATCCTTAGGAACTTGCAGCGGG-3′ and 5′-CACTTGGGATCCT TAATCTTCATATGCTTC-3′, were used with the VP2 sense primer to generate ΔC13VP2 and ΔC29VP2 DNA fragments by PCR, respectively. The PCR products were subsequently cloned into the bacterial expression vector, pGEX-4T (Pharmacia), for protein expression.

Expressions of the full-length VP2 (Figure 1**A**, lane 1), ΔC13VP2 (Figure 1**A**, lane 2), and ΔC29VP2 (Figure 1**A**, lane 3) in *E. coli* were confirmed by Western blotting using rabbit anti-JCV VP2 serum. Southwestern blotting was performed for DNA-binding assay (Chang *et al*, 1993). The *E. coli*–expressed VP2 proteins were resolved on a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) gel and subsequently electrotransferred onto a nitrocellulose membrane (MSI, MA, USA). The VP2 proteins were renatured with renaturing buffer (10 mM Tris-HCl, pH 6.7, 50 mM NaCl, 1 mM EDTA, 0.02% bovine serum albumin [BSA], 0.02% Ficall, and 0.02% polyvinyl pyrrolidone) (Chang *et al*, 1993). The membrane was incubated with nick-translated ^{32}P-labeled JCV genomic DNA at a final concentration of 10^5 cpm/ml. After 1 h of DNA probing at room temperature, the membranes were washed three times with renaturing buffer. Autoradiography was performed using x-ray film. The results showed that the full-length VP2 was able to bind DNA (Figure 1**B**, lane 1). In contrast, VP2 with the last 13 amino acids truncated (ΔC13VP2) (Figure 1**B**, lane 2) and VP2 with the last 29 amino acids truncated (ΔC29VP2) (Figure 1**B**, lane 3) completely lost their DNA-binding activities. These results indicate that the last 13 amino acids serve as a DNA-binding sequence for JCV VP2.

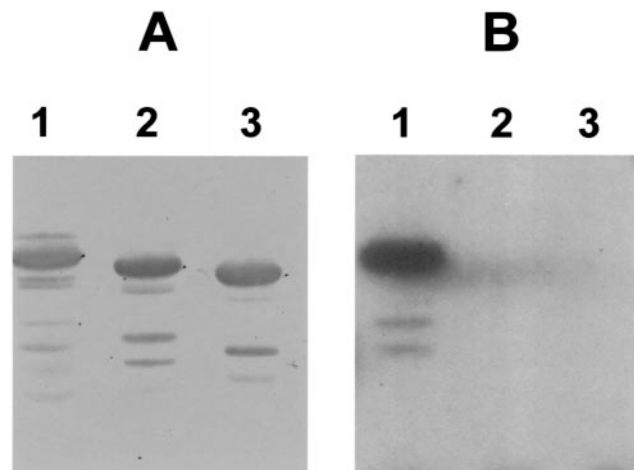

Figure 1 DNA-binding activities of wild-type and truncated VP2. (**A**) Western blot. (**B**) Southwestern blot. Lane 1, wild-type VP2; lane 2, ΔC13VP2; lane 3, ΔC29VP2.

To further determine the important amino acids for DNA-binding activity in the last 13 amino acids, point mutations were constructed by changing the coding codons. The 13 amino acids in the DNA-binding sequence were replaced by noncharged amino acids and designated P1- to P13-VP2 (Figure 2**A**). The mutants were cloned into the pGEX-4T expression plasmid and expressed in *E. coli*. The mutated codons were confirmed by DNA sequencing (ABI). Expressions of the VP2 mutant proteins were identified by Western blot analysis. Approximately equal amounts of VP2 mutant proteins were used for DNA-binding assay using Southwestern blotting with nick-translated ^{32}P-labeled JCV genomic DNA. The results of DNA-binding assay showed that the DNA-binding activities of Lys^{332} substituted with Ile (P1-VP2) and Lys^{336} substituted with Met (P5-VP2) were drastically reduced (Figure 2**B**, lanes 1 and 5). Therefore, Lys^{332} and Lys^{336} appeared to be the crucial amino acids of JCV VP2 for DNA binding.

The virus particle of polyomavirus is composed of an icosahedral capsid made up of 72 pentamers of the major capsid protein, VP1, two minor capsid proteins, VP2 and VP3, and viral genomic DNA (Tooze, 1981). X-ray diffraction difference maps of polyomavirus showed that there are 72 prongs of VP2 or VP3, which radiate from a central core into

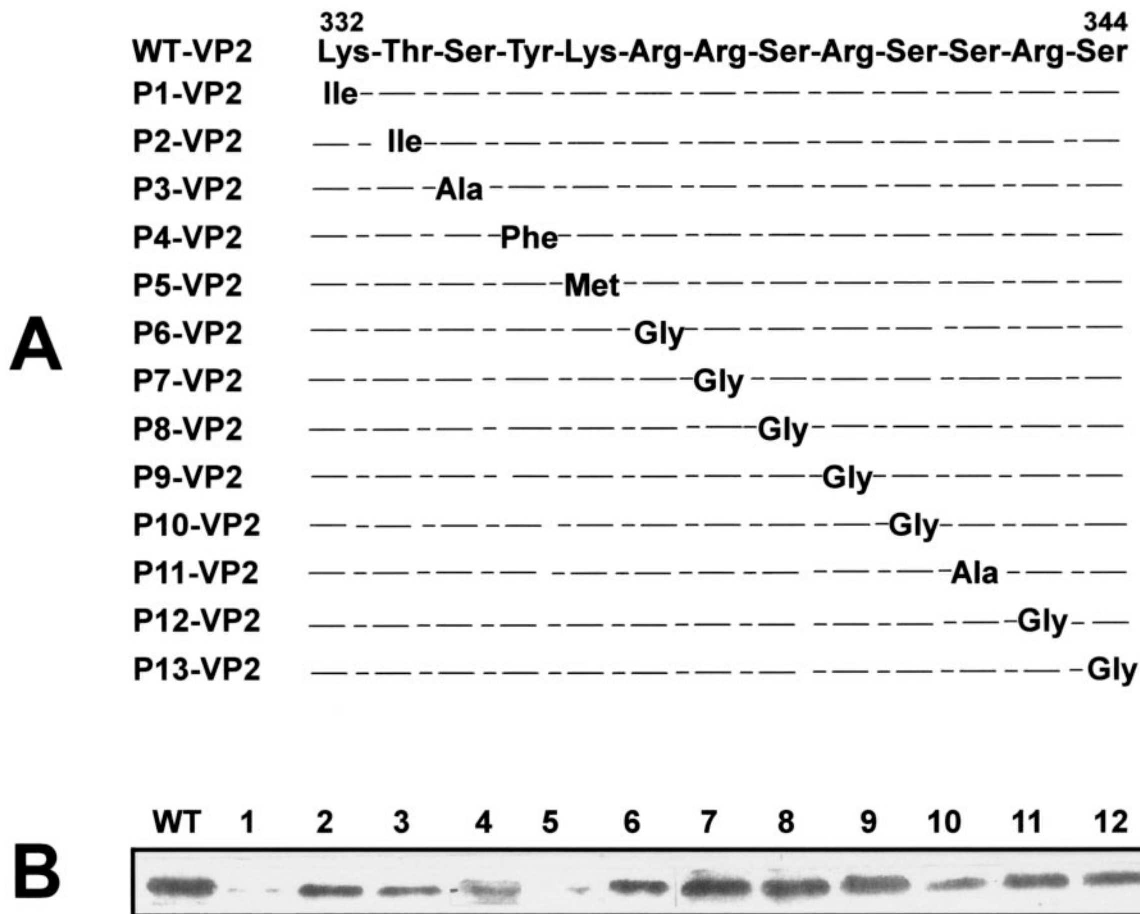

Figure 2 Point mutations of VP2 and DNA-binding activities. (**A**) Various point mutations generated within the last 13 amino acids of VP2. (**B**) Southwestern blots of various point mutations of VP2. Lanes 1 to 13 represent mutant P1-VP2 to P13-VP2.

the axial cavities of the VP1 pentamers (Liddington *et al*, 1991; Griffith *et al*, 1992). These findings indicate that the minor capsid proteins are packaged inside the capsid particle with viral genomic DNA. Our previous study demonstrated that the first 12 amino acids at the N-terminus of JCV VP1 are responsible for DNA binding and DNA packaging (Ou *et al*, 2001b). According to the simulated three-dimensional structure of JCV VP1 (Ou *et al*, 2001a), the N-terminus of the VP1 polypeptide is cryptic in the capsid. The findings of VP1-DNA interaction are in agreement with the structural conformation. The present study further demonstrates that the minor capsid protein VP2 of the JCV has DNA-binding activity, and that the DNA-binding domain is located among the last 13 amino acids of the C-terminus. Because the other minor capsid protein, VP3, shares two thirds of the VP2 polypeptides at the C-terminus, it is proposed that the DNA-binding domain of VP3 is located in the same region as that of VP2 (Clever *et al*, 1993).

The mechanism of assembly of the viral capsid proteins and viral DNA is still uncertain. Virion assembly may involve a sequential addition of viral capsid proteins to viral DNA in the nucleus (McMillen and Consigli, 1974; Garcea and Benjamin, 1983). It is not clear whether the three structural proteins are transported into the nucleus independently (Chang *et al*, 1992a, 1992b; Ishii *et al*, 1996) or are associated with each other in the cytoplasm (Cai *et al*, 1994; Shishido-Hara *et al*, 2000) prior to nuclear entry to interact with viral DNA for assembly. Therefore, further information regarding the interactions of the structural proteins with the viral genomic DNA appears to be a prerequisite for fully understanding the mechanism of JCV assembly.

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