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Fv-1 Restriction of Murine Leukemia Virus May Not Necessarily Be at Cytoplasmic-Nuclear Transport Phase

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Fv-1 restriction of murine leukemia virus (MuLV) determined by two amino acids in p30 (CA) of the viral protein (1-2) is considered to occur during the post-penetration phase of infection (3-5). Since the formation of circular proviral DNA, which is considered to reflect the entrance of pre-integration complex into the nucleus has been seen to be impaired in the restrictive cells (6-7), it has been speculated that the cytoplasmic-nuclear transport of the provirus was inhibited in restrictive cells. However, whether Fv-1 acts in cytoplasm, in the nucleus, or in both is still unknown.

I report here that an MuLV, which was constructed by replacing the *gag* region of NB-tropic Moloney MuLV with N-tropic WN1802N-derived *gag*, N-MoF (8), did not show any inhibition of formation of the circular intermediate, though its titration pattern was that of a typical N-tropic virus.

Figure 1 shows the structures of N-MoF and a similar B-tropic construct, B-MoFdA (9). In XC assay (10), both N-MoF and B-MoFdA showed the expected titration pattern of N-tropic and B-tropic viruses (Fig.2).

In order to examine the formation of linear and circular proviruses in the infected cells, cells plated in an amount of 2×10^6 cells/10 cm dish were infected with the viruses in the presence of 0.8 μg/ml of polybrene for 5 h. At indicated periods after the start of infection, DNAs were extracted according to Hirt's method (11). One third of the DNAs extracted from each plate was electrophoresed in 0.8% agarose gel, and analyzed by Southern blotting using an Xba1-Xba1 (#5766-#8113) fragment of Moloney MuLV *env* as a probe.

As shown in Figure 3-A, though formation of the circular proviral DNA of WN1802N was inhibited in C57BL/6-derived B-type YH-7 cells, that of N-MoF was not. That of B-MoFdA in N-type NIH3T3 cells was inhibited as expected (Fig. 3-B).

The above observation showed that the Fv-1 restriction could take place without inhibition of the formation of circular DNA. The circular form is not considered a pre-integration molecule itself but rather a by-product (12). Therefore, this discrepancy may not be surprising. However, as the circular

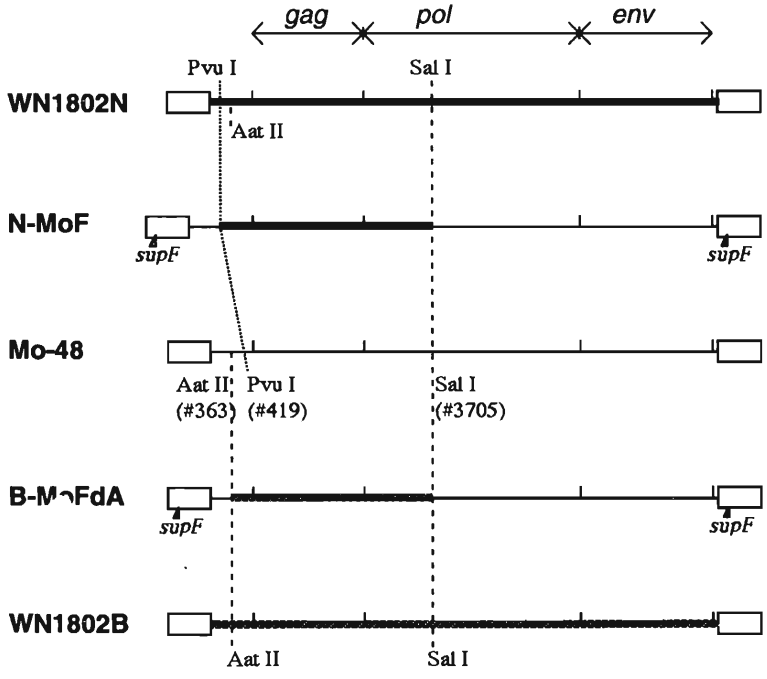

Fig. 1. Structure of recombinant viruses (See refs. 8 and 9).
Thick filled portion is derived from WN1802N, thick shaded portion from WN1802B, and thin portion from Moloney MuLV Mo-48.

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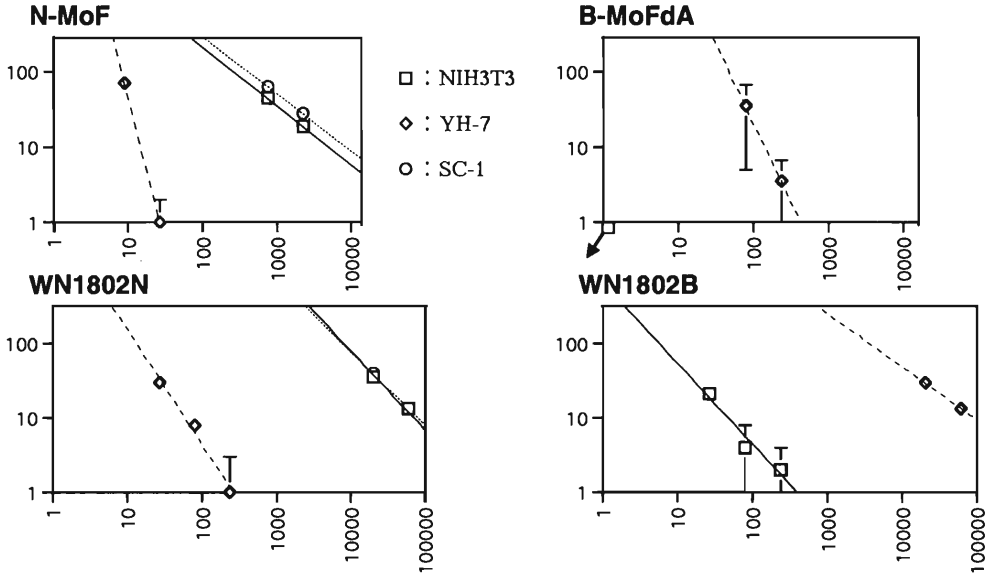

Fig. 2. Titration of N-MoF, B-MoFdA, and WN1802N/B on N-type NIH3T3, B-type YH-7, and Fv-1 unrestrictive SC-1 cells.
The linear curve fitting was done by CA-Cricket Graph III.
Vertical axis: number of plaques per plate.
Horizontal axis: dilution of viruses.

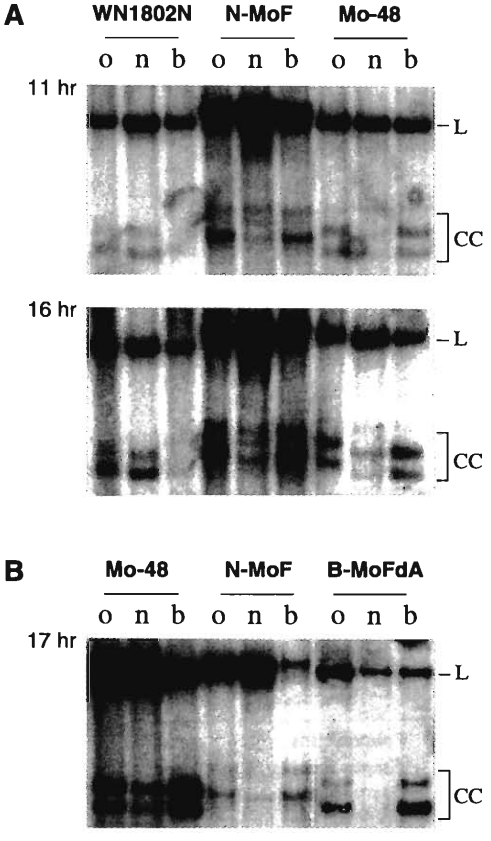

Fig. 3. Formation of extrachromosomal proviral DNA after infection. Each virus stock of comparable titer on Sc-1 cells was infected to SC-1 (o), NIH3T3 (n), or YH-7 (b) cells. At the indicated hours after infection, extrachromosomal DNAs were extracted according to Hirt's method (11). The DNAs were electrophoresed in 0.8% agarose gel, and analyzed by Southern blotting using Moloney-MuLV *env* probe. L: linear form. CC: closed circular form.

form is strictly nuclear (13), the restriction of N-MoF was exerted probably after entrance to the nucleus; i.e., it was inferred that Fv-1 restriction can occur in the nuclear phase. As WN1802N and N-MoF had *gag* regions with the same sequence, it is speculated that a region other than *gag* is able to determine the impaired formation of circular DNA.

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Surveillance of Poliovirus-Isolates in Japan, 1999

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In 1999, 11 polioviruses were isolated from nine clinical patients in four prefectures. Samples of the viruses were sent to National Institute of Infectious Diseases (NIID) and subjected to the intratypic differentiation by PCR-restriction fragment length polymorphism method developed by Radu Crainic (1). As shown in Table 1, all of the examined polioviruses were vaccine-derived strains. Type 3 poliovirus was isolated from the stool specimen of a poliomyelitis patient in Kyoto, and examined for intratypic differentiation in 1999. Since the onset of the paralysis in the Kyoto case occurred in November 1998,

Table 1. Characterization of poliovirus isolates in 1999

Code	Age	Sex	Date of vaccination		Date of onset	Date of sampling	Clinical diagnosis	Serotype	Intratypic differentiation
			in the patient	in the area					
99-kyoto-1	2Y	M	98-10-28(2nd)		98-11-19	98-11-19	Poliomyelitis	Polio-3	Vaccine-like
99-kyoto-2	7M	F	98-4-16		98-5-2	98-5-2	Aseptic meningitis	Polio-2	Vaccine-like
99-Niigata-1	1Y	F	98-10-23	99-4-16	99-4-5	99-4-9	Gastroenteritis	Polio-1&2	Vaccine-like
99-Niigata-2	2Y	M	98-11-2	99-4-5	Unknown	99-4-12	Erythema infectiosum	Polio-1	Vaccine-like
99-Fukushima-1	5Y	M	Unknown	98-10-30	99-2-17	99-2-17	*URD	Polio-1	Vaccine-like
99-Fukushima-2	7M	M	None	99-4-15	99-4-30	99-4-30	**ITP	Polio-2	Vaccine-like
99-Niigata-3	3Y	F	97-5-9(2nd)	99-5-14	99-5-31	99-5-31	URD	Polio-1	Vaccine-like
99-Niigata-4	7Y	F	Unknown	99-4-13	99-6-24	99-6-25	Herpangina	Polio-1	Vaccine-like
99-Nara-1	8M	F	None	99-10-26	99-11-24	99-11-24	Diarrhea	Polio-2&3	Vaccine-like

* : Upper respiratory disease
**: Idiopathic thrombocytopenic purpura

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