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Genome organization of retroviruses. III. Restriction endonuclease cleavage maps of mouse sarcoma virus double-stranded DNA synthesized in vitro

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ABSTRACT

Genome length complementary DNA (cDNA) transcripts were synthesized *in vitro* by using purified virions of a cloned isolate of mouse sarcoma virus (MSV Clone 124). The cDNA transcripts were converted to double-stranded form by utilizing DNase-digested calf thymus DNA primers and *E. coli* DNA polymerase I. Restriction endonucleases *Sal* I, *Hind* III, *Hpa* I, *Bgl* II and *Xba* I were found to cleave the MSV double-stranded DNA once to generate two fragments, whereas restriction endonucleases *Bgl* I and *Hae* II cleaved twice to generate three fragments. Restriction endonucleases *E. coli* R_I and *Bam* HI did not cleave MSV double-stranded DNA. The order of the restriction fragments was determined in relation to the 5' and 3' ends of the genomic RNA.

INTRODUCTION

Murine sarcoma viruses can transform fibroblasts *in vitro* and induce tumor formation *in vivo* (1). However, they are unable to replicate in the absence of a helper virus (1). A cloned isolate of murine sarcoma virus, Clone 124, isolated by Ball and colleagues (2) has been studied extensively. Its genome is about 5 to 6 kb long and appears to contain both helper virus-specific and unique nucleotide sequences (3). The relationship between the genome of MSV 124 and helper MLV has been elucidated by electron microscopic analysis of heteroduplexes formed between MSV 124 genomic (30S) RNA and long Moloney MLV (M-MLV) cDNA transcripts (4). We have been interested in studying the organization of MSV genomes in the infected cell. In order to study this problem at a molecular level, we decided to construct a detailed physical map of the MSV genome. Due to relative paucity of the proviral DNA isolated from MSV-infected cells, we decided to synthesize viral DNA *in vitro*. Genome length cDNA transcripts were synthesized by using purified virions of MSV 124. The cDNA transcripts were then converted to double-stranded form by employing *E. coli* DNA polymerase I and DNase-digested calf thymus DNA primers. The double-stranded DNA was then

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fractionated on neutral sucrose gradients and used for restriction endonuclease analysis. In this communication, we report the construction of a physical map of MSV genome by several restriction endonucleases.

RESULTS AND DISCUSSION

Figure 1 shows the patterns of double-stranded DNA after treatment with

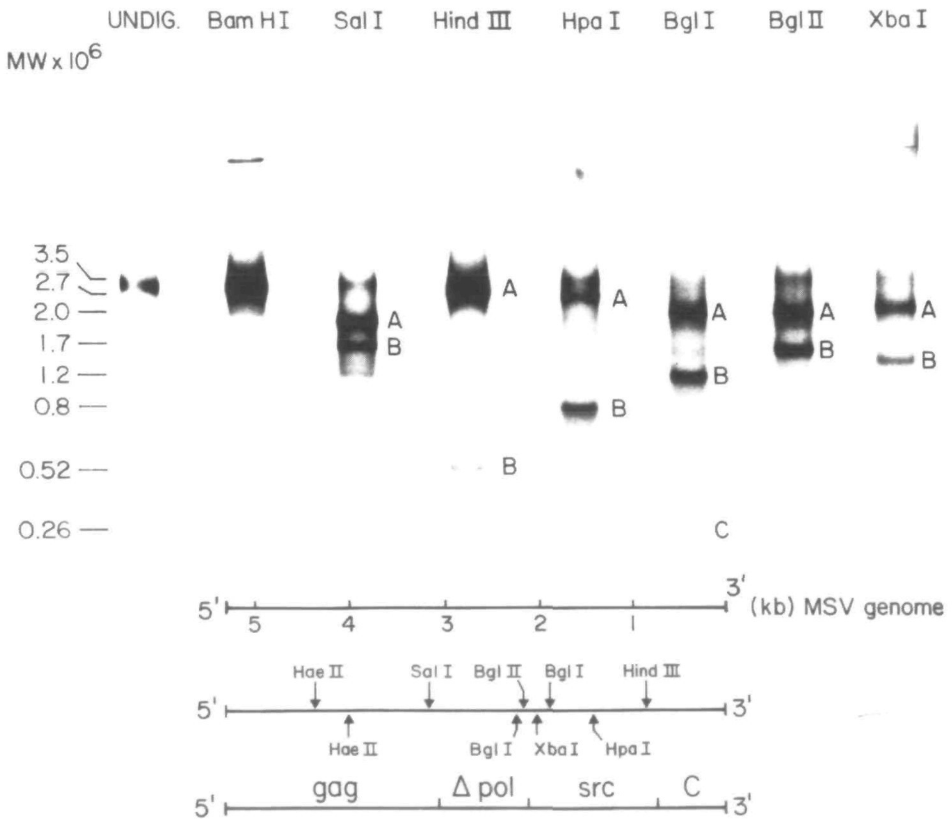

FIGURE 1. Patterns of MSV 124 double-stranded DNA generated by digestion with restriction endonucleases. *In vitro* double-stranded DNA was synthesized from purified virions of MSV 124 as described (5). The peak fraction was digested with the restriction endonucleases as described (5), and analyzed on 1.2% neutral agarose gels as described (5). The detailed molecular weights of the cleaved fragments have previously been described (5). The *Hae* II map was generated by redigestion of *Sal* IA, *Sal* IB, *Bgl* IIA and *Bgl* IIB fragments. Δ *pol* = truncated polymerase gene.

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restriction endonucleases, Bam HI, Sal I, Hind III, Bgl I, Bgl II, Hpa I and Xba I. The molecular weights and number of restriction fragments generated by these enzymes are listed in Table 1. Bam HI does not cleave MSV double-stranded DNA (data not shown). The order of the DNA fragments generated by digestion with restriction endonucleases in relation to the 5' and 3' ends of genomic RNA was established by two methods: a) cleavage of less than genome length double-stranded DNA as described before (5), and b) redigestion of DNA fragments obtained after digestion with restriction endonucleases, Sal I and Bgl II. A composite cleavage map of MSV double-stranded DNA is shown in Fig. 1. This physical map is in good agreement with those proposed by other groups (6,7).

A diagrammatical sketch of a heteroduplex formed between MSV Clone 124 viral

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TABLE 1. Molecular Weight Estimations of Various DNA fragments Generated by Digestion of MSV 124 DNA By Restriction Endonucleases

Restriction Endonuclease	Fragments Generated	Mol. Wt. of Fragments ($x 10^6$)	Nucleotide Base Pairs (kb)	Order of the Fragments 5'---3'
Undigested	---	3.5	5.3	---
Sal I	A	2.05	3.1	B-A
	B	1.45	2.2	
Hind III	A	3.0	4.52	A-B
	B	0.52	0.78	
Hpa I	A	2.7	4.09	A-B
	B	0.8	1.21	
Bgl II	A	2.1	3.18	A-B
	B	1.4	2.12	
Xba I	A	2.2	3.33	A-B
	B	1.3	1.97	
Bgl I	A	2.0	3.10	A-C-B
	B	1.21	1.81	
	C	0.26	0.39	
Hae II	A	2.65	4.0	B-C-A
	B	0.65	0.98	
	C	0.23	0.35	
Bam HI	---	3,5	5.3	---
E. coli R_1	---	3.5	5.3	---

RNA and M-MLV genome length cDNA transcripts is shown in Fig. 2. Since the genome of MLV is about 9 kb and that of MSV is only 6 kb, the most consistent interpretation of the heteroduplex in Fig. 2 will be as follows: a) About 0.69 kb at the 3' end of the two viral genomes is similar; b) The 1.36 kb substitution loop appears to be "src" specific, whereas the longer arm of the substitution loop appears to be the env gene of the M-MLV. The 1.36 kb "src" substitution loop starting at 0.69 kb from the 3' end of the MSV 124 genomic RNA has been independently confirmed by RNase T_I oligonucleotide fingerprinting (3), hybridization analysis (8) and recently by transfection studies (7); c) The two large deletion loops of approximately 1.15 kb and 1.6 kb appear to represent the pol gene of M-MLV. Murine sarcoma viruses have been shown to be deficient in reverse transcriptase; d) A large portion of the "gag" gene appears to be similar in the two viruses. There appear to be several restriction enzymes which cleave in or near the substitution loop. In a very elegant study, Anderson et al. (7) have shown that a DNA fragment cleaved with restriction endonucleases Sal I and Hind III is able to transform fibroblasts.

Fig. 2 compares the physical map of MSV 124 double-stranded DNA with that of M-MLV double-stranded DNA (5). Restriction endonuclease Sal I cleaves M-MLV double-stranded DNA once to generate two fragments. MSV 124 double-stranded DNA is also cleaved once to generate two fragments. There appears to be a 0.57 kb region of sequence homology between the two deletion loops. This region contains the Sal I sites. However, there appears to be a Bam I site in M-MLV, approximately 0.36 kb toward the 3' end of the genome which is not present in MSV double-stranded DNA, since the latter is not cleaved by Bam HI. Thus the

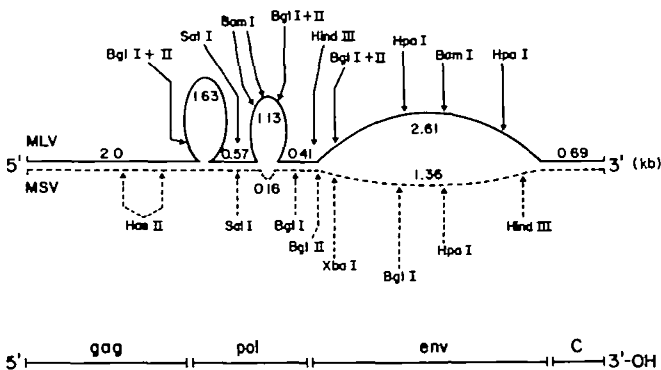

FIGURE 2. Comparison of physical maps of MSV 124 and M-MLV double-stranded DNAs in relation to heteroduplex analysis. The solid line represents M-MLV and the broken line represents MSV genomes.

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region of homology between M-MLV and MSV genomes can be localized by restriction enzymes. This type of analysis also points toward the notion that MSV is generated by deletion of M-MLV genome and recombination with cellular sequences. The Hind III site in MSV double-stranded DNA is most likely present in the "src" sequences as no corresponding Hind III site in M-MLV has been identified.

The physical maps of MSV 124 double-stranded DNA should prove to be very helpful in studying its integration into cellular DNA.

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Editor's note: Publication of this paper represents an experimental format for presentation of endonuclease cleavage site maps of exceptional interest and broad applicability. The referees were provided with the more extensive data from which the maps were constructed.

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