# Integration Sites for Moloney Murine Leukemia Virus DNA in Infected Mouse Cells

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The integration of viral DNA in mouse cells infected with Moloney murine leukemia virus (M-MuLV) has been studied by the "blotting" technology introduced by Southern (1975). We were interested in determining if there is a single site in the chromosomal DNA at which M-MuLV viral DNA is integrated, a small number of sites, or a large number of possible integration sites. The technique used involved cleavage of infected cell DNA with a sequence-specific restriction endonuclease, resolution of the resulting DNA fragments by electrophoresis in 0,6% agarose gels, and blot transfer of the separated fragments to nitrocellulose filters. Detection of DNA fragments with sequence homology to M-MuLV was achieved by hybridization with $^{32}$P-labeled M-MuLV complementary DNA (cDNA). The labeled cDNA (approximately $2 \times 10^8$ cpm/μg) was synthesized in an endogenous reaction using purified virus and added calf thymus oligo-deoxynucleotide primers in order to generate a uniformly representative probe (Fan and Verma, 1978).

The interpretation of the results of these experiments depends upon a knowledge of the cleavage pattern of viral DNA itself by each restriction endonuclease used. Fig. 1 shows cleavage of unintegrated M-MuLV linear proviral DNA by several restriction endonucleases. The molecular weight of the linear form of proviral DNA is $5{,}8 \times 10^6$ daltons. Most important for subsequent experiments is the fact that *Eco* RI endonuclease *does not* cleave unintegrated proviral DNA. Thus, cleavage of infected cell DNA should result in the appearance of virus-specific DNA fragments with molecular weights greater than $5{,}8 \times 10^6$ daltons, the exact size depending on the spacing of *Eco* RI cleavage sites in the host cell DNA surrounding the integrated M-MuLV DNA.

Fig. 2A shows the result when infected and uninfected cells DNAs are cleaved with RI and analyzed. It is immediately apparent that a large number of DNA fragments with sequence homology to M-MuLV are present in uninfected mouse cells. This is not entirely unexpected since mouse cells contain a family of sequences present in about 10 copies/haploid genome, which have sequence homology to about 50% of the genome of M-MuLV (Jaenisch et al., 1975). It is presumably these related sequences which are being detected in uninfected cell DNA. There are, however, additional bands present only in infected cell DNA, and these are tentatively identified as containing the M-MuLV DNA integrated during infection. Fig. 2B shows that the uninfected cell M-MuLV-related RI fragments are unique to mouse cells, and

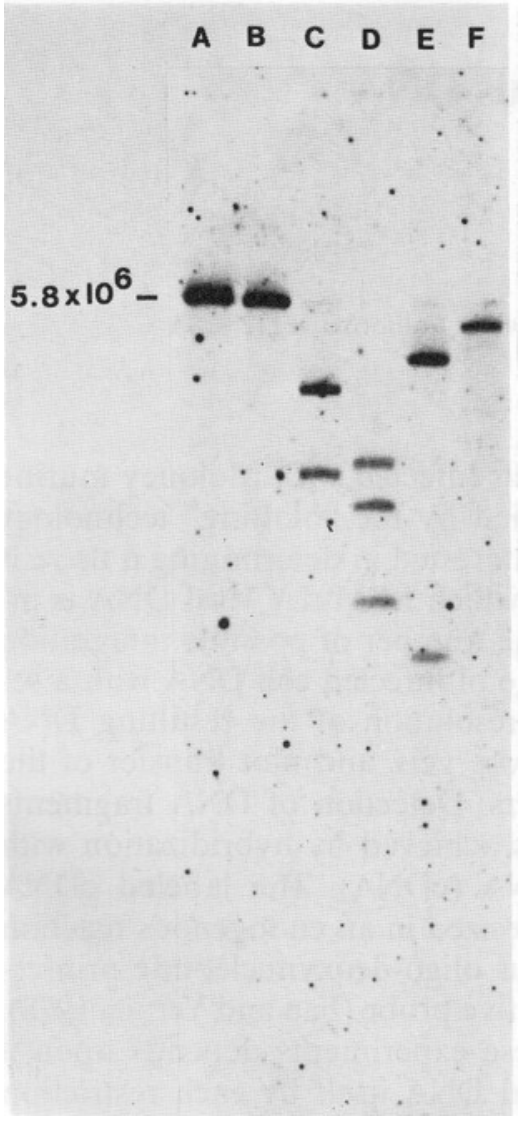


**Fig. 1.** Restriction enzyme cleavage pattern of unintegrated linear proviral DNA. Proviral DNA was isolated from 3T6 cells 20 hr after infection with Clone 1 M-MuLV. DNA was extracted from the cytoplasm and digested with various restriction enzymes. The DNA fragments were separated by electrophoresis in 0.6% agarose gels and transferred to nitrocellulose filters. The filters were hybridized with $^{32}$P-labeled M-MuLV cDNA. An autoradiogram of the washed filter is shown. The molecular weight of linear proviral DNA is $5.8 \times 10^6$ daltons. *A* – Untreated proviral DNA. *B* – RI-treated. *C* – *Hind* III-treated. *D* – *Bam* HI-treated. *E* – *Hpa* I-treated. *F* – *Pst* I-treated

not detected in rat DNA. All hybridization can be competed by an excess of purified 38S genomic viral RNA (Fig. 2C). Thus, all of the fragments detected by hybridization to $^{32}$P M-MuLV cDNA are related to viral DNA sequences, rather than related to possible nonviral sequences contaminating the $^{32}$P cDNA probe.

Fig. 3 shows the results obtained with RI cleaved cellular DNA from uninfected and M-MuLV-infected NIH and BALB/c cells. The pattern of M-MuLV related bands in the two uninfected cell lines is similar, but some differences also exist. In each infected cell line, at least one additional DNA fragment not found in the uninfected parent can be identified and we tentatively conclude that these unique fragments contain the integrated M-MuLV DNA.

The molecular weights of the M-MuLV-specific RI fragments unique to infected cells are different in each different cell line examined (10 different lines examined). In addition, none of the M-MuLV-containing fragments from exogenously infected fibroblasts co-migrate with the unique M-MuLV-containing RI fragment from cell lines carrying an endogenous M-MuLV (Jaenisch, 1976; Bacheler and Fan, 1978). Thus, we conclude that many dif-

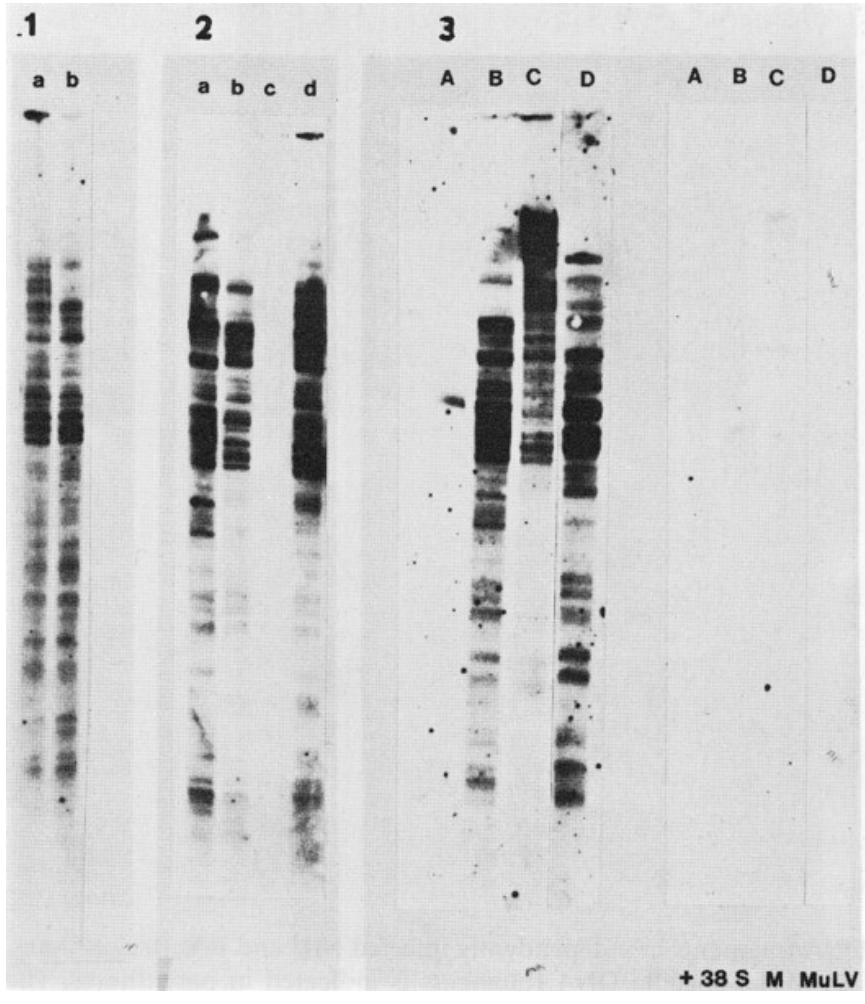


**Fig. 2.** M-MuLV related DNA fragments in infected and uninfected cell DNA. RI cleaved cell DNA from infected and uninfected lines was analyzed as in Fig. 1.
1. RI cleaved mouse cell DNA. *a* M-MuLV-infected NIH cell. *b* Uninfected NIH cell
2. RI cleaved mouse and rat cell DNA. *a* 3T6 uninfected NIH mouse cell line. *b* 3T3 uninfected NIH mouse cell line. *c* NRK uninfected rat cell line. *d* A9 M-MuLV infected NIH mouse cell line
3. RI cleaved mouse cell DNA. Replicate blots were hybridized in the presence or absence of an excess of unlabeled. purified 38S M-MuLV genomic RNA. *A* – Proviral DNA. *B* – G Clone 1 cell line from BALB/Mo mouse with an endogenous M-MuLV. *C* – A9 M-MuLV infected NIH cell line. *D* – 3T3 uninfected NIH cell line

ferent sites of integration for M-MuLV are possible upon exogenous infection of fibroblasts. This conclusion is based only upon the spacing of RI cleavage sites on either side of the integrated M-MuLV and it is possible that a small DNA region is specifically recognized during integration.

In each infected cell line examined, we have been able to detect at least one unique *Eco* RI fragment which presumably contains the integrated M-MuLV proviral DNA. Further evidence for the presence of an integrated M-MuLV DNA copy in each of these lines is provided by the results of digestions with *Bam* HI. *Bam* HI cleaves linear proviral DNA twice, resulting in three fragments of molecular weights of 2,5, 1,9 and $1{,}25 \times 10^6$ daltons. Verma and McKennett (1978) have shown that the $1{,}9 \times 10^6$ dalton B-fragment is an

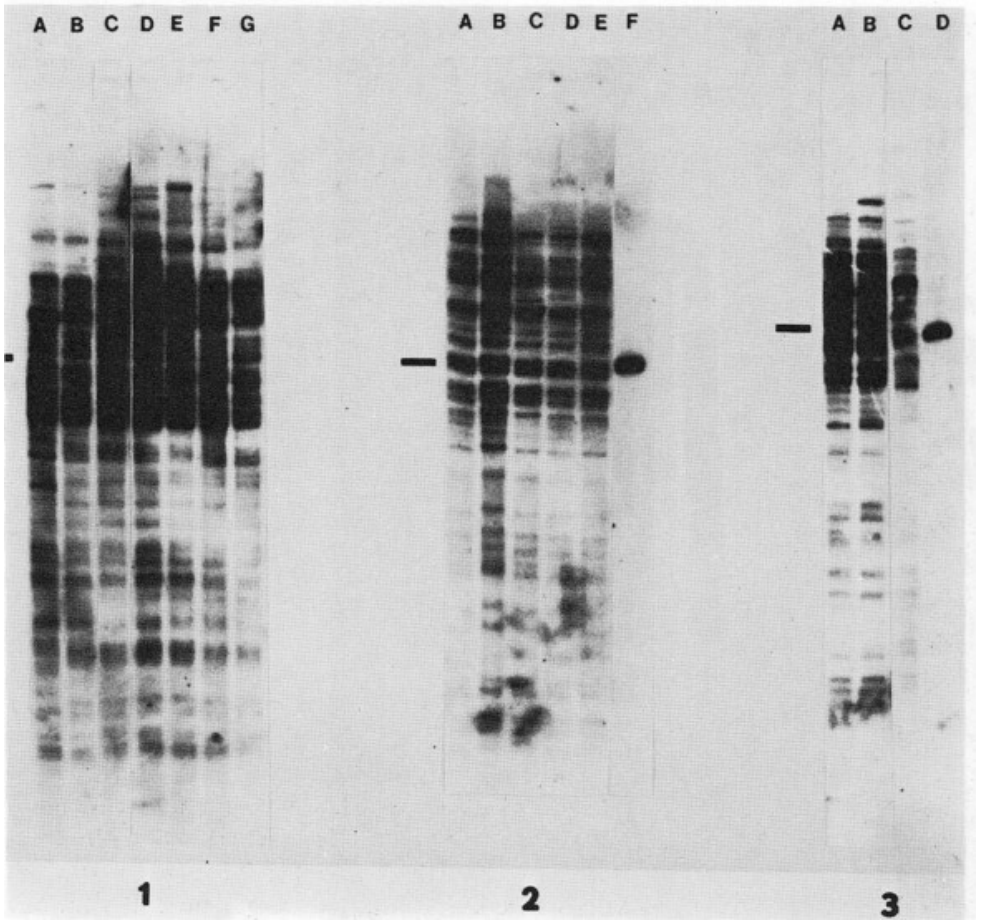


**Fig. 3.** M-MuLV related DNA fragments in independently infected NIH and BALB/c cell lines. The molecular weight of M-MuLV-specific DNA fragments is indicated in parentheses. The position of full length unintegrated proviral DNA is indicated by a bar.
1. *A* – 3T6 uninfected NIH. *B* – 3T3 uninfected NIH. *C* – A9 M-MuLV infected NIH (23–32. 21. 12.2. 8.7). *D* – B7 M-MuLV infected NIH (28–32. 21. 12.2. 8.7). *E* – E7 M-MuLV infected NIH (18.5). *F* – Clone 1 M-MuLV infected NIH (30. 21. 18). *G* – Clone 4A M-MuLV infected NIH (16.5).
2. *A* – M-MuLV infected SVT2 (17.6). *B* – M-MuLV infected SVT2 (18. 16.7). *C* – M-MuLV infected SVT2 (8.0). *D* – M-MuLV infected SVT2 (8.3). *E* – Uninfected SVT2. *F* – Proviral DNA (5.8).
3. *A* – Fibroblast line from BALB/c mice. *B–G* – Clone 1 fibroblast line from BALB/Mo mice carrying an endogenous M-MuLV (16.5–17). *C* – 2° MEF. from BALB/Mo mice (16.5–17). *D* – Proviral DNA (5.75)

internal fragment from the "middle" of viral DNA. Cleavage of each of the infected cell lines, but not uninfected cell DNA, results in the release of a $1.9 \times 10^6$ dalton fragment which co-migrates with the *Bam* HI-B fragment (Fig. 4). Fragments corresponding to the end fragments A and C are not seen. Thus, in each infected cell line, at least one M-MuLV viral DNA sequence arrangement retaining the internal cleavage sites for *Bam* HI is found. The sequence organization of the internal region of unintegrated proviral DNA is therefore retained upon integration.

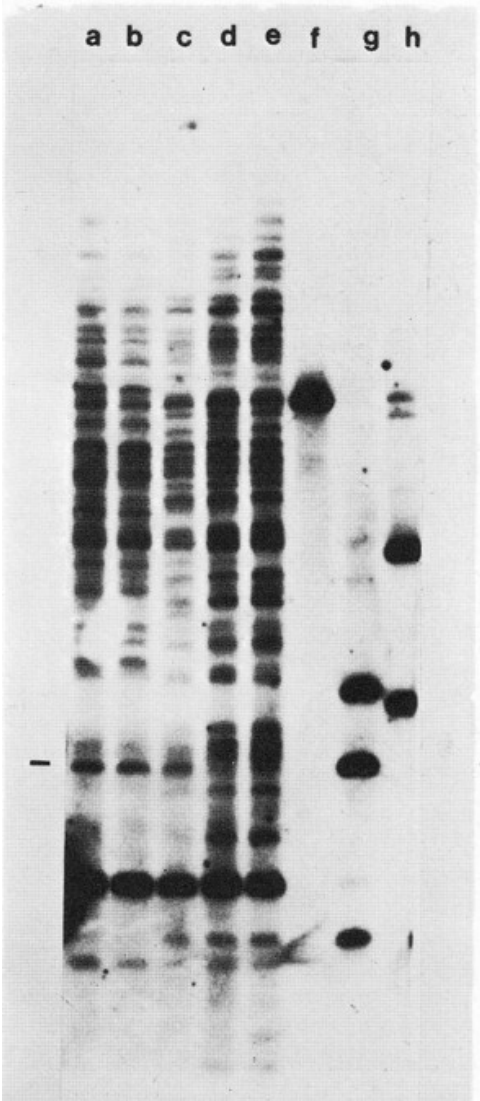


**Fig. 4.** M-MuLV related DNA fragments in infected and uninfected cell DNA cleaved with *Bam* HI. *A* – Clone 4A M-MuLV-infected NIH. *B* – Clone 3A M-MuLV-infected NIH. *C* – G Clone 1 BALB/Mo cell line carrying an endogenous M-MuLV. *D* – H uninfected BALB/c cell line. *E* – A31 uninfected BALB/c cell line. *F* – Proviral DNA. RI-treated. *G* – Proviral DNA. *Bam* HI-treated. *H* – Proviral DNA. *Hind* III-treated. The bar indicates the position of the $1.9 \times 10^6$ dalton *Bam* HI-B fragment

## References

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