JOURNAL OF VIROLOGY, Oct. 1983, p. 300–303
0022-538X/83/100300-04$02.00/0

Vol. 48, No. 1

Genetic Mapping of a Mouse Chromosomal Locus Required for Mink Cell Focus-Forming Virus Replication

CHRISTINE A. KOZAK

Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20205

Received 8 April 1983/Accepted 23 June 1983

Mouse-hamster somatic cell hybrids were used to show that the recombinant mink cell focus-forming murine leukemia viruses and their ecotropic virus progenitors require different mouse chromosomes for replication. Mouse chromosome 1 was shown to carry the genetic information necessary for the replication of six different mink cell focus-forming isolates, and this gene, designated *Rmc-1*, was tentatively positioned at the distal end of the chromosome.

Interspecific somatic cell hybrids have been effectively used to identify and chromosomally map genes required for the replication of murine leukemia viruses (MuLVs) in their natural host. Such studies have shown that cellular functions necessary for the replication of ecotropic and amphotropic MuLVs are coded by loci on chromosomes 5 and 8, respectively (4), and that the chromosome 5 locus codes for a specific cell surface viral receptor (15, 17). Another class of MuLVs, the mink cell focus-forming (MCF) viruses, represents de novo recombinants between infectious ectropic MuLVs and nonecotropic endogenous sequences. These viruses vary considerably with respect to the extent of genetic recombination and in a series of biological criteria. However, MCF viruses can all be distinguished from their replication-competent ecotropic progenitors by properties such as dualtropic host range, induction of mink cell foci, and reactivity in interference and neutralization assays (8, 16). In the experiments described here, mouse-hamster hybrids were used to demonstrate that ecotropic and MCF MuLVs also differ in the mouse chromosomal genes needed for replication in hybrid cells. Evidence is also presented that the major genetic determinant required for sensitivity to MCF virus infection is present on mouse chromosome 1 and that this locus may reside in a subchromosomal region known to be rich in retroviral sequences.

Somatic cell hybrids were made between mouse peritoneal cells or spleen cells and cells of the Chinese hamster line E36. The three sets of hybrids used here were generated by using cells from BALB/c, A, and NFS.*Akv-2* congenic mice and have been described previously (11–13). Specific primary and secondary hybrid lines were tested for their sensitivity to ecotropic, xenotropic, and MCF viruses. The virus stocks used were obtained from J. Hartley (National Institutes of Health, Bethesda, Md.) and included the NB-tropic Moloney ecotropic MuLV, the xenotropic isolate AKR-6, the Friend MCF virus, the MCF AKR-247 virus, and the MCF AKR-13 virus (2). C58/J Th-1 MCF virus was isolated by J. Hartley from the thymus of a 9-month-old C58/J mouse. Moloney-HIX MCF virus was originally obtained from P. Fischinger (National Institutes of Health, Bethesda, Md.) (3), and an MCF virus isolate from an HRS/J mouse, PTV-1, was obtained from R. Schwartz (Tufts Medical School, Boston, Mass.) (6). A parallel culture of each virus-challenged hybrid line was characterized for loss or retention of specific mouse chromosomes by testing for 13 mouse isozyme markers as described previously (14). A subset of these hybrids was selected for complete karyotypic analysis by the sequential use of Giemsa-trypsin banding and Hoechst 33258 (10).

A total of 45 hybrids were tested for susceptibility to both ecotropic and MCF MuLVs (Table 1). Of these, 28 (62%) were susceptible to one MuLV type but not both, demonstrating that different chromosomes are required for productive infection by ecotropic and MCF MuLVs. Furthermore, these data discount the possibility that the ecotropic receptor on chromosome 5 is necessary but not sufficient for MCF virus susceptibility, since the majority (19 of 26) of the MCF virus-sensitive hybrids were refractory to infection by the ecotropic virus.

A comparison of mouse isozyme expression and virus replication showed that only peptidase-3 (PEP-3) had a high degree of concordance with susceptibility to Moloney-HIX MCF virus. This was seen in hybrids of all three strains (Table 2). The two phenotypes were concordantly expressed or lost in all 45 primary hybrids. The expression of all other mouse isozyme phe-

TABLE 1. Comparison of ecotropic and MCF MuLV replication in somatic cell hybrids[a]

Hybrid series	No. of hybrids with ecotropic replication/MCF replication				% Discordant[b]
	+/+	−/−	+/−	−/+	
BALB/c	5	6	5	11	59
A	2	2	4	5	69
NFS.*Akv-2*	0	2	0	3	60

[a] Cells in subconfluent growth were treated with polybrene (16 μg/ml; Abbott Laboratories) and infected with the NB-tropic Moloney ecotropic MuLV or the Moloney HIX-MCF virus (10^2 to 10^4 PFU or focus-forming units per 0.2 ml, respectively). Cultures were washed 24 h later. After 4 days, cells were exposed to UV irradiation. The ecotropic virus-infected cultures were overlaid with SC-1 cells; the MCF virus-infected cultures were overlaid with mink lung cells (CCL64). Ecotropic virus was assayed 4 days later by the XC test. MCF virus infected cultures were passaged after 4 days to dishes containing cover slips; mink infectious virus was assayed by the fluorescent-antigen focus assay, using a fluorescein-conjugated anti-MuLV (7) antibody. In negative clones and the Chinese hamster parental line, virus replication was not detectable. Virus replication was generally less efficient (1 to 2 logs lower plating efficiency) in virus-sensitive hybrid lines, than in mouse embryo fibroblasts. This decrease may reflect some chromosomal heterogeneity, altered availability of receptors on hybrid cells, or restrictions in subsequent stages of virus replication.

[b] Total percentage of discordant hybrids, 62%.

notypes was highly discordant with virus replication (>30% discordance). Consistent with previous observations, ecotropic virus sensitivity correlated with the expression of PGM-1 and PEP-7, isozymes coded by chromosome 5 loci.

Ten primary clones which were MCF virus sensitive and expressed mouse PEP-3 activity were subcloned. Characterization of the subclones for the two phenotypes again showed that loss of chromosome 1 was almost completely consistent with loss of MCF virus sensitivity (Table 2). Of the four exceptional subclones, the subclone of BALB/c hybrid 14 was lost soon after initial testing and could not be characterized further. The three remaining exceptional clones were subclones of the same BALB/c hybrid; all three were sensitive to MCF virus but lacked PEP-3 activity. These lines were tested for expression of another chromosome 1 marker, *Bxv-1*, the xenotropic MuLV induction locus present at the distal end of chromosome 1 in BALB/c mice (14). All three subclones were induction positive, thus confirming the association of MCF virus sensitivity with chromosome 1 markers.

Karyotypic analysis was done on 27 hybrids, including 13 BALB/c hybrids, 10 A hybrids, and 4 NFS.*Akv-2* hybrids. Sensitivity to MCF virus showed a positive correlation with chromosome 1 (Table 3). Two discrepancies were identified. One hybrid, BM34, represents one of the three subclones noted above which was permissive for MCF virus and carried *Bxv-1*, although it lacked PEP-3 activity. The second discrepant clone, 7-2.9-2, was the product of five successive cycles of subcloning done in an attempt to isolate the BALB/c chromosome 1. After the fifth cycle of subcloning, hybrid 7-2.9-2 retained no mouse chromosomal markers except *Pep-3* and *Bxv-1*; it was also susceptible to MCF virus. Karyotypic analysis of hybrids BM34 and 7-2.9-2 indicated that no intact mouse chromosome 1 could be identified in either hybrid by Giemsa banding. No major chromosomal rearrangements were revealed in either hybrid by staining for mouse centromeres with Hoechst 33258; no mouse centromeres were present in 7-2.9-2. These data suggest that both hybrids carry noncentromeric fragments of chromosome 1.

Results of these karyotypic analyses support the conclusions that mouse chromosome 1 carries genetic information necessary for MCF virus replication and that no additional mouse chromosomes need be present. The identification of two MCF virus-sensitive hybrids which apparently carry only the distal portion of chromosome 1 further suggests that this sensitivity locus maps to the distal segment of chromosome 1. It is striking that a number of endogenous

TABLE 2. Correlation of Moloney-HIX MCF virus replication with mouse PEP-3 activity in somatic cell hybrids[a]

Hybrid series	No. of hybrids with MCF replication/PEP-3 activity			
	+/+	−/−	+/−	−/+
Primary clones				
BALB/c	12	7	0	0
A	7	6	0	0
NFS.*Akv-2*	7	4	0	0
Secondary clones				
BALB/c 1	1	1	0	0
BALB/c 12	1	1	0	0
BALB/c 2	1	1	0	0
BALB/c 5	11	1	0	0
BALB/c 14	1	4	1	0
BALB/c 3	3	0	3[b]	0
BALB/c 7-2	7	3	0	0
A 3	10	2	0	0
A 6	3	0	0	0
NS 12	7	2	0	0

[a] Ten replication-sensitive primary clones were used to generate secondary clones. The total percentages of discordant hybrids were 0% for primary clones and 6% for secondary clones.

[b] All three were positive for another chromosome 1 locus, *Bxv-1* (see text).

TABLE 3. Correlation of mouse chromosomes and MCF virus replication in 27 somatic cell hybrids

Mouse chromosome	No. of hybrid clones with chromosome retention/MCF replication				% Discordant
	+/+	−/−	+/−	−/+	
1	9	16	0	2	7
2	6	8	8	5	48
3	6	13	3	5	30
4	4	14	2	7	33
5	0	15	1	11	44
6	5	12	4	6	37
7	8	5	11	3	52
8	4	14	2	7	33
9	4	14	2	7	33
10	4	15	1	7	30
11	0	16	0	11	41
12	6	6	10	5	56
13	5	12	4	6	37
14	3	14	2	8	37
15	10	6	10	1	41
16	5	11	5	6	41
17	9	9	7	2	33
18	7	13	3	4	26
19	6	8	8	5	48
X	7	12	4	4	30
Y	0	16	11	0	41

proviral sequences homologous to xenotropic MuLV (M. D. Hoggan and C. A. Kozak, unpublished data) or mouse mammary tumor virus (19; J. I. MacInnes, V. L. Morris, W. F. Flintoff, and C. A. Kozak, unpublished data) have also been mapped to this same region. The close physical association of these loci warrants further study and suggests that there may be chromosomal regions rich in genetic information related to retroviruses.

Biological data from interference assays (16) and the biochemical characterization of MuLV genomes (1) suggest that the envelope glycoproteins of different MCF virus isolates are similar and that they are related to xenotropic MuLV envelope glycoproteins. Therefore, additional experiments were done to determine whether xenotropic MuLVs, as well as other MCF virus isolates, also replicate only in hybrids carrying chromosome 1. A panel of 10 hybrids was tested for sensitivity to five additional MCF viruses: AKR-247, AKR-13, Friend MCF, PTV-1, and C58/J Th-1. Only the five hybrids with chromosome 1 were sensitive to all isolates. Similarly, four MCF virus-sensitive hybrids with few mouse chromosomes, including 7-2.9-2, were challenged with the xenotropic isolate AKR-6. None of these hybrids was sensitive to infection. This negative result is not considered conclusive, however, since it has been suggested that there are several levels of resistance to xenotropic MuLV in mouse cells (5, 9, 18). Therefore, the MCF virus-sensitive, xenotropic MuLV-resistant hybrid cells may still retain mouse genetic information that specifically blocks xenotropic MuLVs.

The loci for sensitivity to ecotropic and amphotropic MuLVs have been designated *Rec-1* and *Ram-1*. Consistent with this nomenclature, I recommend naming the MCF virus sensitivity locus *Rmc-1*. Although it is probable that *Rmc-1*, like *Rec-1*, codes for a cell surface receptor, the nature of this locus has not been determined here. However, the isolation of chromosomes required for replication of each class of MuLV in permanent hamster hybrid lines is of practical value in the characterization of these loci and their gene products. Such hybrid lines should also provide an efficient means of isolating or identifying new MCF viruses.

I thank C. Corey for technical assistance and S. Grove for helping in preparing the manuscript.

This work was supported in part by National Institutes of Health contract N01-CP-33288.

LITERATURE CITED

1. **Chattopadhyay, S. K., M. W. Cloyd, D. L. Linemeyer, M. R. Lander, E. Rands, and D. R. Lowy.** 1982. Cellular origin and role of mink cell focus-forming viruses in murine thymic lymphomas. Nature (London) **295**:25–31.
2. **Cloyd, M. W., J. W. Hartley, and W. P. Rowe.** 1979. Cell-surface antigens associated with recombinant mink cell focus-inducing murine leukemia viruses. J. Exp. Med. **149**:702–712.
3. **Fischinger, P. J., S. Nomura, and D. P. Bolognesi.** 1975. A novel murine oncornavirus with dual ecotropic and xenotropic properties. Proc. Natl. Acad. Sci. U.S.A. **72**:5150–5155.
4. **Gazdar, A. F., H. Oie, P. Lalley, W. Moss, J. D. Minna, and U. Francke.** 1977. Identification of mouse chromosomes required for murine leukemia virus replication. Cell **11**:949–956.
5. **Gazdar, A. F., E. K. Russell, and J. D. Minna.** 1974. Replication of mouse tropic and xenotropic strains of murine leukemia virus in human × mouse hybrid cells. Proc. Natl. Acad. Sci. U.S.A. **71**:2642–2645.
6. **Green, N., H. Hiai, J. H. Elder, R. S. Schwartz, R. H. Khiroya, C. Y. Thomas, P. N. Tsichlis, and J. M. Coffin.** 1980. Expression of leukemogenic recombinant viruses associated with a recessive gene in HRS/J mice. J. Exp. Med. **152**:249–264.
7. **Hartley, J. W., and W. P. Rowe.** 1976. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. J. Virol. **19**:19–25.
8. **Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe.** 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. Proc. Natl. Acad. Sci. U.S.A. **74**:789–792.
9. **Ishimoto, A., J. W. Hartley, and W. P. Rowe.** 1977. Detection and quantitation of phenotypically mixed viruses: mixing of ecotropic and xenotropic murine leukemia viruses. Virology **81**:263–269.
10. **Kozak, C. A., J. B. Lawrence, and F. H. Ruddle.** 1977. A sequential staining technique for the chromosomal analysis of interspecific mouse/hamster and mouse/human somatic cell hybrids. Exp. Cell Res. **105**:109–117.
11. **Kozak, C. A., E. Nichols, and F. H. Ruddle.** 1975. Gene linkage analysis in the mouse by somatic cell hybridization: assignment of adenosine phosphoribosyltransferase to chromosome 8 and α-galactosidase to the X chromosome. Somatic Cell Genet. **1**:371–382.

12. **Kozak, C. A., and W. P. Rowe.** 1979. Genetic mapping of ecotropic murine leukemia virus-inducing locus of BALB/c mouse to chromosome 5. Science **204**:69–71.
13. **Kozak, C. A., and W. P. Rowe.** 1980. Genetic mapping of the ecotropic virus-inducing locus (*Akv-2*) of the AKR mouse. J. Exp. Med. **152**:1419–1423.
14. **Kozak, C. A., and W. P. Rowe.** 1980. Genetic mapping of xenotropic murine leukemia virus-inducing loci in 5 mouse strains. J. Exp. Med. **152**:219–228.
15. **Oie, H. K., A. F. Gazdar, P. A. Lalley, E. K. Russell, J. D. Minna, J. DeLarco, G. J. Todaro, and U. Francke.** 1978. Mouse chromosome 5 codes for ecotropic murine leukemia virus cell-surface receptor. Nature (London) **274**:60–62.
16. **Rein, A.** 1982. Interference grouping of murine leukemia viruses: a distinct receptor for the MCF-recombinant viruses in mouse cells. Virology **120**:251–257.
17. **Ruddle, N. H., B. S. Conta, L. Leinwand, C. Kozak, F. Ruddle, P. Besmer, and D. Baltimore.** 1978. Assignment of the receptor for ecotropic murine leukemia virus to mouse chromosome 5. J. Exp. Med. **148**:451–456.
18. **Scolnick, E. M., and W. P. Parks.** 1974. Host range studies on xenotropic type C viruses in somatic cell hybrids. Virology **59**:168–178.
19. **Traina, V. L., B. A. Taylor, and J. C. Cohen.** 1981. Genetic mapping of endogenous mouse mammary tumor viruses: locus characterization, segregation, and chromosomal distribution. J. Virol. **40**:735–744.