Genetic Transmission of Moloney Leukemia Virus: Mapping of the Chromosomal Integration Site

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Abstract

Mice genetically transmitting the exogenous Moloney leukemia virus (Balb/Mo) have been previously derived. These animals carried one copy of Moloney virus DNA (M-MuLV) in their germ line and transmitted the virus as a single Mendelian gene to the next generation.

Homozygous BALB/Mo mice were used to genetically map the M-MuLV locus. Embryo fibroblasts were fused to established Chinese hamster cells and somatic cell hybrids were selected. Segregation of mouse chromosomal markers in the hybrids was correlated to the loss of M-MuLV-specific sequences as detected by molecular hybridization. Of 15 isozymes located on different mouse chromosomes only triosephosphate isomerase segregated syntenic with the M-MuLV gene, suggesting that the virus was integrated on chromosome No. 6. This was confirmed by sexual genetic experiments analyzing segregation of Moloney viremia and two markers on chromosome 6 and 15, respectively. The results show that M-MuLV expression is linked to wa-1 on chromosome 6 at a distance of about 30 map units. These data define a new genetic locus, *Mov-1*, representing the structural gene of M-MuLV in BALB/Mo mice.

Introduction

Endogenous C-type viruses are transmitted genetically from one generation to the next [1,2,3] and thus are present in every cell of the animal. In contrast, exogenous C-type viruses, which are transmitted by horizontal infection, are not part of the germ line of an animal and thus are not transmitted genetically. The apparent resistance of the germ line to infection with leukemia virus may be explained by the "organtropism" of leukemia viruses; only certain "target" tissues such as spleen or thymus cells are susceptible to infection, whereas most other non-target tissues cannot be infected by leukemia virus [4,5].

It has been possible to overcome the "organtropism" restriction of the exogenous Moloney leukemia virus (M-MuLV) by infecting mice at the 4–8 cell preimplantation stage before differentiation into "target" or "non-target" cells has taken place. These embryos developed into normal adult animals, which carried M-MuLV-specific sequences in all organs including cells of the germ line [5]. Thus the exogenous virus, M-MuLV, was established as an

endogenous virus in these mice (= BALB/Mo). Genetic and biochemical evidence indicated that these mice were heterozygous for a single Mendelian locus with one proviral copy integrated at this site. Mice, homozygous at this locus, were derived and molecular hybridization studies have demonstrated a somatic amplification of these virus sequences in target cells during the process of leukemogenesis [5,6].

BALB/Mo mice develop a specific thymus-dependent leukemia similar to the disease observed in AKR mice. A number of genetic factors appear to control the development of leukemia by endogenous C-type viruses and/or the susceptibility to leukemic transformation by exogenous C-type viruses. Attempts to elucidate genetic factors involved in virus replication and transformation include the use of sexual genetic experiments and the use of interspecies somatic cell hybrids preferentially segregating the chromosomes of one species. The following genes related to replication or regulation of MuLV have been assigned to individual mouse chromosomes: Fv-1 to chromosome No. 4 [7], Rec-1 to No. 5 and Ram-1 to No. 8 [8], Fv-2 to No. 9 [9] and Rgv-1 to No. 17 [10]. In human cells a locus controlling the replication of Baboon endogenous type-C virus has been assigned to chromosome No. 6 [11]. So far, Akv-1 represents the only genetically defined locus representing the proviral genome of an endogenous virus. The Akv-1 locus has been mapped by sexual genetics on chromosome 7 near Gpl-1 [12,13]. Another proviral genome in the AKR strain, Akv-2, has not been assigned to a particular mouse chromosome.

In this paper we summarize our experiments to genetically map the integration site of M-MuLV in BALB/Mo mice.

Methods

All methods used to quantitate virus-specific sequences by molecular hybridization and to measure virus expression in animals have been described previously [4–6]. The M-MuLV cDNA used was selected against uninfected mouse DNA to remove all sequences which cross-hybridize with endogenous mouse viruses [6]. Cell fusion experiments and isoenzyme tests were performed essentially as described elsewhere [14–17]. Mice were bred in our laboratory or purchased from the Jackson Laboratory.

Results

We have used both sexual and somatic cell genetic approaches to map the M-MuLV locus in the mouse genome. First sexual genetic experiments utilizing crosses between BALB/Mo and C57BL mice excluded five chromosomes as potential integration sites. We then utilized interspecies somatic cell hybrids, which segregated specifically mouse chromosomes to identify the chromosome syntenic with the M-MuLV locus. Evidence described in the following section suggested chromosome No. 6 as integration site for M-MuLV. This was confirmed in subsequent sexual genetic experiments using appropriate linkage test strains of mice.

A. Linkage Studies Using Somatic Cell Hybridization

Fibroblast cultures were established from BALB/Mo embryos homozygous for the M-MuLV locus. Fibroblasts are non-target cells for M-MuLV and therefore spontaneous virus expression is repressed [4]. As expected, molecular hybridization experiments showed the presence of 2 M-MuLV copies per diploid mouse genome. However, when these cultures are superinfected with M-MuLV, they produce high titers of virus and virus production is accompanied by an amplification of M-MuLV-specific DNA sequences (unpublished).

These mouse fibroblasts were fused to established Wg 3-h-O Chinese hamster cells [17] in the presence of polyethylene glycol. It has been shown previously that somatic cell hybrids isolated after fusion of secondary mouse fibroblasts and established Chinese hamster cells segregate mouse chromosomes [18]. Hybrid clones were selected in HAT medium and analyzed: (i) for the presence of mouse chromosomes by isozyme determinations, (ii) for the presence of M-MuLV-specific DNA sequences by molecular hybridization, and (iii) for virus production by assaying for infectious virus by the XC assay. The latter test was important since it has been observed that fusion of mouse macrophages to human cells readily activates endogenous mouse viruses [19]. Activation and production of the endogenous M-MuLV could possibly lead to reintegration of the virus in either mouse or hamster chromosomes and thus prevent identification of the original integration site by segregation analysis. Therefore, all hybrid clones were tested for the presence of infectious virus. None of 30 clones tested produced virus, indicating that expression of integrated M-MuLV in BALB/Mo fibroblasts was under tight control and that M-MuLV was not activated upon fusion of BALB/Mo fibroblasts to hamster cells.

So far, we have analyzed 30 independent mouse-Chinese hamster clones segregating mouse chromosomes. Table 1 is a summary of our observations correlating M-MuLV-specific sequences with the presence of isozyme markers for 15 mouse chromosomes enzyme markers were assayed for chromosomes 3, 13, 15, 16 and 17. The expression of mouse triosephosphate isomerase, whose gene has been recently assigned to chromosome No. 6 [20], was concordant in 93% of the clones with the presence of M-MuLV specific sequences. Only one out of 30 hybrid clones had lost the enzyme but retained M-MuLV-specific sequences, and one clone had retained the enzyme but lost the virus sequences. Karyotype analyses have been performed on these two exceptions: no chromosome No. 6 could be identified by morphological criteria. This suggests that chromosome No. 6 was fragmented and transposed to another chromosome in these clones. All other enzyme markers showed a highly asyntenic segregation with the M-MuLV gene. It should be emphasized here that our experiments using molecular hybridization with a specific cDNA probe identify the structural gene of M-MuLV and not some regulatory locus for virus expression. These data therefore suggest, but do not prove, that M-MuLV in BALB/Mo mice is integrated on chromosome No. 6. So far, we cannot rule out that chromosomes 3, 13, 15, 16 or 17 were retained

Table 1. Correlation between mouse isozymes and M-MuLV-specific DNA sequences in mouse-chinese hamster hybrid clones

Mouse isozyme	Mouse chromosome	Isozyme retention/ M-MuLV DNA present (% asyntenic segregation)
Dipeptidase-1	1	27
Adenylate kinase 1	2	40
Phosphoglucomutase-2	4	53
Phosphoglucomutase-1	5	50
Trisephosphate isomerase	6	7
Glucosephosphate isomerase	7	22
Adenine phosphoribosyl-transferase	8	30
Mannosephosphate isomerase	9	40
Tripeptidase-1	10	27
Galactokinase	11	50
Acid phosphatase	12	43
Esterase-10	14	30
Dipeptidase-2	18	43
Glutamate-oxalacetate transaminase	19	57
Hypoxanthine phosphoribosyl-transferase	X	37

Thirty independent somatic cell hybrid clones were analyzed for the presence of 15 mouse chromosomes by isozyme determinations and for the presence of M-MuLV-specific sequences by molecular hybridization.

in many hybrids and thus could possibly carry the M-MuLV gene. However, results obtained in sexual genetic experiments described in the next section strongly support the notion that the M-MuLV gene is integrated on chromosome No. 6.

B. Assignment of the M-MuLV Locus by Sexual Genetic Experiments

In our first genetic experiments to map the M-MuLV locus we mated BALB/Mo mice with C57BL mice and backcrossed the F-1 animals to C57BL. The resulting BC-1 (= backcross 1) animals were tested for correlation or lack of correlation between the presence of M-MuLV sequences and inheritance of 6 markers on 5 different chromosomes. Table 2 summarizes these data. It is evident that the M-MuLV gene was not linked to any of the markers tested. This included chromosome 7 on which the Akv-1 locus was mapped near the Gpl-1 marker [12]. These results are in agreement with the segregation data using somatic cell hybrids described above.

Since the somatic cell hybrid experiments suggested chromosome No. 6 as a possible integration site for M-MuLV, we crossed BALB/Mo with ABP/J mice. This strain is a linkage test strain carrying the morphological marker wa-1 (curled whiskers) on chromosome No. 6 and the coat colour marker bt (white belt) on chromosome 15. Since both markers are recessive, the viremic F-1 mice show straight whiskers and no belt (F-1 genotype: $Mo^+ + +/Mo^-$ bt wa-1). F-1 males were backcrossed to ABP females and the presence of M-MuLV in the serum was correlated to the expression of both recessive mark-

Table 2. Segregation analysis of cross between BALB/Mo × C57BL

Marker tested	Mouse chromosome	Total No. of BC-1 animals	Segregation of M-MuLV-specific sequences and markers in BC-1 generation	
			Parental	Recombinant
Agouti	2	94	53	41
MUP I	4	94	46	48
GPI-1	7	90	38	52
Hbb	7	94	45	49
Es-1	8	94	54	40
Mod-1	9	64	33	31

BALB/Mo animals were mated with C57BL mice. F-1 mice were backcrossed to C57BL and the resulting BC-1 animals analyzed for M-MuLV-specific DNA sequences in their liver DNA and for the indicated markers [16].

ers. A study of the segregation of the Mo^+ and wa-1 markers shows that 69% of the BC-1 animals were of parental genotype and 31% recombinants (Table 3A). This suggests strongly that the M-MuLV locus segregates together with the wa-1 locus. The frequency of recombinants assigns the M-MuLV locus 31 map units from wa-1 on chromosome No. 6.

As controls the segregation of the M-MuLV locus and the bt locus as well as the segregation of bt and wa-1 are given in Table 3B and C. About equal proportions of recombinant and parental genotypes are found, indicating independent segregation of these loci. These results confirm the assignment of the M-MuLV locus to chromosome 6 and argue against chromosome 15 as the Moloney virus carrying chromosome (see discussion).

Discussion

Integration of exogenous RNA tumor virus sequences into the chromosomal DNA of infected cells has been demonstrated in many systems [1,30,31]. The exogenous Moloney leukemia virus has been established as an endogenous virus in mice by infection at the 4–8 cell preimplantation stage and integration of viral specific information into single blastomeres [5]. The subline of mice derived from such an infection transmitted the M-MuLV gene in only one chromosomal locus, and this virus gene was maintained in the colony by paternal transmission from heterozygous males mated with normal females. The integration of M-MuLV into the paternal chromosome complement had no detectable effect on normal embryonal and postnatal development of the animals. Further experiments were performed to determine whether homozygosity at the M-MuLV locus was compatible with normal development. Molecular hybridization and genetic experiments were used to identify homozygous offspring derived from matings of heterozygous parents [6]. The results indicated that homozygosity at the M-MuLV locus has no effect on

Table 3. Segregation analysis of cross between BALB/Mo × ABP

P-1 $Mo^+ + +/Mo^+ + +$ × Mo^-wa-1 bt/Mo^-wa-1 bt P-2

F-1 $Mo^+ + +/Mo^-$wa-1 bt × Mo^-wa-1 bt/Mo^-wa-1 bt P-2

BC-1

A. Segregation of Mo^+ and wa-1

BC-1	$Mo^+ +$	Mo^- wa-1	Mo^+ wa-1	$Mo^- +$
No. of animals	74	80	37	33
	69% parental		31% recombinant	

B. Segregation of Mo^+ and bt

BC-1	$Mo^+ +$	Mo^- bt	Mo^+ bt	$Mo^- +$
No. of animals	52	65	56	46
	53% parental		47% recombinant	

C. Segregation of bt and wa-1

BC-1	+ +	bt wa-1	+ wa-1	bt +
No. of animals	54	68	49	54
	54% parental		46% recombinant	

Homozygous BALB/Mo were mated with ABP mice. The resulting F-1 animals were backcrossed to ABP mice and the segregation of the markers wa-1, bt and Mo^+ was determined.

the normal development of the mice. Furthermore, it was observed that the development of leukemia is not influenced by the genotype of the mice because mice homozygous or heterozygous for the M-MuLV locus or normal animals infected at birth with virus developed disease at similar rates. The latter animals carry M-MuLV-specific sequences only in their target tissue and never transmit the virus genetically [5,6].

We have shown recently that BALB/Mo mice express virus-specific RNA sequences or virus-specific proteins in target tissues only, whereas the virus gene remains repressed in non-target tissues. Similarly, a somatic virus gene amplification during the process of leukemogenesis is observed in target tissues only [5,21,32]. It has been suggested that the observed tissue specificity

of expression and transformation of different RNA tumor viruses may be due to different but virus-specific integration sites into the host chromosomes [22]. As a first attempt to approach this question, we performed experiments to identify the mouse chromosome carrying the M-MuLV locus. It was of special interest to investigate whether M-MuLV was integrated into chromosome 15, since a trisomy of this chromosome has been shown to occur frequently in M-MuLV or AKR virus-induced lymphomas [23,24].

Somatic cell hybrids between BALB/Mo fibroblasts and hamster cells were analyzed for segregation of specific mouse chromosomes and M-MuLV-specific DNA sequences. Our preliminary experiments analyzing isozyme markers on 15 mouse chromosomes showed that only triosephosphate isomerase segregates syntenic with M-MuLV-specific sequences. The gene coding for this enzyme has been assigned recently to mouse chromosome No. 6 [20], and thus our experiments suggested that M-MuLV in BALB/Mo mice is located on this chromosome. This preliminary assignment was confirmed by sexual genetic experiments. We used two independently segregating markers, wa-1 on chromosome 6 and bt on chromosome 15, to study possible linkage of virus induction in BC-1 animals. The results in Table 3 show that M-MuLV was linked to the wa-1 marker and unlinked to the bt marker. The frequency of recombinants suggested that the M-MuLV locus is approximately 31 map units away from the wa-1 locus on chromosome No. 6. On the basis of these data we propose to call this gene *Mov-1* denoting Moloney virus in BALB/Mo mice.

The first structural gene comprised of the proviral DNA sequences of an endogenous virus, the AKR leukemia virus, was assigned to the *AKv-1* locus on chromosome No. 7 [12,13]. Another, unlinked proviral genome in AKR mice, *AKv-2*, has not been localized yet. The similarities between the AKR virus-induced and the M-MuLV-induced disease should be emphasized here. Both AKR and Moloney virus induce a thymus-derived leukemia with a frequent chromosome 15 trisomy. In both cases a somatic amplification of virus-specific sequences is observed during leukemogenesis, and in both cases virus gene expression seems to be restricted to the target organs [5,21,32]. The only two virus structural genes mapped so far, which induce thymus-dependent leukemia, *Mov-1* and *Akv-1*, are clearly non-allelic. We consider two alternatives to explain the molecular events during virus-induced thymus-dependent leukemogenesis. In the first hypothesis, leukemia viruses can integrate at a few virus-specific chromosomal sites to induce tissue-specific virus expression and virus gene amplification, which is followed by transformation of specific target cells [22]. Alternatively, the virus could integrate at one or a few out of a large number of possible integration sites. These original integration sites would not be specific for the virus, but the observed specificity of expression and transformation may depend on specific secondary integrations which occur during leukemogenesis. Only those lymphatic cells which carry additional virus sequences at transformation-specific sites might be selected for during the process of leukemogenesis.

In order to decide between these hypotheses, it will be necessary to develop new mouse lines which carry M-MuLV in their germ line and to map the

integration sites. It also would be interesting to study if *AKv-2* is integrated at a site homologous to *Mov-1*. These studies will contribute to our understanding of how a virus can transform a specific target cell.

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