Molecular analysis of the Mov 34 mutation: transcript disrupted by proviral integration in mice is conserved in Drosophila

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Summary

The Mov 34 mutation is a recessive embryonic lethal mutation caused by retroviral integration in the murine germline. This integration disrupts a transcription unit that appears to encode a novel protein. The Mov 34 proviral integration is located on mouse chromosome 8 and the human homolog of this gene has been mapped to chromosome region 16q23-q24. An evolutionary conserved syntenic relationship exists between this region of human chromosome 16 and a region of mouse chromosome 8 that also contains oligosyndactyly (Os), another recessive lethal mutation. Genetic studies have ruled out Os as residing at the same locus as the Mov 34 integration.

The Mov 34 transcript is conserved in evolution, and a Drosophila homolog appears to encode a protein with 62% amino acid identity to the murine protein. In situ hybridization to Drosophila polytene chromosomes localizes the Drosophila homolog to 60B,C on chromosome 2. Several Drosophila lethal mutations also map to this region.

Key words: Mov 34, insertional mutation, Drosophila homolog, embryonic lethal.

Introduction

Despite the long history of the collection and analysis of mutant mice, the number of mouse mutations in which the primary defect is understood at the molecular level is still small (Balling et al. 1988; Roach et al. 1985; Stoye et al. 1988; Veres et al. 1987). This is due primarily to the large genome size of the mouse and the limited resolution afforded by traditional genetic crosses. An alternative approach has been to generate new mouse mutations by the experimental introduction of exogenous DNA into the mouse germ line (for review, see Gridley et al. 1987; Jaenisch, 1988). The introduced DNA then serves as a tag for the cloning of the affected locus. A number of such insertional mutants have been produced and analyzed (Covarrubias et al. 1987; Covarrubias et al. 1986; Jaenisch et al. 1983; Mahon et al. 1988; Mark et al. 1985; McNeish et al. 1988; Schnieke et al. 1983; Spence et al. 1989; Wilkner and Palmiter, 1987; Woychik et al. 1985).

We have previously described a mutant mouse strain, Mov 34, in which integration of a Moloney murine leukemia provirus into the germ line has resulted in a recessive embryonic lethal mutation (Soriano et al. 1987). Embryos homozygous for the Mov 34 proviral integration die shortly after implantation into the uterus. Here we describe the chromosomal location of this integration and a genetic complementation and mapping analysis with a previously described embryonic lethal mutation on that chromosome. We also report the cDNA sequence analysis of a transcript disrupted by the proviral integration, and the cloning and analysis of a Drosophila homolog of this gene.
Materials and methods

Chromosomal mapping of Mov 34 integration site
Southern analysis of 18 Chinese hamster×mouse and one rat×mouse somatic cell hybrid lines with partial complements of mouse chromosomes and of 16 Chinese hamster×human and one mouse×human hybrid cell line was carried out by standard methods as described (Barton et al. 1986). The hybrids were derived from 10 different series as summarized recently (Francke et al. 1986). The Mov 34 sequence on the mouse X chromosome was sublocalized with a hybrid containing the 16 T derivative of Searle's T(X;16)16H translocation (Francke and Taggart, 1979). The human Mov 34 sequences on chromosomes 16 and 17 were further mapped by using hybrid lines carrying only region 16pter-16q22 (Barton et al. 1986) or region 17q21-pter, respectively (Francke and Bushby, 1975). Discordancy analysis was carried out individually for each of 3 EcoRI fragments in the mouse and 5 EcoRI fragments in the human against each of the mouse and human chromosomes in the hybrids segregating the appropriate species' chromosomes. The Southern blots shown in Fig. 1 were hybridized with pC2. Similar results were obtained after hybridization with pC28.

In situ hybridization of pC2, nick-translation with 3H-TdR, to a human peripheral blood chromosome preparation from a normal female was carried out as described previously (Francke et al. 1986). Cells with 1 to 5 grains over chromosomes were randomly selected. Grain localizations were recorded on standard human chromosome ideograms.

Library screening
Genomic and cDNA libraries were screened, the hybridizing phage plaque purified, and the inserts subcloned by standard procedures (Maniatis et al. 1982). For the isolation of additional murine Mov 34 cDNA clones, a Balb/c day 16 embryo cDNA library (gift of A. Stacey) was screened with a 4.8 kb HindIII fragment of the genomic Mov 34 locus (probe B in Soriano et al. 1987). For analysis and sequencing, inserts were subcloned into either pGem4 (Promega) or pBl30 (International Biotechnologies, Inc.). The Drosophila genomic DNA library (gift of T. Maniatis) was screened with the Mov 34 cDNA clone pC28. The 12 kb insert of the Drosophila genomic clone isolated was used as a probe on a Northern blot (gift of M. Rosbash) of isolated heads and bodies of adult flies. The 12 kb Drosophila genomic insert was then used to screen a Drosophila head cDNA library (gift of G. Rubin).

In situ hybridization in Drosophila
Salivary gland chromosome squashes were prepared from ryb third instar larvae as described (Pardue, 1986). A plasmid with a 3 kb BamHI fragment from the Drosophila genomic homolog of the murine Mov 34 gene was used as a probe following labelling with biotinylated dUTP. The nick-translation and hybridization were carried out by the standard protocol of Engels, using an alkaline phosphatase detection system (Engels et al. 1986).

Nucleotide sequencing and analysis
For sequencing, nested deletions of the plasmid inserts were constructed by the exonuclease III method (Henikoff, 1984). DNA sequencing was performed on single-stranded or double-stranded plasmid DNA, by the dideoxy chain termination method (Sanger et al. 1977), using the Sequenase enzyme (US Biochemical Corp.). Areas exhibiting cross-reactions were also sequenced with dITP in place of dGTP. In addition, some areas were sequenced using oligonucleotide primers deduced from previously sequenced regions. Sequences were compiled and analyzed using the GCG programs (Devereux et al. 1984). Searching of the NBRF (Nucleic Acid, Release 34.0; Protein, Release 20.0) and Genbank (Release 59.0) databases was performed with the FASTA and TFASTA programs (Pearson and Lipman, 1988).

Results

Chromosomal mapping of the Mov 34 proviral integration
A cDNA clone with a 1450 bp insert, designated pC2, of a transcript located near the site of the Mov 34 proviral integration (Soriano et al. 1987) was used for chromosomal mapping of the murine and human genes in mouse–hamster and human–hamster somatic cell hybrid lines. On Southern blots of EcoRI-digested DNA from the mouse–Chinese hamster lines (Fig. 1A), two bands (3.5 kb and 1.2 kb) were concordant with mouse chromosome 8, while a third fragment (4.3 kb), which hybridizes more weakly at high stringency (Soriano et al. 1987), cosegregated with the X chromosome (Table 1A). It was assigned to region D-F with translocation hybrids (see Methods). On Southern blots of EcoRI-digested DNA from the human–Chinese hamster lines, five hybridizing human-specific fragments were localized to three chromosomal sites (Fig. 1B). Only fragment 4 still hybridized after a high-stringency wash (Soriano et al. 1987). This fragment segregated with human chromosome 16 (Table 1B). Hybrids with an abnormal chromosome 16 lacking region 16q22-pter were negative, placing the gene detected by pC2 into this region. Of the hybridizing bands present after a low-stringency wash, the three largest EcoRI fragments segregate with human chromosome 1, while the smallest EcoRI fragment was mapped to chromosome region 17q21-qter.

After hybridization of tritium-labelled pC2 to metaphase chromosomes from normal human 46,XX female cells, autoradiography and staining to reveal a G-banding pattern for chromosome identification, the location of 291 silver grains on chromosomes in 143 cells (average 2.0 grains/cell) was determined. The most striking accumulation was found at bands 16q23-q24 with 24 (8%) grains at this site in 17 (12%) of the randomly selected cells (Fig. 2, left). Thirteen (9%) cells had label at 17q21-22 with 16 (5%) grains at this site (Fig. 2, right). No distinct peak was found on chromosome 1, only 7 grains (2.4%) at 1p32-p31 which were suggestive but not significant (not shown). No other chromosomal site was labelled above background.

Only the site on human chromosome 16/mouse chromosome 8 falls into a syntenic group known to be conserved between the two species. In both cases, this was also the region that hybridized on Southern blots with the pC2 probe at highest stringency. Furthermore, the site on 16q23-q24 was the major site of in situ hybridization of pC2 to human chromosomes. The second major site on 17q21-q22 falls in a region of homology with mouse chromosome 11 that did not
Analysis of the Mov 34 mutation

Fig. 1. EcoRI-digested DNA of Chinese hamster (CH), mouse (M) and human (H) origin and of interspecies somatic cell hybrids was Southern blotted and probed with pC2. (a) CH×M hybrids had either the mouse 4.3 kb fragment alone (lanes 3 and 6), all three mouse fragments (lane 4), only the 2 smaller bands (not shown) or none (lane 5). Mouse 3.5 and 1.2 kb fragments were always present or absent together, but independently of the 4.3 kb band. (B) Of the 5 human fragments, all of them larger than the rodent bands, only band 4 remained after high-stringency washes. CH×H hybrids (lanes 5-9) and CH×M hybrids (lanes 4 and 10) were positive for various combinations of the human bands or contained none (lane 7). In hybrid lines with defined parts of human chromosomes, bands 1 and 2 were mapped to chromosome 1q11-q32, band 3 to 1pter-p32, band 4 to 16q22-q24 and band 5 to 17q21-qter.

hybridize with this probe. Analyses performed subsequent to the initiation of these experiments showed that the 5' end of the pC2 cDNA may be an artifact of the cDNA cloning (see below), and may be encoded elsewhere in the genome than the remainder of the pC2 insert. Therefore, the Southern blotting experiments were repeated with probe pC28, which contains a larger insert of the Mov 34 cDNA (see below). Hybridization with probe pC28 identifies the same restriction fragments as probe pC2, as well as additional fragments that co-segregated with human chromosome 16 and mouse chromosome 8 (not shown). Therefore, we believe that the more weakly hybridizing sequences are not due to an unrelated gene but may represent related sequences or processed pseudogenes. Thus, we felt that human 16q/mouse 8 was the most likely location of the Mov 34 locus, which was confirmed by the breeding experiment described below. This region contains the loci APRT, GOT2, TAT, HP, MT1, MT2, and CTB on the distal half of the long arm of human chromosome 16. The homologous mouse genes are distributed over the central third of mouse chromosome 8 (region B-C). A developmental mutant in this region of mouse chromosome 8 is oligosyndactyly (Os). No human disease gene is known to map in the syntenic region on human chromosome 16.

The Mov 34 mutation is not allelic to oligosyndactyly Os is a radiation-induced mutation, identified because it causes fusion of the digits of the fore- and hind-paws in mice heterozygous for the mutation (Grueneberg, 1956). Os in the homozygous state results in lethality at the time of implantation (Van Valen, 1966), approximately the same time as homozygous Mov 34/Mov 34 embryos. This lethality is apparently due to mitotic arrest in homozygous embryos (Magnuson and Epstein, 1984).

To determine if the Mov 34 mutation is allelic to Os, heterozygous Mov 34/+ mice were mated to heterozygous Os/+ mice. The two mutations fully complemented each other, since 6 out of 8 progeny containing the Os chromosome also contained the Mov 34 chromosome. To determine the genetic distance of Mov 34 from Os, the double heterozygotes (Mov 34/Os) were backcrossed to wild-type Balb/c mice (Table 2). The Mov 34 integration was linked to the Os mutation, confirming the localization indicated by the somatic cell hybridization experiments. The Mov 34 provirus is approximately 7 cM distant from Os (5/69 recombinant progeny). Since only these two markers were segregating in the cross, we do not know on which side of Os the Mov 34 locus maps.
Table 1.

(A) Mapping of Mov 34 in mouse by Southern analysis of 19 somatic cell hybrid lines

<table>
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<tr>
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<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
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<td>53</td>
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<td>39</td>
<td>39</td>
<td>50</td>
<td>50</td>
<td>56</td>
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<td>39</td>
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<td>53</td>
<td>21</td>
<td>24</td>
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<td>21</td>
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<td>21</td>
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<td>29</td>
<td>29</td>
<td>32</td>
<td>53</td>
<td>26</td>
<td>29</td>
<td>69</td>
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(B) Mapping of EcoRI fragments hybridizing with Mov 34 probe in 17 somatic cell hybrid lines

<table>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<td>24</td>
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</table>

Fig. 2. Distribution of silver grains after hybridization of labelled pC2 to normal human chromosomes. In 143 random cells scored, two sites of significant label were detected: The major one at 16q23-q24 (left) and a secondary one on 17q21-q22 (right).

Table 2. Recombination between Os and Mov 34

<table>
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<th>Genotype</th>
<th>Observed</th>
<th>Class</th>
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<td>Os+/+, +/+</td>
<td>36</td>
<td>Parental</td>
</tr>
<tr>
<td>+/+, M34/+</td>
<td>28</td>
<td>Parental</td>
</tr>
<tr>
<td>Os+/+, M34/+</td>
<td>3</td>
<td>Recombinant</td>
</tr>
<tr>
<td>+/+, +/+</td>
<td>2</td>
<td>Recombinant</td>
</tr>
</tbody>
</table>

Double heterozygotes (Os+/+, M34+/+) were backcrossed to wild-type Balb/c mice. Os+/+ heterozygotes were scored by the presence of fused digits in the fore- and hindpaws of mice containing this mutation. The chromosome containing the Mov 34 proviral integration was scored by the detection of an allele-specific band in Southern blots of tail DNA of the progeny mice.

Isolation and sequence analysis of additional Mov 34 cDNA clones

The cDNA clone, pC2, was isolated from a brain cDNA library (Soriano et al. 1987). Comparative restriction enzyme mapping and nucleotide sequence analysis indicated that approximately 300 bp at the 5' end of the pC2 cDNA may be an artifact of the cDNA cloning (not shown). For sequence analysis, additional cDNA clones were isolated from a day 16 mouse embryo cDNA library using a genomic probe which identifies the 1.7 kb Mov 34-associated transcript.

Four cDNA clones were isolated, with insert sizes ranging from 0.5 kb to 1.4 kb. Restriction enzyme mapping and partial nucleotide sequence analysis revealed that these four clones overlap, and differ only in the amount of sequence at the 5' end of the cDNA. When the insert of the longest clone, pC28, is hybridized to Southern blots of the cloned genomic Mov 34 locus, hybridization is observed on both sides of the proviral integration site (not shown). This indicates that the proviral integration has physically disrupted this transcription unit, and is presumably the reason for the approximately 2-fold reduction in steady-state levels of this transcript in Mov 34/+ heterozygous animals compared to wild-type littermates (Soriano et al. 1987). Nucleic acid sequence analysis of genomic and cDNA clones indicate that the proviral integration has occurred in an intron of this gene.

The nucleotide and predicted amino acid sequence of
Cloning of a Drosophila homolog of the Mov 34 gene

We had previously demonstrated that the Mov 34 gene is conserved in evolution, and that a band hybridizing to a Mov 34 cDNA could be detected in Southern blots of Drosophila DNA (Soriano et al. 1987). To determine the extent of conservation of the Mov 34 gene, and hopefully to gain some insight into its function, we decided to clone the Drosophila homolog of the murine Mov 34 gene. A library of Drosophila genomic DNA was screened with the Mov 34 cDNA clone pC28. A phage containing a hybridizing 12 kb insert was isolated and analyzed. When the entire 12 kb insert was used as a probe on a Northern blot, RNA from both heads and bodies of Drosophila adults revealed a hybridizing 1.4 kb band (not shown). A CDNA library of RNA isolated from Drosophila heads was screened with the 12 kb genomic Mov 34 insert. Several hybridizing phage were isolated, and the longest insert (p1.4D) was subcloned and sequenced. The nucleotide and predicted amino acid sequence of clone p1.4D is presented in Fig. 4. Clone p1.4D is also

Fig. 4. Nucleotide and predicted amino acid sequence of murine cDNA clone pC28. Nucleotide numbering is shown to the left of the figure, predicted amino acid numbering is shown to the right.
not a full-length clone. Clone pl.4D is 1356 nucleotides long and contains a 375 amino acid open reading frame, which begins at the extreme 5' end of the cDNA and continues to nucleotide 1125. This open reading frame would encode a protein with a relative molecular mass of $42 \times 10^3$. Fig. 5 compares the predicted amino acid sequence of the *Drosophila* homolog with the predicted amino acid sequence of the murine *Mov 34* cDNA. The sequences are 62% identical at the amino acid level.

**Table 3. Drosophila mutations localized in region 60B,C**

<table>
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<tr>
<th>Gene</th>
<th>Abbreviation</th>
<th>Cytological localization</th>
<th>Genetic map position $\dagger$</th>
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<tr>
<td>abbreviated</td>
<td>abb</td>
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<td>2-105.5</td>
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<tr>
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<td>NA</td>
<td>2-106.3</td>
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<tr>
<td>purple</td>
<td>pd</td>
<td>59E2-60B10*</td>
<td>2-106.4</td>
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<tr>
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<td>ll</td>
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<td>2-106.7</td>
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<td>mr</td>
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</tr>
<tr>
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<td>l(2)ax</td>
<td>60B*</td>
<td>2-106.9</td>
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<td>Foa</td>
<td>59E3-60B10*</td>
<td>2-107</td>
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<td>Dat</td>
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<td>2-107</td>
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<td>sp</td>
<td>60B13-60C5*</td>
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<td>bs</td>
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<td>Pxe</td>
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</table>

* (Lindsley and Grell, 1968).
† (Marsh and Wrigh, 1980).
$\dagger$ (Lindsley and Zimm, 1986).
NA, not available.

In situ hybridization analysis of *Drosophila* Mov 34 homolog

A subclone of the 12 kb *Drosophila* genomic fragment was used for in situ hybridization of *Drosophila* polytene chromosomes. A single locus of hybridization was observed at region 60 distal B,C on the right arm of chromosome 2 (not shown). Among the genes which have been localized to this region of chromosome 2 (Table 3) are several embryonic and larval lethal mutations, including l(2)NS, l(2)ax, Forkoid, Plexate, and Pin.

**Discussion**

Insertional mutagenesis in mice is a useful approach to dissect molecular controls of mammalian embryogenesis, as it allows the isolation and characterization of genes whose mutant phenotype is known. The previously isolated *Mov 34* mutation is a recessive embryonic lethal mutation caused by integration of a Moloney murine leukemia provirus into the germline. Mouse embryos homozygous for the *Mov 34* integration complete preimplantation development normally but die just after implantation into the uterus (Soriano et al. 1987). In an effort to understand the function of the *Mov 34* gene in preimplantation mouse development, we have made use of the high evolutionary conservation of the gene, which allowed the isolation and chromosomal mapping of the *Drosophila* homolog. We furthermore have determined the chromosomal localization of the *Mov 34* gene in the mouse and human genome, and have determined the nucleotide sequences of both murine and *Drosophila* cDNA clones.

**Chromosomal localization**

Somatic hybrid analysis and in situ hybridization
demonstrated that the Mov 34 proviral integration has occurred on mouse chromosome 8. In humans, the homolog of the disrupted gene is on chromosome 16q23-q24. A previously identified mouse mutation, Os, is also located on chromosome 8 in the region known to contain genes that have counterparts on human 16q. Homozygous Os/Os embryos die at a similar time as homozygous Mov 34/Mov 34 embryos (Van Valen, 1966). It is known that insertional mutations caused by endogenous retroviruses (Jenkins et al. 1981; Stoye et al. 1988) or by experimental introduction of DNA into the germ line (Woychik et al. 1985) may correspond or be allelic to previously identified spontaneous mouse mutations. We therefore considered Mov 34 a candidate gene for the Os mutation. Complementation tests revealed, however, that Mov 34 and Os are not allelic, and backcross experiments mapped the Mov 34 locus approximately 7 cM from Os on chromosome 8.

The Mov 34 protein is highly conserved in evolution. Extensive database searching with both the nucleotide and the translated amino acid sequence of the Mov 34 cDNA clone pC28 has revealed no significant homology to any known nucleotide or amino acid sequence. The sequenced clone is not full length, but the majority of the protein-coding region is present. There is no region that appears to be a transmembrane domain, nor are there any N-linked glycosylation sites. The one striking positive and negative charges.

Analysis of the sequence of the presumptive Mov 34 protein is the carboxy-terminal end, which consists of a very hydrophilic domain of amino acids with alternating positive and negative charges.

References


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Analysis of the Mov 34 mutation

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