Development 109, 235-242 (1990)
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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 evolutionarily 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.* 1986; Covarrubias *et al.* 1987; Jaenisch *et al.* 1983; Mahon *et al.* 1988; Mark *et al.* 1985; McNeish *et al.* 1988; Schnieke *et al.* 1983; Spence *et al.* 1989; Wilkie 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-qter, respectively (Francke and Bushby, 1975). Discordancy analysis was carried out individually for each of 3 *Eco*RI fragments in the mouse and 5 *Eco*RI 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-translated with ^{3}H-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 *Hin*dIII 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 pIBI30 (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 ry^{506} third instar larvae as described (Pardue, 1986). A plasmid with a 3 kb *Bam*HI fragment from the *Drosophila* genomic homolog of the murine *Mov34* 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 either 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 compressions 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).

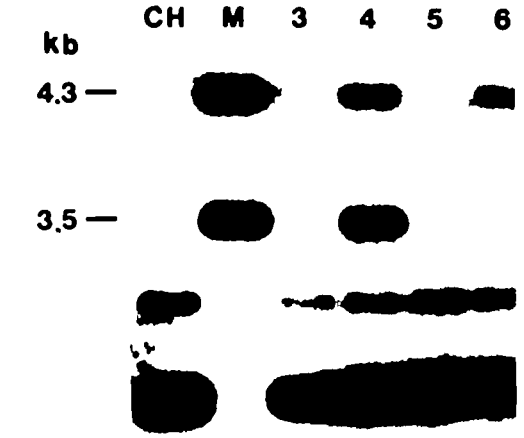
Results

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 *Eco*RI-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 *Eco*RI-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-qter were negative, placing the gene detected by pC2 into this region. Of the hybridizing bands present after a low-stringency wash, the three largest *Eco*RI fragments segregate with human chromosome 1, while the smallest *Eco*RI 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-q22 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



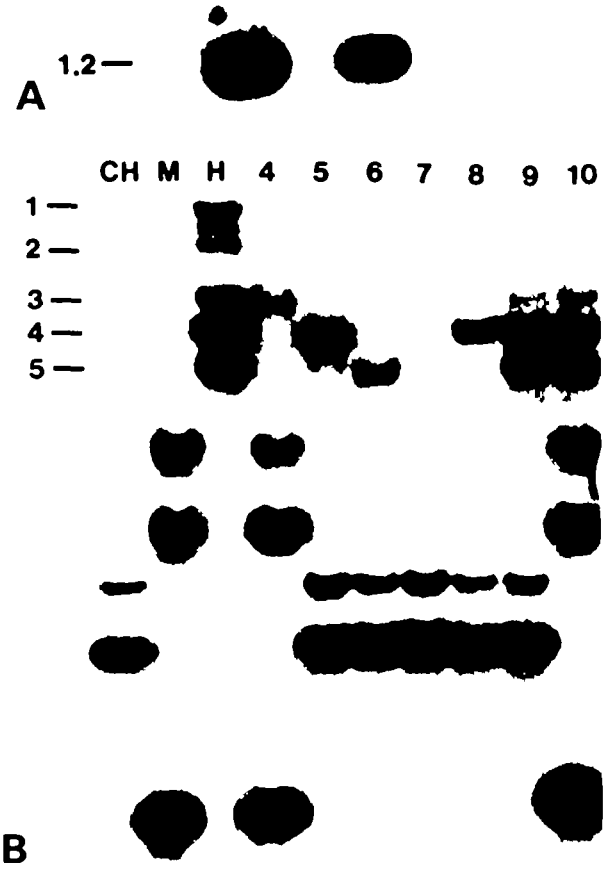


Fig. 1. *Eco*RI-digested DNA of Chinese hamster (CH), mouse (M) and human (H) origin and of interspecies somatic cell hybrids was Southern blotted and probed with pC2. (a) CH×M hybrids had either the mouse 4.3 kb fragment alone (lanes 3 and 6), all three mouse fragments (lane 4), only the 2 smaller bands (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 *CTRB* 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 (Grüneberg, 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*

	Fraction (%) of discordant hybrids for mouse chromosome																		
RI fragments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	X
4.3 kb	31	32	53	53	65	47	39	50	50	56	84	39	71	53	21	24	26	53	0
3.5/1.2 kb	37	21	11	21	29	24	28	0	50	50	58	44	29	29	32	53	26	29	69

(B) *Mapping of* Eco*RI fragments hybridizing with* Mov 34 *probe in 17 somatic cell hybrid lines*

	Fraction (%) of discordant hybrids for human chromosome																						
RI fragments	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
1, 2, 3	0	25	40	21	38	44	33	31	31	41	40	24	65	40	35	27	29	38	18	41	31	40	40
4	31	44	33	21	44	38	60	44	38	35	53	29	47	40	41	0	47	31	41	47	44	33	40
5	31	31	60	50	19	63	27	25	31	24	27	41	47	60	41	47	0	44	35	24	50	47	30

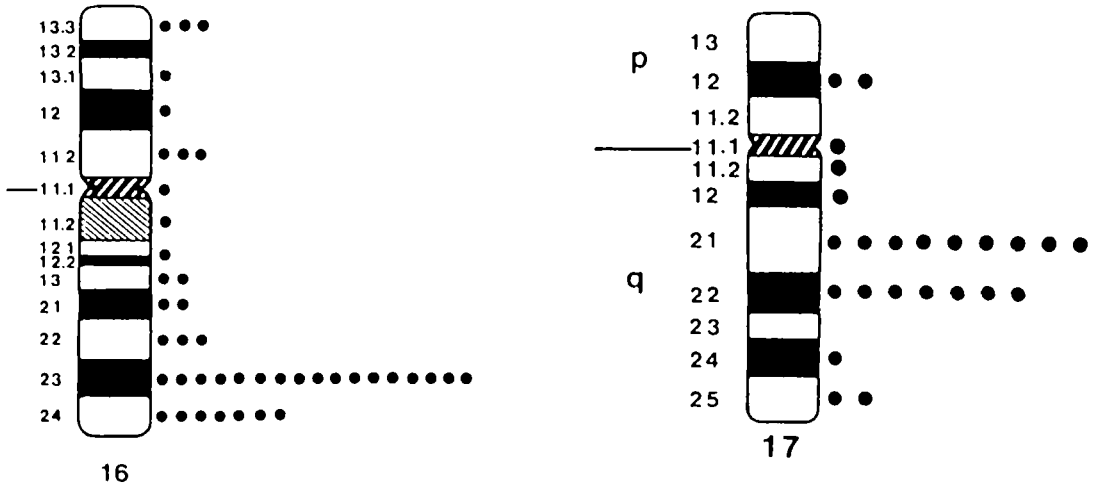


Fig. 2. Distribution of silver grains after hybridization of labelled pC2 to normal human chromosomes. In 143 random cells scored, two sites of significant label were detected: The major one at 16q23-q24 (left) and a secondary one on 17q21-q22 (right).

Table 2. *Recombination between* Os *and* Mov 34

Genotype	Observed	Class
Os/+, +/+	36	Parental
+/+, *M34*/+	28	Parental
Os/+, *M34*/+	3	Recombinant
+/+, +/+	2	Recombinant

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

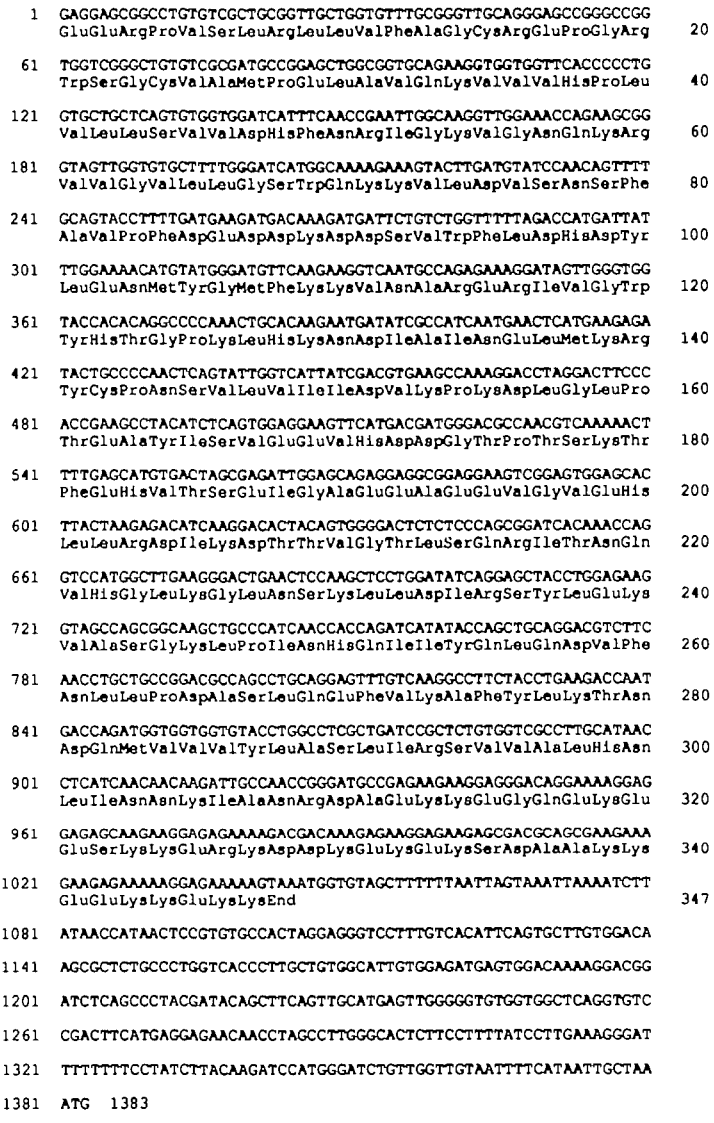


Fig. 3. Nucleotide and predicted amino acid sequence of murine cDNA clone pC28. Nucleotide numbering is shown to the left of the figure, predicted amino acid numbering is shown to the right.

clone pC28 is presented in Fig. 3. pC28 is not a full-length clone of the 1.7 kb *Mov 34*-associated transcript. pC28 is 1383 nucleotides long and contains a 347 amino acid open reading frame, which begins at the extreme 5′ end of the cDNA and continues to nucleotide 1041. This open reading frame would encode a protein with a relative molecular mass of 39×10^3. A search of the protein and nucleic acid databases with the sequence of pC28 revealed no significant homology to any known protein or nucleotide sequence. One striking feature of the translated amino acid sequence of pC28 is the carboxy-terminal end, which consists of a very hydrophilic domain of amino acids with alternating positive and negative charges.

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 at low stringency 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 *Drosophila* 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

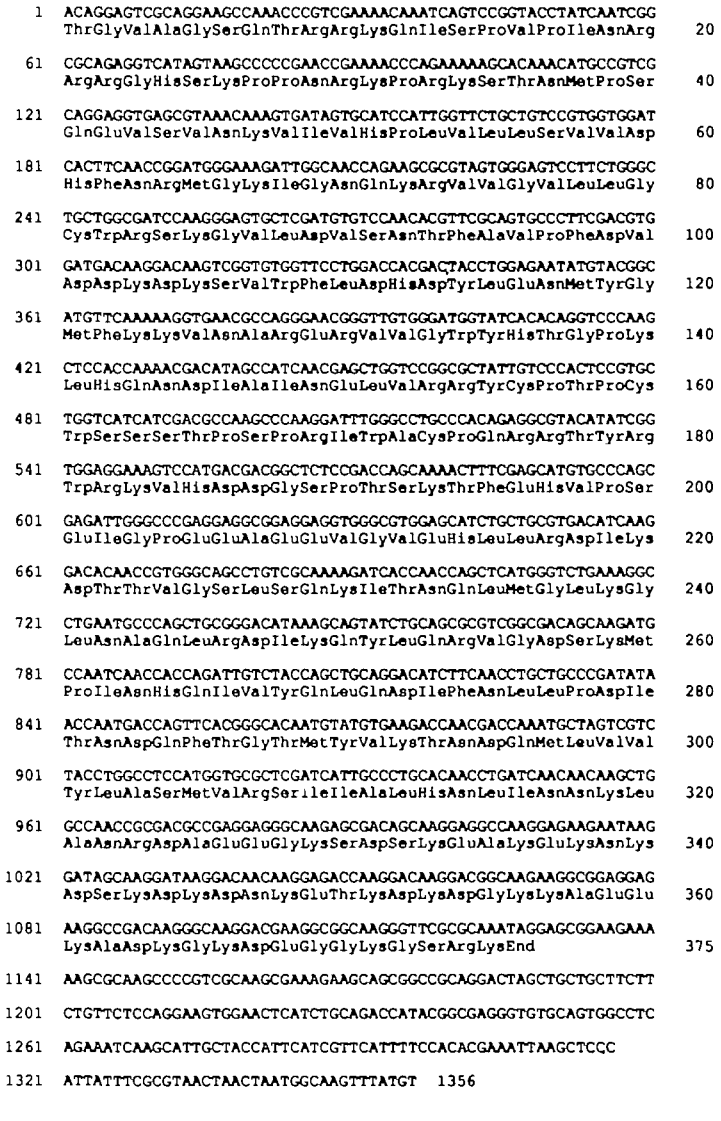


Fig. 4. Nucleotide and predicted amino acid sequence of *Drosophila* cDNA clone p1.4D. Nucleotide numbering is shown to the left of the figure, predicted amino acid numbering is shown to the right.

not a full-length clone. Clone p1.4D 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 42×10^3. 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.

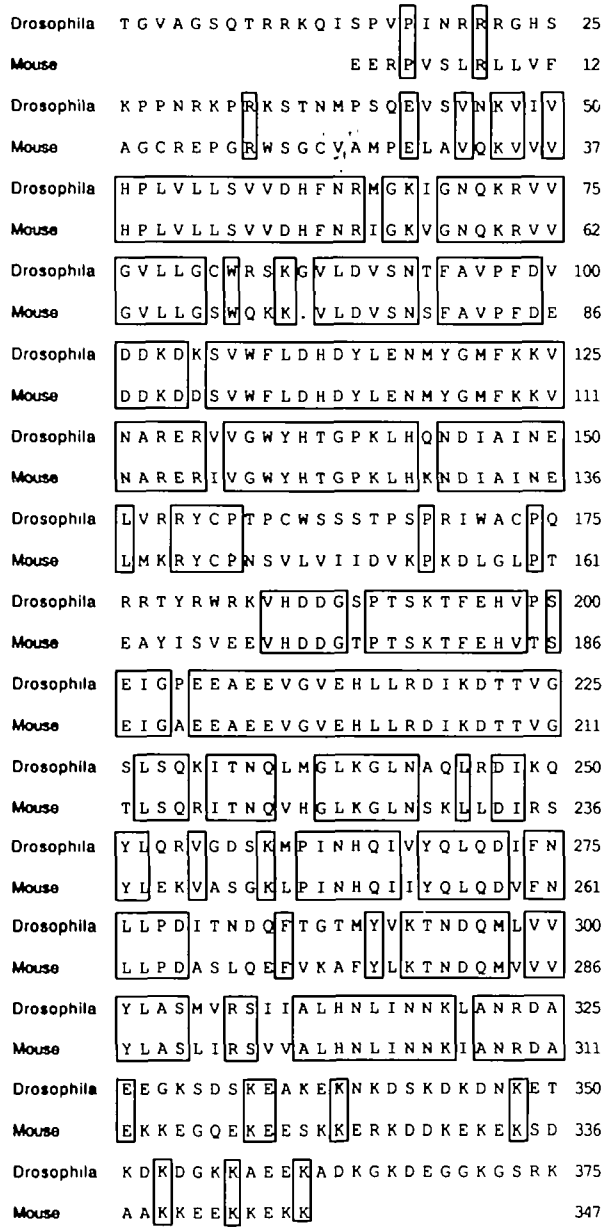


Fig. 5. Comparison of the predicted amino acid sequences of the murine cDNA clone pC28 and the *Drosophila* cDNA clone p1.4D. A single amino acid gap (.) has been inserted into the mouse sequence at amino acid position 73 to maximize homology. Identical amino acid residues are boxed.

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

Gene	Abbreviation	Cytological localization	Genetic map position‡
abbreviated	*abb*	59E2-60B10*	2-105.5
slight	*slt*	NA	2-106.3
purpleoid	*pd*	59E2-60B10*	2-106.4
lanceolate	*ll*	59E2-60B10*	2-106.7
morula	*mr*	59E2-60B10*	2-106.7
lethal(2)ax	*l(2)ax*	60B*	2-106.9
Forkoid	*Fo*	58E3-60B10*	2-107
dopamine acetyltransferase	*Dat*	60B1-10†	2-107
lethal(2)NS	*l(2)NS*	60B10-12*	2-107.0
speck	*sp*	60B13-60C5*	2-107.0
orange	*or*	NA	2-107.2
blistered	*bs*	60C5-60D2*	2-107.3
Pin	*Pin*	60C5-60D2*	2-107.3
balloon	*ba*	60C5-60D2*	2-107.4
Plexate	*Px*	60C6-60D1*	NA

* (Lindsley and Grell, 1968).
† (Marsh and Wright, 1980).
‡ (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 structural feature 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.

Analysis of the sequence of the presumptive *Mov 34* protein did not give any obvious indication of its function. Sequence analysis of gene homologs in different organisms can be useful in identifying conserved domains important for protein function. A number of *Drosophila* homologs of mammalian proto-oncogenes have been cloned and analyzed in this fashion (for review see Shilo, 1987). Therefore, we cloned a *Drosophila* homolog of the murine *Mov 34* gene. The genomic *Drosophila* clone hybridizes to a 1.4 kb RNA, similar in size to the 1.7 kb mouse RNA. Analysis of a *Drosophila* cDNA clone reveals that the murine and *Drosophila Mov 34* proteins are strongly conserved, with 62% identity at the amino acid level (Fig. 5). The amino acid sequence conservation is distributed throughout the length of the two proteins. This level of sequence conservation is similar to that seen between mammalian and *Drosophila* homologs of most proto-oncogenes (see Table 1 in Pribyl *et al.* 1988). Comparison of the hydropathy profile (Kyte and Doolittle, 1982) of the murine and *Drosophila* proteins reveals that the hydropathic characteristics of the two proteins are strongly conserved, including the extremely hydrophilic domain at the carboxy terminus.

In situ hybridization localized the *Drosophila Mov 34* homolog to 60B,C on the right arm of chromosome 2. Among the genes that have been localized to this region of chromosome 2 (Table 3) are several homozygous lethal mutations, including *I(2)NS*, *I(2)ax*, *Forkoid*, *Plexate*, and some alleles of *Pin*. Also present in band 60B are genes affecting bristle or wing morphology or color (*abb*, *ba*, *bs*, *ll*, *mr*, *slt*, and *sp*), female sterility (*mr*), eye color (*pd*, *or*) and the structural gene for dopamine-*N*-acetyltransferase (*Dat*).

The function of the *Mov 34* protein and its time of activation during early cleavage is not known. Because region 60B,C on *Drosophila* chromosome 2 has not been saturated by previous mutational analysis, it is possible that the *Mov 34* homolog does not correspond to a known allele but has still to be mutated. The availability of the full sequence of the mouse gene and the *Drosophila* homolog should allow us to raise antibodies to both proteins. This will enable us to pursue a systematic analysis of the role this gene plays in early development of both mice and flies.

We would like to thank Drs T. Maniatis, G. Rubin, A. Stacey, and M. Rosbash for providing libraries and materials, and Dr T. Magnuson for providing *Os/+* mice. The technical assistance of D. Grotkopp, R. Curry and J. Du is gratefully acknowledged. T.G. was supported by a postdoctoral fellowship from the American Cancer Society. U.F. is an investigator of the Howard Hughes Medical Institute. This work was supported by grants from the National Cancer Institute (OIG) 5 R35 CA44339 to R.J., and from the NIH (GM26105) to U.F.

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(*Accepted 7 February 1990*)