

Random cloning of genes from mouse chromosome 17

(testis-specific gene/*t* complex)

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ABSTRACT We describe a method for isolating cosmid clones randomly from mouse chromosome 17. A cosmid library was constructed from the mouse–Chinese hamster cell line R4 4-1 that contains a limited amount of mouse DNA (chromosomes 17 and 18 and some other unidentified material) on a Chinese hamster background. The library was screened with the murine repetitive sequence probe pMBA14, which selectively hybridizes with mouse DNA. The mouse-derived cosmid clones thus identified were individually hybridized with DNA from the mouse–Syrian hamster cell line JS17 containing all mouse chromosomes except chromosome 17 on a Syrian hamster background. We deduced that the cosmid clones that contained sequences absent in JS17 were derived from mouse chromosome 17. One of the chromosome 17-derived cosmid clones, 3-4-1 (located proximal to the *T122/T66C* segment) was found to be highly polymorphic among European wild-mouse populations and may be a useful probe to elucidate the evolution and migration of *Mus* species. The randomly isolated mouse-derived cosmid clones can also be screened for the presence of functional genes. Using testicular cDNA as a probe, a testis-specific gene was cloned from mouse chromosome 17.

The isolation of DNA clones from one particular mammalian chromosome can be achieved in three ways: (i) by constructing a chromosome-specific genomic library from sorted metaphase chromosomes (1); (ii) by microdissection cloning (2); and (iii) by constructing a genomic library from an interspecies cell hybrid and subsequently screening the library with a species-specific repetitive sequence probe (3).

We used the third approach to isolate DNA clones from mouse chromosome 17. Here, we describe the method we used and the initial characterization of two cosmid clones, one of which carries a testis-specific gene.

MATERIALS AND METHODS

Animals. All mice used in this study were obtained from our animal colony at the Max Planck Institute for Biology. Breeding pairs of strains carrying partial *t* haplotypes *t*^{h49}, *t*^{lowH}, and *t*^{h2} were supplied by Mary F. Lyon, Medical Research Council Radiobiology Unit, Harwell, Oxford, England. Strains carrying additional *t* haplotypes (*t*^{Tuw} strains) were established as described (4). Chinese and Syrian hamsters were purchased from the Zentralinstitut für Versuchstierzucht, Hannover, F.R.G.

Hybrid Cell Lines. R4 4-1 is a mouse–Chinese hamster hybrid line that contains only one mouse chromosome, termed M1, on a Chinese hamster background (5). The p arm of M1 is chromosome 17, whereas its q arm consists of chromosome 18 and some additional unidentified material (6). JS17 is a mouse–Syrian hamster hybrid containing all mouse chromosomes except chromosome 17 on a Syrian hamster background (7). JS16 is a mouse–Syrian hamster

hybrid containing a full complement of mouse chromosomes (7).

Construction and Screening of a Cosmid Library. DNA was isolated according to the method of Blin and Stafford (8) with modifications. A cosmid library was constructed, essentially as described by Steinmetz *et al.* (9), from high molecular weight DNA isolated from R4 4-1. The library contained a total of 4×10^5 independent clones. To distinguish between mouse- and Chinese hamster-derived cosmid clones, the library was screened as described (9) with the murine repetitive sequence probe pMBA 14 (see ref. 10), which hybridizes with mouse, but not with Chinese hamster DNA (data not shown). This probe is specific for a BAM5 family of repetitive sequences present in 0.5×10^5 copies per haploid mouse genome (10). After hybridization, filters were washed with $0.1 \times$ SSC ($1 \times$ SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0)/0.1% NaDodSO₄ at 60°C.

RNA Isolation and cDNA Synthesis. Cytoplasmic RNA was isolated from various mouse organs as described by Chirgwin *et al.* (11). Poly(A)⁺ RNA was selected by two passages over oligo(dT)-cellulose. ³²P-labeled cDNA of high specific activity (1.5×10^8 cpm/ μ g) was synthesized from BALB/c testis poly(A)⁺ RNA according to the method described by Davis *et al.* (12), with minor modifications.

Southern and RNA Gel Blot Hybridization. Restriction enzyme digests of genomic DNA were size-fractionated on 0.6% agarose gels, transferred to nitrocellulose filters (13), and hybridized with either nick-translated (14) or oligolabeled (15) probes. Prehybridization and hybridization were carried out as described (9). The washing conditions were $0.1 \times$ SSC/0.1% NaDodSO₄ at 60°C. For RNA gel blots, total cytoplasmic RNA (20 μ g) was denatured in 6.6% (vol/vol) formaldehyde/50% (vol/vol) formamide at 60°C for 5 min and electrophoresed on a 1% agarose gel containing 6.6% (vol/vol) formaldehyde. RNA was transferred to nitrocellulose filters (16) and hybridized as described for Southern blot hybridization. Washing conditions were $0.1 \times$ SSC/0.1% NaDodSO₄ at 50°C.

RESULTS AND DISCUSSION

Isolation of Cosmid Clones from Mouse Chromosome 17. To isolate chromosome 17-derived cosmid clones, we first screened the R4 4-1 cosmid library with pMBA14—a repetitive sequence probe selectively hybridizing with mouse DNA. This screening has yielded ≈ 3000 pMBA14-positive, and hence mouse-derived, cosmid clones. However, not all of them originate from mouse chromosome 17, since R4 4-1 contains some non-chromosome 17-derived mouse DNA. To identify the chromosome 17-derived fragments, the pMBA14-positive clones were digested with *Eco*RI and hybridized with full genomic DNA. Fragments free of repet-

Abbreviation: RFLP, restriction fragment length polymorphism.

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itive sequences were selected, nick-translated, and used as probes to screen Southern blots containing digested DNA from JS16 and JS17 cell lines, as well as DNA isolated from BALB/c mice. The JS16 line contains a full complement of mouse chromosomes, whereas the JS17 line lacks chromosome 17. Cosmids that hybridized with JS16 and BALB/c DNA but did not hybridize with JS17 were selected for further characterization. Fig. 1 gives one example of such a clone. Here, a 3-kilobase (kb) *EcoRI* fragment isolated from cosmid 1-2-4 was used as a probe for *Bam*HI digests of DNA from the indicated sources. The absence of hybridizing bands in JS17 indicates that the probe contains a sequence not present in any mouse chromosome other than chromosome 17. We initially isolated 26 pMBA14-positive cosmid clones and found 8 of them to be derived from mouse chromosome 17. Thus, approximately one-third of the pMBA14-positive cosmid clones originate from mouse chromosome 17.

Isolation of a Highly Polymorphic Cosmid Clone from the Centromeric Portion of Mouse Chromosome 17. Cosmid clone 3-4-1, one of the eight mouse chromosome 17-derived cosmid clones described above, was found to hybridize to sequences that are highly polymorphic among European wild mouse populations. A 9.5-kb *EcoRI* fragment (3-4-1E1-3) isolated from 3-4-1 was used as a probe for Southern blots of DNA extracted from mice listed in Tables 1 and 2. Fig. 2 shows the eight distinct restriction fragment length polymorphism (RFLP) patterns (1 through 8) found in this study. Excluding patterns 1 and 2, which were seen too rarely for us to draw any conclusions, all the other RFLP patterns showed characteristic geographic distributions. Pattern 3 was found in a large area extending from Greece, Italy, and Germany to the Orkney Islands (Scotland); pattern 4 mainly in an area surrounding the Mediterranean Sea (Egypt, Israel, Spain, Italy, and Greece); pattern 5 primarily in Western Europe (Spain, France, Germany, and Northern Italy); pattern 6 in Eastern Europe including the Soviet Union; pattern 7 in Southern Germany and Northern Italy; and pattern 8 in laboratory mice. The following strains of laboratory mice were tested: A, BALB/c, C57BL/6, C57BL/10, DBA/2, C3H, B10.S, B10.SM, B10.BR, T^{hp}. Pattern 6 appears to be characteristic of *Mus musculus*, the eastern form of the house mouse that is distinct from the western form, *Mus domesticus*.

Mice are believed to have been introduced into Europe via two routes from the Near East (17): one is via the Mediterranean countries, including Spain and Italy, and the other is via Turkey and Russia. The geographical distribution of patterns 4 and 6 coincides with these two hypothetical routes of mouse migration, indicating that RFLP patterns defined with 3-4-1E1-3 reflect the evolutionary history of European wild mice. Furthermore, all laboratory mice tested, including *t*⁶ haplotype mice, gave us pattern 8, which is consistent with the hypothesis that they are all closely related in their origin

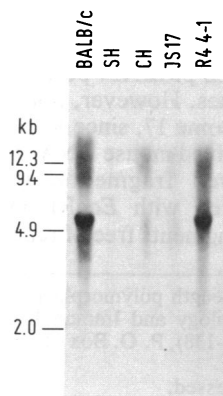


FIG. 1. Localization of cosmid clone 1-2-4 to mouse chromosome 17. A 3-kb *EcoRI* fragment was isolated from a cosmid clone 1-2-4, radiolabeled with ³²P, and used as a probe for Southern blots of *Bam*HI-digested DNA from BALB/c, Syrian hamster (SH), Chinese hamster (CH), JS17, and R4 4-1. Since the fragment used as the probe was contaminated with small amounts of repetitive sequence, hybridization was done in the presence of high molecular weight mouse DNA at a concentration of 50 μ g/ml.

Table 1. Distribution of 3-4-1E1-3 RFLP patterns in wild mice

Strain	Origin	RFLP patterns*
DHL2415	Helgoland, F.R.G.	3
DGK2856	Giessen, F.R.G.	3
DGK2858	Giessen, F.R.G.	3
DML3064	Maria Laach, F.R.G.	3 + 5 [†]
DVO2248	Vöhl, F.R.G.	5
DVO2263	Vöhl, F.R.G.	5
DPF3067	Pfalzfeld, F.R.G.	5
DPF3069	Pfalzfeld, F.R.G.	5
DHH2347	Herzhausen, F.R.G.	5
DLF3251	Laubach/Fehrenz, F.R.G.	5 + 7
DLF3252	Laubach/Fehrenz, F.R.G.	3 + 7
DLF3253	Laubach/Fehrenz, F.R.G.	7
DOH2009	Ottersheim, F.R.G.	7
PEN2618	Penedes, Spain	5
PEN2655	Penedes, Spain	4
PEN2625	Penedes, Spain	4 + 5
PEN2624	Penedes, Spain	4 + 5
IPG2206	Gello, Pisa, Italy	3 + 5 [†]
IGR2343	Mariano, Grosseto, Italy	4 + 5
GAT2531	Thebes, Greece	3 + 4
GAT2463	Thebes, Greece	3 + 4
GAT2536	Thebes, Greece	3
GPK10	Patras, Greece	4
GPA274/85	Patras, Greece	4
ISL2717	Jerusalem, Israel	4
ISL2730	Jerusalem, Israel	5
YCN1	Zagreb, Yugoslavia	6
YHL2458	Zagreb, Yugoslavia	6
PWK	Prague, Czechoslovakia	6

*Heterozygosity for two RFLP patterns.

[†]Although RFLP patterns 3 + 5 and 5 + 8 are indistinguishable (Fig. 2), 3 + 5 was assigned since pattern 8 was found exclusively in laboratory mice.

(18). Thus, the cosmid 3-4-1 appears to be very helpful in elucidating the evolution and migration of *Mus* species.

To localize 3-4-1 further into a specific region of mouse chromosome 17, DNA from mice carrying partial *t* haplotypes, *t*^{h49}, *t*^{lowH}, and *t*^{h2}, was digested with *Taq* I, Southern-blotted, and hybridized with 3-4-1E1-3 (Fig. 3). Since *t*^{h49} and *t*^{lowH} are recombinants obtained by crossing *T*(*t*^{lowH})/*tf*/*t*^{w5} with +*tf*/+*tf* (19) and *t*^{h2}*tf*/*Tt*^{h17} with +*tf*/+*tf* (20), respectively, the three strains, *t*^{h49}, *t*^{lowH}, and *t*^{h2}, share the region distal to and including DNA loci *T122/T66C* defined by Fox *et al.* (21). The fact that *t*^{h49} shares no band with either *t*^{h2} or *t*^{lowH} places 3-4-1 proximal to *T122/T66C* (i.e., to the centromeric portion of mouse chromosome 17). Since the *t* complex occupies most of the centromeric region of mouse chromosome 17, we next tested 3-4-1E1-3 against a panel of *t* mice captured at various locations in Europe (Table 2). No RFLP patterns specific for *t* mice were found; on the contrary, *t*-bearing mice captured at a given location showed the same RFLP pattern as mice without *t* chromosomes. For example, *t* mice captured in Spain showed pattern 4 or 5, as did their non-*t* counterparts, and the same was true in the case of pattern 6, and so on. Thus, 3-4-1 is located in a region where the recombination between *t* and non-*t* chromosomes can occur freely. This, as well as the fact that it maps proximally to *T122/T66C*, places 3-4-1 centromerically from the two inversions in the *t* complex (22), and most likely outside of the *t* complex.

Isolation of a Mouse Chromosome 17-Derived Cosmid Clone Containing a Testis-Specific Transcript. The *t* complex is believed to be involved in sperm differentiation. With the aim of cloning the *t* complex genes, we isolated pMBA14-positive cosmid clones at random from the R4 4-1 cosmid library and searched for cosmids containing testicular transcripts. Brief-

Table 2. Distribution of 3-4-1E1-3 RFLP patterns in *t* mice

<i>t</i> mice	Strain	Origin	RFLP patterns
<i>t</i> ^{Tuw10}	EDY589	Eday, Orkney Islands, U.K.	3*
<i>t</i> ^{Tuw27}	ROD1455	Dudelhof, F.R.G.	7
<i>t</i> ^{Tuw28}	ERP1465	Erpenhausen, F.R.G.	4
<i>t</i> ^{Tuw29}	BRW942	Aulendorf, F.R.G.	7
<i>t</i> ^{Tuw24}	LGN925	Langenargen, F.R.G.	4
<i>t</i> ^{Tuw25}	OBL984	Oberer Lindenhof, F.R.G.	1
<i>t</i> ^o	—	Paris, France	5
<i>t</i> ¹²	—	Paris, France	5
<i>t</i> ^{Tuw12}	LRA410	La Roca, Spain	5
<i>t</i> ^{Tuw15}	MOY336	Moya, Spain	4
<i>t</i> ^{Lub1}	—	Alpic Drobic, Italy	5
<i>t</i> ^{Lub4}	—	Cremona, Italy	7
<i>t</i> ^{Lub7}	—	Tortona, Italy	3
<i>t</i> ^{Lub9}	—	Calcinato, Italy	3
<i>t</i> ^{w73}	—	S. Jutland, Denmark	6
<i>t</i> ^{Tuw6}	BRU382	Brno, Czechoslovakia	6
<i>t</i> ^{Tuw26}	PLD826	Bialowieza, Poland	6
<i>t</i> ^{Tuw20}	MSW251	Ryazan, Astrachan, U.S.S.R.	6
<i>t</i> ^{Tuw32}	ISL33	Haifa, Israel	4
<i>t</i> ^{Tuw7}	CRO435	Nahya, Giza Governate, Egypt	4
<i>t</i> ^{Tuw8}	CRO437	Nahya, Giza Governate, Egypt	2
<i>t</i> ^{w1}	—	New York or Philadelphia, USA	4
<i>t</i> ^{w2}	—	New York or Philadelphia, USA	1
<i>t</i> ^{w5}	—	New York, USA	4
<i>t</i> ^{w12jf}	—	Oakland, USA	5
<i>t</i> ^{Tuw11}	GPC882	Buin, Chile	3
<i>T</i> ⁶	—	Laboratory	8

*Most of the *t* mice tested are *t*/+ heterozygotes. RFLP patterns contributed by *t* haplotypes are shown here.

ly, cosmid DNA was digested with one or two restriction enzymes, blotted to duplicates of nitrocellulose filters, and probed with (i) total mouse DNA and (ii) cDNA synthesized from BALB/c testis poly(A)⁺ RNA. Restriction fragments, which were negative with total mouse DNA (and thus free of highly repetitive sequences) but positive with testicular cDNA, were isolated and subjected to RNA gel blotting analyses.

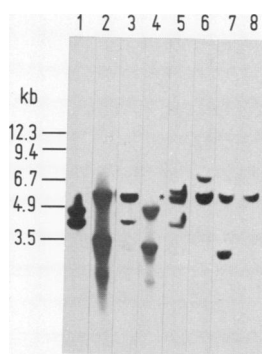


FIG. 2. Prototype RFLP patterns detected with 3-4-1E1-3. *Taq* I digests of DNA isolated from mice listed in Tables 1 and 2 were Southern-blotted and hybridized with 3-4-1E1-3 (a 9.5-kb *Eco*RI fragment of 3-4-1). Hybridization was carried out including high molecular weight mouse DNA at a concentration of 50 μ g/ml. The various patterns are shown in the lane of the same number. The mice from which the RFLP patterns were obtained are as follows: pattern 1, *t*^{w2}; pattern 2, CRO437; pattern 3, *t*^{Lub9}; pattern 4, CRO435; pattern 5, *t*^{w12jf}; pattern 6, MSW251; pattern 7, ROD1455; and pattern 8, B10.BR. The band marked with an asterisk in pattern 5 (identical with a band found in pattern 8) is contributed by laboratory mice that were crossed to maintain the *t* line *t*^{w12jf}. In fact, pattern 5 has only two bands. Pattern 8 consists of at least two bands that are hardly separable under normal electrophoretic conditions.

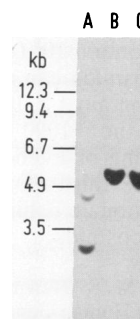


FIG. 3. Mapping of cosmid clone 3-4-1 proximal to *T122/T66C*. DNA from mice carrying partial *t* haplotypes was digested with *Taq* I, subjected to Southern blotting, and hybridized with 3-4-1E1-3 as in Fig. 2. Lanes: A, *t*^{H9}; B, *t*^{H2}; C, *t*^{lowH}.

One cosmid clone, designated 1-1-1, was found to contain a repetitive sequence-free *Hind*III fragment (0.95 kb) hybridizing strongly with testicular cDNA. This fragment (1-1-1H4) was used as a probe for Southern (Fig. 4A) and RNA gel blot (Fig. 4B) analyses. The fact that 1-1-1H4 hybridizes with BALB/c and R4 4-1 DNA, but not with JS17 DNA (Fig. 4A), localizes 1-1-1 to mouse chromosome 17. Fig. 4B demonstrates tissue specificity of the 1-1-1H4 transcript. Among the eight tissues examined (testis, ovary, brain, liver, kidney, spleen, lung, and heart), only testis RNA showed positive hybridization, indicating that 1-1-1 contains a gene specifically transcribed in the testis. The size of the testicular transcript was 2 kb.

Concluding Remarks. We were able to develop a method of isolating cosmid clones randomly from mouse chromosome 17. The R4 4-1 library contains 3000 pMBA14-positive cosmid clones, 1000 of which are from mouse chromosome 17, which provides us with a large number of potentially useful DNA markers for mouse chromosome 17. If we

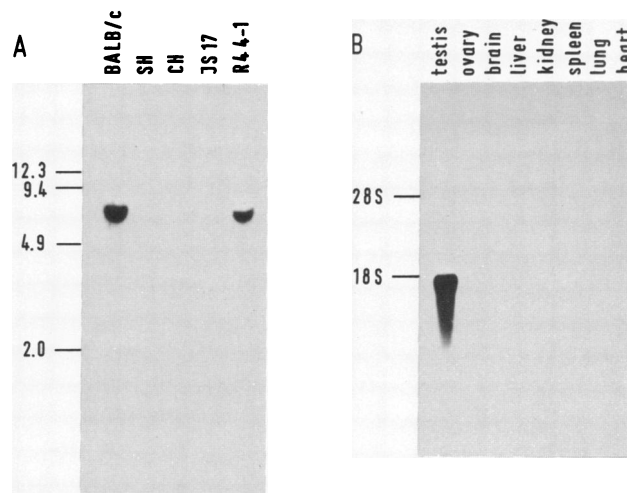


FIG. 4. Southern and RNA gel blot hybridization analyses of cosmid clone 1-1-1. (A) Localization of cosmid clone 1-1-1 to mouse chromosome 17. A 0.95-kb *Hind*III fragment (1-1-1H4), isolated from 1-1-1, was subcloned into pUC18 and used as a probe for Southern blots of *Bam*HI-digested DNA from BALB/c, Syrian hamster (SH), Chinese hamster (CH), JS17, and R4 4-1. (B) Tissue distribution of the 1-1-1 transcript. 1-1-1H4 was used as a probe for RNA gel blots of total cellular RNA (20 μ g) from the indicated sources. RNA was obtained from BALB/c mice, with the exception of ovary RNA that was isolated from CBA mice. The film was developed after overnight exposure with a Kodak intensifying screen. Even after a 5-day exposure, no bands showed up in the lanes other than testes (data not shown).

assume that chromosome 17 occupies $\approx 3.5\%$ of the whole mouse genome, it will contain 10^5 ($= \approx 0.035 \times 3 \times 10^6$) kb of nucleotides. Since cosmids can accept inserts of 40 kb, ≈ 1000 of cosmids will contain 4×10^4 kb of DNA. Although we can expect some of the ≈ 1000 chromosome 17-derived cosmids to be overlapping or even identical, they will still cover approximately one third of mouse chromosome 17. There is, therefore, a rationale behind the systematic search for transcripts in these cosmids.

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