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cDNA sequence and chromosomal localization of the mouse parvalbumin gene, *Pva*

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Summary

In the homozygous condition, the mutation *adr* (*arrested development of righting response*) of the mouse causes a myotonia and a drastic reduction of the Ca^{2+}-binding protein parvalbumin (PV) in fast muscles. Using a rat PV probe, a mouse cDNA clone was isolated from a λgt11 wild-type fast-muscle library and its nucleotide sequence was determined. The protein coding and the 3′ non-translated regions of the mouse gene show extensive homology with the rat PV gene. The result of Southern blot hybridization is consistent with a single copy gene for parvalbumin. Restriction fragment length polymorphisms (RFLPs) between *Mus musculus domesticus* (e.g. C57BL/6) and *Mus spretus* (SPE) were detected with the enzymes *Eco* RI, *Pst* I, and *Sst* I. The restriction fragment patterns of DNA samples from 65 individual offspring of (C57BL/6 × SPE)F1 × C57BL/6 backcrosses were tested with the PV probe and matched, for linkage detection, to pre-existing patterns established with various RFLP probes on the same samples. A co-distribution of PV-RFLPs with *Pvt-1* and *Mlvi-2*, which had been localized on chromosome 15, was detected. Thus, the structural gene for PV, designated *Pva*, maps to chromosome 15 of the mouse whereas the *adr* mutation shows no linkage with markers on this chromosome. Gene locus homology between chromsome 15 of the mouse and chromosome 22 of man (which carries the human PV gene) is discussed.

1. Introduction

Parvalbumin (PV), a high-affinity Ca^{2+}-binding protein, is synthesized in high amounts in fast contracting/relaxing muscles of small mammals (Heizmann *et al.* 1982). It is also expressed in certain non-muscle tissues such as brain. The expression of PV in muscle is neurally and developmentally regulated (Leberer & Pette, 1986; Müntener *et al.* 1987; Berchtold, 1988). Northern blot analysis in rat and mouse revealed two PV mRNA species of 1100 and 700 nt of which the smaller species is more abundant (Epstein *et al.* 1986; Kluxen *et al.* 1988)

The PV content of muscle is reduced in certain hereditary neuromusclar diseases of the mouse e.g. '*dystrophia muscularis*', DY^{2J} (Klug *et al.* 1985) and '*arrested development of righting response*', ADR* (Stuhlfauth *et al.* 1984; Jockusch *et al.* 1988*a*). The ADR syndrome has been characterized as a myotonia (Reininghaus *et al.* 1988) caused by a hyperexcitability of muscle fibres (Mehrke *et al.* 1988). This condition can be phenotypically cured by tocainide (Reininghaus *et al.* 1988), a drug that stabilizes the membrane potential. In tocainide treated animals the PV protein and mRNA levels are partially restored (Jockusch *et al.* 1988*a*; Kluxen *et al.* 1988). This indicates that the myotonic phenotype interferes with the regulation of PV synthesis.

The genomes of mouse and man are far more extensively characterized than those of other vertebrates. A number of mutations are known in mouse that may serve as models for hereditary diseases of man. In addition, regions of homology between human and mouse chromosomes have been identified (Peters, 1988; Kaplan *et al.* 1987). Therefore, based on the knowledge of the rat PV gene (Berchtold *et al.* 1987), we have begun to characterize the PV gene of the mouse.

Here we present the isolation of a parvalbumin cDNA clone from a mouse fast-muscle library, its nucleotide sequence and the deduced amino-acid sequence. By the identification and use of RFLPs for PV we were able to map this gene in the mouse

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* Lower case mutant symbols designate alleles, upper case symbols designate phenotypes.

genome. A preliminary report of part of this work has been given (Zühlke *et al.* 1988).

2. Material and methods

(i) *Animals*

In addition to standard laboratory mouse strains, wild-type (+/*adr*?) and homozygous mutant (*adr*/*adr*) individuals of the A2G inbred strain (Watkins & Watts, 1984) were used for DNA and RNA isolation. Genomic DNA was also prepared of the *Mus spretus* strains SPE (Guénet, 1986) and SEG (kindly provided by Dr O. v. Deimling, Freiburg).

(ii) *Isolation of mRNA*

Fast muscles were obtained from adult A2G wild-type mice. The tissue was frozen in liquid nitrogen immediately after dissection and stored at −70 °C. For the preparation of total RNA, the tissue was homogenized in 4 M guanidinium thiocyanate buffer according to Chirgwin *et al.* (1979). The RNA was pelleted by centrifugation through a 3 ml, 5·7 M-CsCl cushion. Poly(dT) paper (Medac, Hamburg) affinity chromatography was used to obtain messenger RNA (Werner *et al.* 1984). A total of 1–5 μg of poly(A)$^+$ RNA was used for cDNA synthesis.

(iii) *cDNA synthesis and cloning*

First strand synthesis starting from the 3′ end of the poly(A)$^+$ RNA was primed by oligo(dT) and elongated by MMLV reverse transcriptase (Maniatis *et al.* 1982). The DNA/RNA hybrids were treated with RNase H, providing 3′OH primers for DNA polymerase I (second-strand synthesis according to Gubler & Hoffmann, 1983). Double-stranded cDNAs were protected from *Eco* RI restriction by methylation (Huynh *et al.* 1985). *Eco* RI linkers were added to the cDNA and cleaved with *Eco* RI to generate cohesive termini. An appropriate cDNA fraction was size selected by low-melting-temperature agarose gel electrophoresis (Maniatis *et al.* 1982). The purified cDNAs were ligated in *Eco* RI digested phage vector λgt11 (Young & Davis, 1983*b*) and packaged *in vitro* according to Maniatis *et al.* 1982.

(iv) *Screening of the λgt11 library*

Overnight (o.n.) cultures (100 μl) of *E. coli* Y1088 (Young & Davis, 1983*a*) were infected with 10^3 p.f.u. of recombinant λgt11, diluted with 3 ml of top-agar, plated on LB-plates and incubated o.n. at 37 °C (Maniatis *et al.* 1982). Plaques were transferred onto nitrocellulose filters and processed according to Davis *et al.* 1980. Hybridization was performed with a rat parvalbumin cDNA probe (Berchtold & Means, 1985). The isolated fragment of the coding region was labelled with [α^{32}P]dCTP by random deoxyoligonucleotide-priming (Feinberg & Vogelstein, 1983). Positive clones were purified by single plaque passages. Phage DNA was isolated (Maniatis *et al.* 1982) and subcloned for sequencing.

(v) *DNA sequencing*

M13mp18 and M13mp19 were used as cloning vectors for DNA sequencing by the dideoxy chain termination method (Sanger *et al.* 1977). Single-stranded DNA was isolated from plaques suspended in 1 ml LB-medium and incubated with 10 μl of an o.n. culture of *E. coli* JM103 for 6 h at 37 °C. Bacteria were pelleted in a microfuge, 250 μl 20% PEG was added to the supernatant, mixed and incubated for 15 min at room temperature. After centrifugation (5 min) the phage pellet was resuspended in 100 μl TES-buffer. The suspended virus particles were extracted twice with phenol and phenol-$CHCl_3$, the aqueous phase was ethanol precipitated and the viral DNA was dissolved in 20 μl TE-Buffer. The 17-mer universal primer (Boehringer, Mannheim), the 15-mer λgt11 primer and the λgt11 reverse primer (Clontec, Palo Alto) were used to prime the sequencing reactions. Deletions of the cDNA were produced by exonuclease III. Double stranded DNA was isolated and cut with *Bam* HI and *Sph* I. Exonuclease III was added and the reaction was stopped after different time intervals. After treatment with S1 nuclease the deleted fragments were blunt ended by the Klenow fragment of *E. coli* polymerase I and ligated to the vector by T4 DNA ligase. Competent JM103 cells were transformed with the ligation product and plated onto agar medium. Recombinant clones were anaysed by digestion with *Eco* RI and *Hin* dIII. Single-stranded DNA of selected clones covering entirely both strands of the cDNA was prepared as described above and used for sequencing.

(vi) *Preparation of mouse genomic DNA, Southern blot and hybridization*

High-molecular-weight DNA was prepared from organs (lung, liver, heart, brain) of adult mice. The organs were frozen in liquid nitrogen, crushed and re-suspended in 5 ml of 1% sarcosyl, 0·1 mM-EDTA. The mixture was incubated with proteinase K and RNase A. DNA was extracted with phenol and phenol-$CHCl_3$ and purified by dialysis (Weydert *et al.* 1983) and 8 μg DNA were digested with 20 units of restriction enzyme, run on 0·8% agarose/TBE gels and stained with ethidium bromide. The DNA was denatured, neutralized and transferred to nitrocellulose or nylon filters according to Southern (1975).

Radioactive probes were prepared by random priming of 100 ng of electrophoretically purified mouse cDNA fragment. Hybridization was carried out at 42 °C in 50% formamide o.n. Blots were

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ATG TCG ATG ACA GAC GTG CTC AGC GCT GAG GAC ATC AAG AAG GCG ATA GGA GCC TTT GCT  +60
Met Ser Met Thr Asp Val Leu Ser Ala Glu Asp Ile Lys Lys Ala Ile Gly Ala Phe Ala

GCT GCA GAC TCC TTC GAC CAC AAA AAG TTC TTC CAG ATG GTG GGC CTG AAG AAA AAG AAC  +120
Ala Ala Asp Ser Phe Asp His Lys Lys Phe Phe Gln Met Val Gly Leu Lys Lys Lys Asn

CCG GAT GAG GTG AAG AAG GTG TTC CAT ATT CTG GAC AAA GAC AAA AGT GGC TTC ATT GAG  +180
Pro Asp Glu Val Lys Lys Val Phe His Ile Leu Asp Lys Asp Lys Ser Gly Phe Ile Glu

GAG GAT GAG CTG GGG TCC ATT CTG AAG GGC TTC TCC TCA GAT GCC AGA GAC TTG TCT GCT  +240
Glu Asp Glu Leu Gly Ser Ile Leu Lys Gly Phe Ser Ser Asp Ala Arg Asp Leu Ser Ala

AAA GAA ACA AAG ACG CTT CTG GCC GCT GGA GAC AAG GAT GGG GAC GGC AAG ATT GGG GTT  +300
Lys Glu Thr Lys Thr Leu Leu Ala Ala Gly Asp Lys Asp Gly Asp Gly Lys Ile Gly Val

GAA GAA TTC TCC ACT CTG GTG GCT GAA ACG TAA GTGGCGCTGACTGCTTGGGTCCCCCACCTCTCCATCCCCA  +373
Glu Glu Phe Ser Thr Leu Val Ala Glu Thr ***

ACGCCCCATCTCAGCCCTTCTCGCGGCCCTCCTGAGTTTCTGTTCAGTTTGTTTGTGTTATTTTTTACTCCCCCATCCTCTATGGCCCTCGGA  +466

TGACGCCATTCTTCTGGAAATGCTGGAGAAACAATAAAGGCTGTACCAATCGGACACC  +524
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Fig. 1. (*a*) cDNA and deduced amino-acid sequence of a mouse parvalbumin cDNA clone in 5′ → 3′ orientation. Numbering refers to the A (+1) in the start codon. A putative polyadenylation signal is underlined.

washed with 2 × SSC, 0·1 % SDS and 0·2 × SSC, 0·1 % SDS at 65 °C (Maniatis *et al.* 1982).

Kodak XAR film or Amersham Hyperfilm-MP were used for autoradiography with two Cronex lightning intensifying screens (Dupont) for 2–8 days at −70 °C.

3. Results and discussion

(i) *Isolation and sequencing of a mouse parvalbumin cDNA clone*

A cDNA library was constructed in λgt11 using as template poly(A)$^+$ RNA from fast muscles, tibialis anterior and vastus, of the A2G wild-type mouse (genotype +/*adr*?). Putative PV clones were identified by several rounds of screening with a 240 bp *Pst* I/*Eco* RI fragment of a rat parvalbumin cDNA (Berchtold & Means, 1985). One clone containing an insert of 0·5 kb, termed *mpv*, was chosen for further characterization.

The identity of clone *mpv* as a PV cDNA was demonstrated by hybridization to electrophoretically separated mRNAs from wild-type and myotonic mouse muscle. Similar hybridization patterns were detected for PV mRNAs (700 and 1000 nt) using the mouse (data not shown) or the rat probe (Kluxen *et al.* 1988).

The parvalbumin cDNA fragment was subcloned and sequenced in both orientations. A stretch of 524 bp containing the coding sequence and a part of the 3′ non-coding region was determined (Fig. 1 *a*). Although the 5′ non-coding region is lacking, the comparison with the rat cDNA sequence (Epstein *et al.* 1986) suggests the ATG (+1) as the initiation codon. Strong homologies of nucleotide (96%) and amino-acid (94·5%) sequences were found between mouse and rat. The 3′ non-coding region of the mRNA sequences is also conserved and putative polyadenylation signals for the short mRNAs are located at identical positions (+498 to +504). In rat, one poly(A)$^+$ site was found 10 nt downstream of the sequence AATAAA. The mouse cDNA clone lacks the poly(A) tail of the mRNA although it contains a stretch of 20 nt beyond a putative polyadenylation signal. It is possible that this clone represents a shortened fragment derived from the longer, less abundant mRNA species.

Comparing the amino-acid sequences of three mammalian species homologies of 85% to 95% were found (Fig. 1 *b*). Out of 110 amino acid residues, 8 are exchanged between mouse and rat, 18 between mouse and rabbit (Enfield *et al.* 1975) and 16 between rat and rabbit. Five residues are identical in rat and rabbit, but differ in mouse PV. This finding suggests a relationship between rodents and lagomorphs. Between carp (Coffee & Bradshaw, 1973) and mammals about 60% of the amino acids are identical. None of the amino-acid residues involved in calcium binding of carp III parvalbumin (Kretsinger & Nockolds, 1973) are exchanged, indicating the strong evolutionary conservation of these Ca^{2+}-binding domains.

(ii) *Identification of RFLPs and mapping of the mouse parvalbumin gene* Pva

Genomic DNA of *Mus spretus*, *Mus musculus domesticus* A2G (+/*adr*? and *adr*/*adr*) and from five additional laboratory strains was isolated and digested with *Eco* RI, *Hin* dIII, *Bam* HI, *Kpn* I, *Pst* I, *Pvu* II, *Sst* I and *Taq* I. After gel electrophoresis the DNA was blotted and hybridized with the mouse cDNA probe. No differences in the hybridization patterns between the laboratory mouse strains could be detected (data not shown). The results of Southern-

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	1																									
Mouse	M	S	M	T	D	V	L	S	A	E	D	I	K	K	A	I	G	A	F	A	A	A	D	S	F	D	H	K
Rat	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
Rabbit	.	A	.	.	E	L	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	E	.	.	.	.	.
Carp	.	.	F	A	G	.	.	N	D	A	.	.	A	A	.	L	E	.	C	K	.	.	.	.	.	.	.	.

	29																									
Mouse	K	F	F	Q	M	V	G	L	K	K	K	N	P	D	E	V	K	K	V	F	H	I	L	D	K	D	K	S
Rat	.	.	.	.	.	.	.	.	.	.	S	A	D	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.
Rabbit	.	.	.	.	.	.	.	.	.	.	.	S	T	E	D	.	.	.	.	.	.	.	.	.	.	.	.	.
Carp	A	.	.	A	K	.	.	.	T	S	.	S	A	.	D	.	.	.	A	.	A	.	I	.	Q	.	.	.

	57																										
Mouse	G	F	I	E	E	D	E	L	G	S	I	L	K	G	F	S	S	D	A	R	D	L	S	A	K	E	T	K
Rat	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Rabbit	.	.	.	.	.	E	.	.	.	F	.	.	.	.	.	.	P	.	.	.	.	.	.	V	.	.	.	.
Carp	.	.	.	.	.	.	.	.	K	L	F	.	Q	N	.	K	A	.	.	.	A	.	T	D	G	.	.	.

	85																									110
Mouse	T	L	L	A	A	G	D	K	D	G	D	G	K	I	G	V	E	E	F	S	T	L	V	A	E	T
Rat	.	.	M	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	S
Rabbit	.	.	M	.	.	.	.	.	.	.	.	.	.	.	.	A	D	.	.	.	.	.	.	S	.	S
Carp	.	F	.	K	.	.	.	S	.	.	.	.	.	.	.	.	D	.	.	T	A	.	.	K	A	–

Fig. 1. (*b*) Comparison of the amino-acid sequences (using the one-letter code) of parvalbumins from four vertebrate species. Only residues differing from those of the mouse are depicted for the other species. Residues marked by asterisks correspond to those involved in calcium binding of carp III parvalbumin (Kretsinger & Nockolds, 1973), missing residues are marked by —.

blot hybridization are compatible with the existence of a single parvalbumin gene (*Pva*) per haploid genome, although a tandem duplication of this chromosomal region with conservation of restriction sites cannot be excluded. The existence of a single copy PV gene has been shown for the rat (Berchtold & Means, 1985).

Restriction fragment length polymorphisms (RFLPs) for *Eco* RI (about 20 kb, the hybridizing fragment in SPE is slightly smaller than in A2G), *Pst* I (A2G: 3 kb, SPE: 8/2·8/1·5 kb) and *Sst* I (A2G: 5/2·5 kb, SPE: 4·5/2·5 kb) were identified between *Mus spretus* substrain SEG from Freiburg and *Mus musculus domesticus* A2G (Fig. 2*a*) and verified for *Mus spretus* substrain SPE from Paris.

Because the genetic polymorphism among laboratory mouse strains is limited, and *Mus spretus* shows the greatest degree of allelic variation compared with other mouse species (Avner *et al.* 1988) the DNA of the offspring of inter-specific crosses between *Mus spretus* and laboratory inbred strains were used for gene localization (for review see Guénet, 1986). DNAs were isolated from individuals of backcrosses between the F1 of an interspecific cross: (C57BL/6 × SPE) × C57BL/6. The restriction fragment length distribution of 65 individual DNA samples was tested using the PV probe (Fig. 2*b*). For linkage detection the PV hybridization pattern was compared to pre-existing patterns of the same samples generated by various mapped RFLP probes. A co-distribution of the RFLPs for *Pva* with *Pvt-1* (Banerjee *et al.* 1985) and *Mlvi-2* (Kozak *et al.* 1985) was detected. Out of 63 tested individual DNAs, 57 were concordant for *Pva*/*Pvt-1*. The concordance for *Pva*/*Mlvi-2* was 33 out of 56 tested animals and 41 out of 59 samples indicated localization of *Pva* to the same linkage group, chromosome 15, as *Pvt-1* and *Mlvi-2*.

No linkage has been found between markers on chromosome 15 and 'myotonia', adr^{mto} (Heller *et al.* 1982) an allele of *adr* (Jockusch *et al.* 1988*b*). The tissue specific reduction of PV in the myotonic ADR mouse is probably due to a secondary effect of the myotonia as indicated by pharmacological experiments (Reininghaus *et al.* 1988; Jockusch *et al.* 1988*a*; Kluxen *et al.* 1988) and not by a mutation in the parvalbumin gene itself.

By *in situ* hybridization *Mlvi-2* has been mapped to band 15A2 near the nucleolus organiser (Adolph *et al.* 1987) and *Pvt-1* to band 15D (Banerjee *et al.* 1985). From our linkage data it may be concluded that the *Pva* gene is located in the terminal chromosomal region (band 15E/F, Fig. 3), as are the genes for arylsulfatase$_1$ A (*As-2*), diaphorase-1 (*Dia-1*) and the proto-oncogene *Sis* (band 15E, Adoph *et al.* 1987). In man, the structural gene for PV has been assigned to chromosome 22 (Berchtold *et al.* 1987) and the genes *SIS*, *ARSA* and *DIA*1 have been mapped to the distal part of the q arm of this chromosome. The human *SIS*

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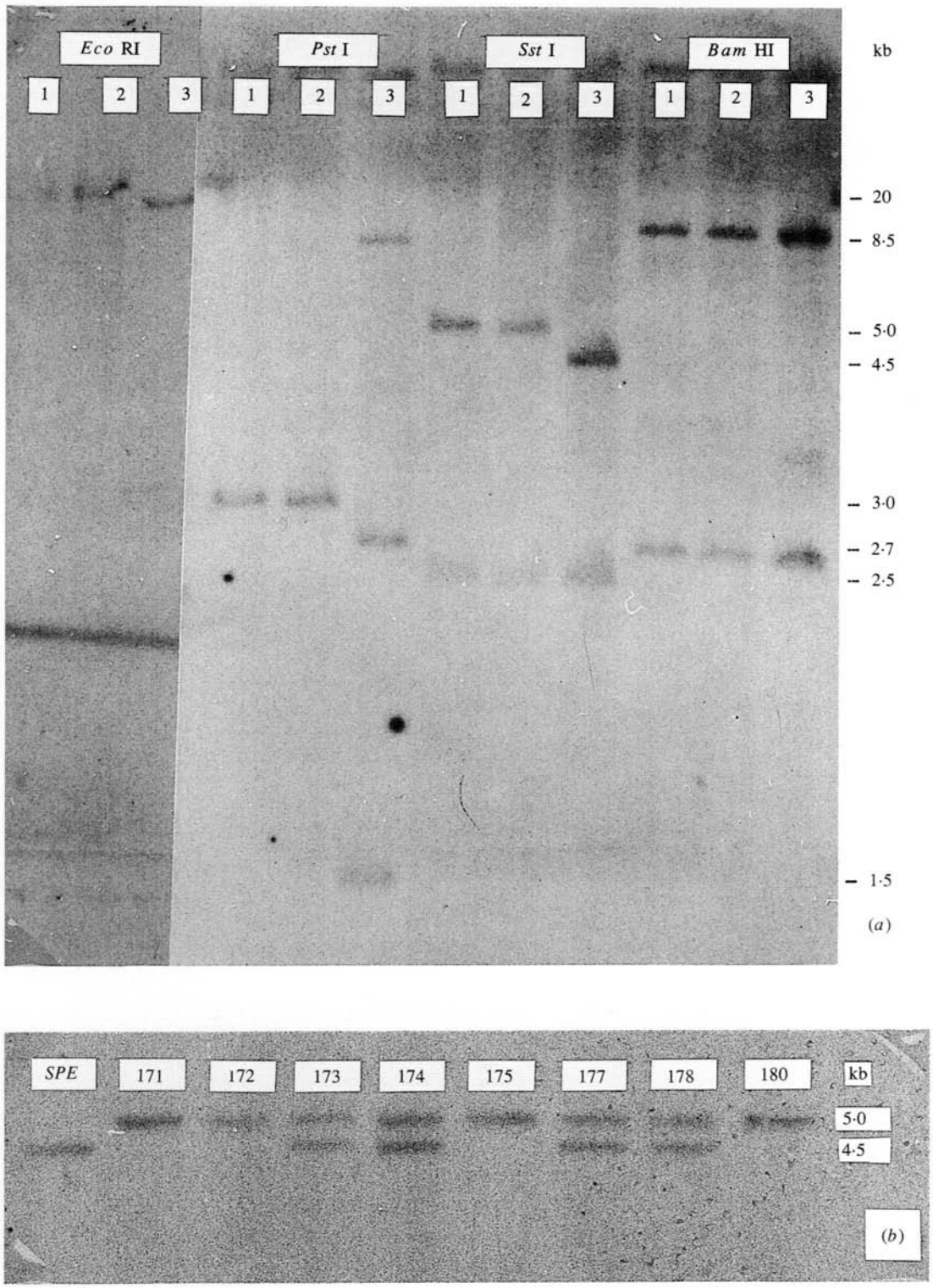

Fig. 2. Restriction fragments of the parvalbumin gene *Pva* in mouse strains and intercrosses. (*a*) Southern blot hybridization of genomic DNA from *Mus musculus domesticus* wild-type (A2G, lanes 2), the myotonic mutant (ADR, lanes 1) and *Mus spretus* (SPE, lanes 3). Genomic DNA, 10 μg respectively, extracted from lungs, were digested with the restriction enzymes *Eco* RI, *Bam* HI, *Sst*$_1$ I or *Pst* I and analysed by Southern blotting. A fragment of the mouse PV gene was labelled and used as a probe. (*b*) RFLPs for *Sst* I in eight individuals of interspecific backcrosses. The DNA from (C57BL/6 × SPE)F1 × C57BL/6 and from *Mus spretus* SPE was digested with *Sst* I, blotted and hybridized with the mouse cDNA probe. Animals homozygous and heterozygous for the *Pva* fragment are characterized by, respectively, 1 and 2 hybridizing fragments.

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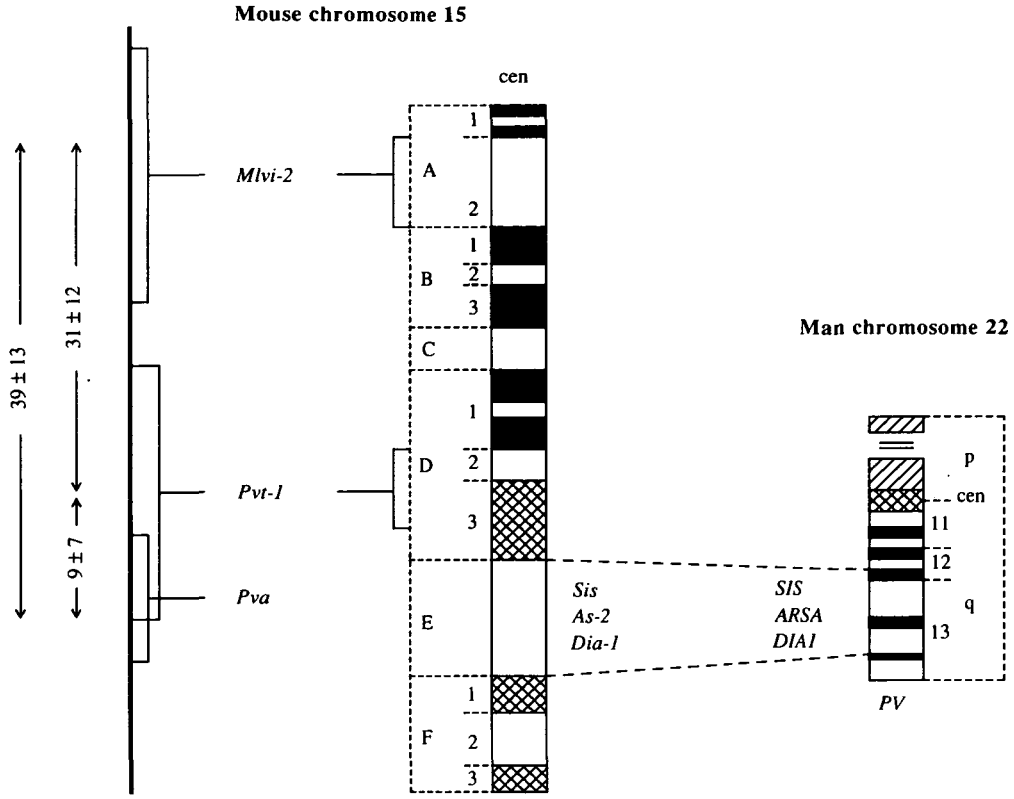

Fig. 3. Schematic representation of linkage and G-banding patterns of mouse chromosome 15 and the G-banding pattern of human chromosome 22. Linkage distances to the marker genes *Pvt*-1 (plasmacytoma variant translocation, Banerjee *et al.* 1985) and *Mlvi-2* (Moloney virus integration site 2, Kozak *et al.* 1985) are given in cM with $P \leqslant 0{\cdot}05$. Chromosomal segments presumed homologous between mouse and man are indicated by dashed lines. The lengths of the mouse and the human chromosomes are not to scale.

proto-oncogene is the most proximal gene in this syntenic group (Kaplan *et al.* 1987). The extent of this man–mouse synteny is further supported by the localization of the gene for cytochrome P450IID and a hemoglobin pseudogene on chromosome 15 of the mouse (Peters, 1988) and chromosome 22 of man (Kaplan & Carritt, 1987).

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References

Adolph, S., Strauss, P. G., Hameister, H. & Vogel, W. (1987). Physical gene map of murine chromsome 15. *Mouse News Letter* **79**, 56.

Avner, P., Amar, L., Dandalo, L. & Guénet, J. L. (1988). Genetic analysis of the mouse using interspecific crosses. *Trends in Genetics* **4** (1), 18.

Banerjee, M., Wiener, F., Spira, J., Babonits, M., Nilsson, M. G., Sumegi, J. & Klein, G. (1985). Mapping of the *c-myc*, *pvt-1* and immunglobulin kappa genes, in relation to the mouse plasmacytoma-associated variant (6;15) translocation breakpoint. *EMBO Journal* **4**, 3183.

Berchtold, M. W. (1988). The rat parvalbumin gene. In *Calcium and calcium binding proteins*, p. 40. Springer-Verlag.

Berchtold, M. W., Epstein, P., Beaudet, A. L., Payne, M. E., Heizmann, C. W. & Means, A. R. (1987). Structural organization and chromosomal assignment of the parvalbumin gene. *Journal of Biological Chemistry* **262**, (18) 8696.

Berchtold, M. W. & Means, A. R. (1985). The Ca^{2+}-binding protein parvalbumin: molecular cloning and developmental regulation of mRNA abundance. *Proceedings of the National Academy of Sciences, USA* **82**, 1414.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. & Rutter, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294.

Coffee, C. J. & Bradshaw, R. A. (1973). Carp muscle calcium-binding protein (I). *Journal of Biological Chemistry* **248**, 3305.

Davis, R. W., Botstein, D. & Roth, J. R. (1980). *Advanced Bacterial Genetics: A Manual for Genetic Engineering*. Cold Spring Harbor Laboratory.

Enfield, D. L., Ericsson, L. H., Blum, H. E., Fischer, E. H. & Neurath, H. (1975). Amino-acid sequence of parvalbumin from rabbit skeletal muscle. *Proceedings of the National Academy of Sciences, USA* **72**, 1309.

Epstein, P., Means, A. R. & Berchtold, M. W. (1986). Isolation of a rat parvalbumin gene and full length cDNA. *Journal of Biological Chemistry* **261**, 5886.

Feinberg, A. P. & Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Analytical Biochemistry* **132**, 6.

Gubler, U. & Hoffman, B. J. (1983). A simple and very efficient method for generating cDNA libraries. *Gene* **25**, 263.

Guénet, J. L. (1986). The contribution of wild derived mouse inbred strains to gene mapping methodology. *Current topics in Microbiology and Immunology* **127**, 119.

Heizmann, C. W., Berchtold, M. W. & Rowlerson, A. M. (1982). Correlation of parvalbumin concentration with relaxation speed in mammalian muscles. *Proceedings of the National Academy of Sciences, USA* **79**, 7243.

Heller, A. H., Eicher, E. M., Hallet, M. & Sidman, R. L. (1982). Myotonia, a new inherited muscle disease in mice. *Journal of Neuroscience* **2**, 924.

Huynh, T. V., Young, R. A. & Davis, R. W. (1985). Construction and screening cDNA libraries in λgt10 and λgt11. In: *DNA Cloning: A Practical Approach* (ed. D. M. Glover). IRL Press.

Jockusch, H., Bertram, K. & Schenk, S. (1988*b*). The genes for two neuromuscular diseases of the mouse, 'arrested development of righting response', *adr*, and 'myotonia', *mto*, are allelic. *Genetical Research*, Cambridge **52**, 203.

Jockusch, H., Reininghaus, J., Stuhfauth, I. & Zippel, M. (1988*a*). Reduction of myosin-light-chain phosphorylation and of parvalbumin content in myotonic mouse muscle and its reversal by tocainide. *European Journal of Biochemistry* **171**, 101.

Kaplan, J.-C., Aurias, A., Julier, C., Prieur, M., Szanert, M.-F. (1987). Human chromosome 22. *Journal of Medical Genetics* **24**, 65.

Kaplan, J.-C. & Carritt, A. (1987). Chromosome 22. Human Gene mapping 9. *Cytogenetics and Cell Genetics* **46**, 260.

Klug, G., Reichmann, H. & Pette, D. (1985). Decreased parvalbumin contents in skeletel muscles of C57/6J(dy^{2J}/dy^{2J}) dystrophic mice. *Muscle and Nerve* **8**, 576.

Kluxen, F. W., Schöffl, F., Berchtold, M. W. & Jockusch, H. (1988). Opposite regulation of the mRNAs for parvalbumin and p19/6.8 in myotonic mouse muscle. *European Journal of Biochemistry* **176**, 153.

Kozak, C. A., Strauss, P. G. & Tsichlis, P. N. (1985). Genetic mapping of a cellular DNA region involved in induction of thymic lymphomas (*Mlvi-1*) to mouse chromosome 15. *Molecular and Cellular Biology* **5**, 894.

Kretsinger, R. H. & Nockolds, C. E. (1973). Carp muscle calcium-binding protein (II). *Journal of Biological Chemistry* **248**, 3313.

Leberer, E. & Pette, D. (1986). Neural regulation of parvalbumin expression in mammalian skeletal muscle. *Biochemical Journal* **235**, 67.

Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982). *Molcular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory.

Mehrke, G., Brinkmeier, H. & Jockusch, H. (1988). The myotonic mouse mutant ADR: electrophysiology of the muscle fiber. *Muscle and Nerve* **11**, 440.

Müntener, M., Rowlerson, A. M., Berchtold, M. W. & Heizmann, C. W. (1987). Changes in the concentration of the calcium-binding parvalbumin in cross-reinervated rat muscles. *Journal of Biological Chemistry* **262**, 465.

Peters, J. (1988). Mouse gene list. *Mouse News Letter* **80**, 7.

Reininghaus, J., Füchbauer, E. M., Bertram, K. & Jockusch, H. (1988). The myotonic mouse mutant ADR: physiological and histochemical properties of muscle. *Muscle and Nerve* **11**, 433.

Sanger, R., Nicklen, S. & Cohen, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences, USA* **74**, 5463.

Southern, E. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *Journal of Molecular Biology* **98**, 503.

Stuhlfauth, I., Reininghaus, J., Jockusch, H. & Heizmann, C. W. (1984). Calcium-binding protein, parvalbumin, is reduced in mutant mammalian muscle with abnormal contractile properties. *Proceedings of the National Academy of Sciences, USA* **81**, 4814.

Watkins, W. J. & Watts, D. C. (1984). Biological features of the new A2G-adr mouse mutant with abnormal muscle function. *Laboratory Animals* **18**, 1.

Werner, D., Chemla, Y. & Herzberg, M. (1984). Isolation of poly(A)$^+$ RNA by paper affinity chromatography. *Analytical Biochemistry* **141**, 329.

Weydert, A., Daubas, P., Caravatti, M., Minty, A., Bugaisky, G., Cohen, A., Robert, B. & Buckingham, M. (1983). Sequential accumulation of mRNAs encoding different myosing heavy chain isoforms during skeletal muscle development *in vivo* detected with a recombinant plasmid identified as coding for an adult fast myosin heavy chain from mouse skeletal muscle. *Journal of Biological Chemistry* **258**, 13867.

Young, R. A. & Davis, R. W. (1983*a*). Yeast RNA polymerase II genes: isolation with antibody probes. *Science* **222**, 778.

Young, R. A. & Davis, R. W. (1983*b*). Efficient isolation of genes by using antibody probes. *Proceedings of the National Academy of Sciences, USA* **80**, 1194.

Zühlke, Ch., Schöffl, F., Jockusch, H., Simon, D., Guénet, J. L. (1988). The parvalbumin gene *Pva* maps to chromsome 15 of the mouse. *Mouse News Letter* **80**, 149.