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Zinc Finger Protein Gene Complexes on Mouse Chromosomes 8 and 11

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Two murine homologs of the *Drosophila* Krüppel gene, a member of the gap class of developmental control genes that encode a protein with zinc fingers, were mapped to mouse chromosomes 8 and 11 by using somatic cell hybrids and an interspecific backcross. Surprisingly, both genes were closely linked to two previously mapped, Krüppel-related zinc finger protein genes, suggesting that they are part of gene complexes.

INTRODUCTION

DNA binding proteins can be classified into two groups on the basis of their binding motif. One group has the helix–turn–helix motif characteristic of procaryotic DNA binding proteins and eucaryotic proteins with the homeobox domain (Pabo and Sauer, 1984; Gehring and Hiromi, 1986; Colberg-Poley *et al.*, 1987). The other group has a DNA binding domain known as a zinc finger. This binding motif is usually represented several times in the protein and is composed of two cysteine residues and one histidine residue that wrap around a zinc ion in a finger-like structure (Berg, 1988; Gibson *et al.*, 1988).

One of the genes encoding a zinc finger protein is the Krüppel gene, which belongs to the gap class of developmental control genes in *Drosophila* (Preiss *et al.*, 1985; Chowdhury *et al.*, 1988). Zinc finger proteins are believed to regulate transcription of genes whose expression is essential during embryonic development (Ginsberg *et al.*, 1984; Rosenberg *et al.*, 1986; Kadonaga *et al.*, 1987; Dressler and Gruss, 1988). Substitution of a single cysteine residue in the zinc binding domain of the Krüppel gene results in loss of wild-type function, i.e., lack of thoracic and five of eight abdominal segments and absence of malpighian tubules (Redemann *et al.*, 1988).

Genes related to Krüppel have been described in the mouse (Schuh *et al.*, 1986; Chowdhury *et al.*, 1987; Chavrier *et al.*, 1988; Dressler *et al.*, 1988; Ashworth *et al.*, 1989) and three have been mapped, *Zfp-1* to Chr 8 (Chowdhury *et al.*, 1989), *Zfp-3* to Chr 11, and *Zfp-4* to Chr 8 (Ashworth *et al.*, 1989). The chromosomal location of these genes may reveal candidate mutations that could be valuable in revealing the function of DNA binding proteins. Mapping one of the murine homologs of the *Drosophila* paired box family is an excellent example of this approach. Gene mapping showed that the *Pax-1* gene was located near the undulated (*un*) mutation on mouse Chr 2 and suggested that *Pax-1* and *un* may be the same (Deutsch *et al.*, 1988). Sequence analysis of the *Pax-1* gene in *un/un* and wild-type mice revealed a substitution that would result in an amino acid replacement in mutant mice (Balling *et al.*, 1988). The *un* mutation may be invaluable for elucidating the function of this murine paired box gene. The present study was undertaken to map the second Krüppel-related gene, *Zfp-2*, reported by Chowdhury *et al.* (1987) and to determine the relative position of *Zfp-1* and *Zfp-4* on Chr 8.

MATERIALS AND METHODS

Mice

Mice were obtained from the research and production colonies of The Jackson Laboratory.

Somatic Cell Hybrids

The panel of hybrids, which was formed by fusing Chinese hamster and BALB/cJ mouse spleen cells, has been described previously (Minna *et al.*, 1975; Popp *et al.*, 1981).

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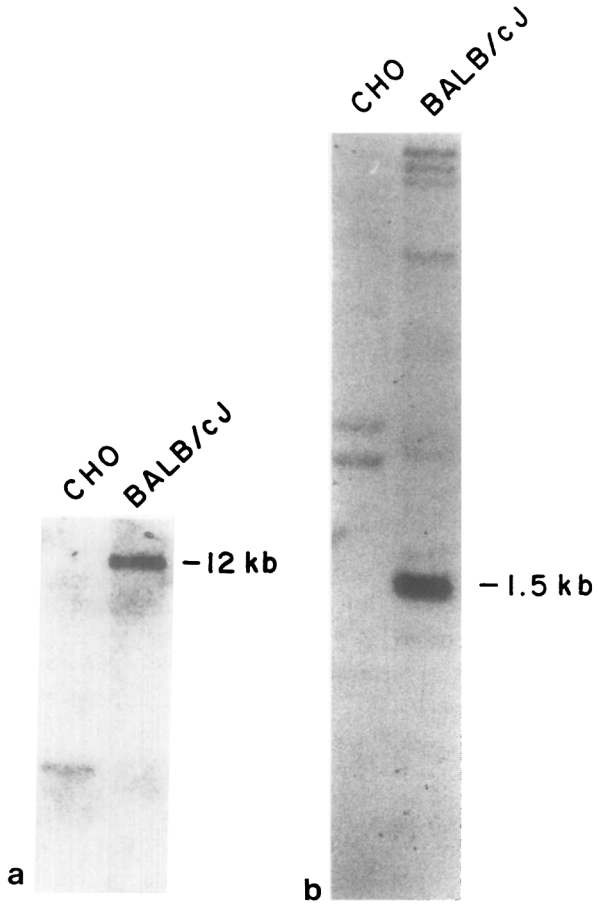

FIG. 1. Restriction fragments in a panel of mouse–Chinese hamster somatic cell hybrids. The size of the fragment used to analyze segregation is indicated. (a) *Zfp-2* (Probe, pKC6; restriction enzyme, *Stu*I). The weakly hybridizing fragments observed in BALB/cJ genomic DNA were not included in the segregation analysis because they could not be scored reliably. (b) *Zfp-1* (Probe, pKC7; restriction enzyme, *Stu*I).

Isozyme Analysis

ES-1 (esterase-1). Kidney samples were diluted with an equal volume of distilled H_2O, homogenized, and centrifuged for 5 min on a MC-150 microfuge with a TMA-S20 head (Tomy). Three microliters of supernatant was applied 1 cm from the cathodal end of a Titan III cellulose acetate plate (Helena Laboratories) with a Zip-Zone applicator (Helena Laboratories). The electrophoresis buffer consisted of 28 m*M* KH_2PO_4 and 24 m*M* Na_2HPO_4, pH 6.8. Electrophoresis was conducted at 140 V for 30 min at room temperature. The stain consisted of 1 ml 2% α-naphthyl acetate (in acetone) and 100 mg Fast Blue RR in 10 ml phosphate tray buffer. This stain was filtered, combined with 10 ml 2% warmed agar, and used as an agar overlay. ES-1 is the most anodal zone of activity (Peters and Nash, 1977). The ES-1 isozyme in C57BL/6J was more anodal than the ES-1 isozyme in *Mus spretus*.

GR-1 (glutathione reductase-1). Titan III cellulose acetate plates were soaked in a Tris–glycine buffer (0.025 *M* Tris, 0.192 *M* ammonia-free glycine, pH 8.5) containing 8 mg dithiothreitol per 150 ml buffer. Three microliters of the kidney supernatant was then applied to the center of a cellulose acetate plate (Helena Laboratories) with a Zip-Zone applicator (Helena Laboratories). The Tris–glycine buffer was also used for electrophoresis, which was conducted at 200 V for 30 min at room temperature. The stain consisted of 7 mg NADPH and 30 mg oxidized glutathione in 10 ml 0.3 *M* Tris–HCl buffer, pH 8.0. This mixture was combined with an equal volume of 2% warmed agar and used as an agar overlay. Enzymatic activity was visualized with uv light after incubating the gel for 30 min at 37°C. GR-1 activity is found between the origin and the anodal end of the gel (Nichols and Ruddle, 1975). The GR-1A isozyme in C57BL/6J migrates more anodally than the GR-1 isozyme found in *M. spretus*.

Genomic DNA

DNAs were either purchased from the Mouse DNA Resource of The Jackson Laboratory or prepared from spleen and brain cells by using standard methods (Phillips and Nadeau, 1984).

Krüppel Probes

The λ mkr1 and λ mkr2 genomic clones were obtained by screening a mouse genomic library with a Krüppel cDNA containing most of the finger-encoding sequences (Chowdhury *et al.*, 1987). The subclone pKC6 contains a 362-bp *Eco*RI fragment of λ mkr2 inserted into the *Eco*RI site in pTZ19R. The subclone pKC7 contains a 1045-bp *Pst*I fragment from λ mkr1 inserted into the *Pst*I site of pSPT18 (Chowdhury and colleagues, unpublished data). The provisional names for genes represented within the insert of pKC6 and pKC7 were mKR2 and mKR1, respectively (Chowdhury *et al.*, 1987). However, standard nomenclature following the recommendations of the Mouse Gene Nomenclature Committee was recently proposed (Chowdhury *et al.*, 1989). The proposed symbols are *Zfp-1* (pKC7, λ mKR1) and *Zfp-2* (pKC6, λ mKR2) for zinc finger protein-1 and zinc finger protein-2, respectively.

To verify the authenticity of these probes, we tested insert size and presence of diagnostic restriction sites within the inserts. pKC6 was digested with *Eco*RI and pKC7 with *Pst*I. These digests released insert fragments whose sizes were approximately 350 bp and 1 kb, respectively. pKC6 has an *Rsa*I site at bp 61

TABLE 1

Segregation of pKC6 and pKC7 Restriction Fragments in a Panel of Mouse–Chinese Hamster Somatic Cell Hybrids[a]

	Mouse chromosome																				
Hybrid	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	pKC6	pKC7
EBS-1	+	+	−	+	F	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+
EBS-2	−	−	+	−	+	+	−	−	+	−	−	+	+	+	+	+	−	?	−	+	−	−
EBS-3	−	+	−	−	−	−	+	−	−	−	−	−	+	−	+	+	−	?	−	+	−	−
EBS-4	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−	−	+	−	−
EBS-5	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	−	+	+	−	+
EBS-9	−	+	+	+	−	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	−	+
EBS-10	+	−	+	−	−	−	+	−	−	+	−	+	+	−	+	−	−	−	−	+	−	−
EBS-11	−	?	−	−	−	−	+	−	−	+	−	+	−	+	+	−	−	−	−	+	−	−
EBS-13	−	+	+	+	−	+	+	+	−	+	−	+	+	−	+	−	+	+	+	+	−	+
EBS-15	−	+	+	+	−	+	+	+	−	+	−	+	+	−	+	−	+	−	+	+	−	+
EBS-17	−	+	−	−	−	−	−	−	+	−	−	−	+	−	+	−	−	−	−	+	−	−
EBS-18	−	+	−	−	−	−	−	−	−	−	−	−	−	−	F	−	−	−	−	+	−	−
EBS-51	−	+	−	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	+	+	−	−
EBS-71	+	+	+	−	−	−	+	−	+	−	−	+	−	−	+	+	−	+	−	+	−	−
EBS-74	−	+	+	−	−	+	−	−	−	−	−	+	−	+	+	+	−	−	−	+	−	−

[a] + indicates that the chromosome was present; −, absent; F, fragment; and ?, uncertainty. The criteria for characterizing the chromosomal constitution of hybrid cell lines are provided by Popp *et al.* (45).

within the 362-bp insert. A double digest of pKC6 with *Eco*RI and *Rsa*I produced the fragments of the expected size. pKC7 has a *Bam*HI site at bp 418 and an *Eco*RI site at position 502 within the 1045-bp insert. Double digests of pKC7 with *Pst*I and either *Bam*HI or *Eco*RI produced fragments of the expected sizes. These results confirmed the authenticity of these two probes.

Probes for *Zfp-3* and *Zfp-4* were described by Ashworth *et al.* (1989). A 4.9-kb *Xba*I fragment was used to follow inheritance of the *Zfp-3* allele from *M. spretus* and an 8.2-kb *Bgl*I fragment to follow inheritance of the *Zfp-4* allele from *M. spretus*.

Trp53 Probe

Probe 27.1a is a *Pst*I–*Bgl*II fragment of the TRP53 cDNA (Jenkins *et al.*, 1984). A 5.0-kb *Bgl*I restriction fragment was used to follow inheritance of the allele from *M. spretus*.

Probes for Ankyrin-1 (Ank-1) and Plasminogen Activator, Tissue (Plat)

These probes and restriction fragment variants in *M. spretus* were described by White *et al.* (1990).

Mitochondrial Uncoupling Protein (Ucp)

This probe was described by Jacobsson *et al.* (1985).

Southern Analysis, Probe Preparation, and Hybridization Conditions

The methods used were described in detail by Nadeau and Phillips (1987).

Mutant Typing

Rex (*Re*) and trembler-J (Tr^J) are dominant mutations that were scored visually. Rex heterozygotes have curly whiskers and a wavy coat (Crew and Auerbach 1939). Trembler-J heterozygotes have rapid tremor beginning at 9 to 10 days of age, a tendency to convulsions, and spasticity in the muscles of the lower back and limbs (Falconer, 1951).

Maximum Likelihood Analysis of Gene Order

The program described by Nadeau *et al.* (1990) was used to identify the most likely gene order and to calculate the lod score for order for multilocus data ($2 < N < 16$). This program is based on maximum likelihood methods described by Bishop (1985).

RESULTS

Synteny of Mouse Krüppel Genes

To establish synteny of *Zfp-2* and to confirm synteny of *Zfp-1*, genomic DNAs from a panel of mouse–Chinese hamster somatic cell hybrids were typed by Southern blot analysis using pKC6 and separately pKC7 as hybridization probes. For *Zfp-2*, none of the hybrids had the 12-kb *Stu*I fragment characteristic of BALB/cJ (Fig. 1a) and none of the hybrids had mouse Chr 11, suggesting that *Zfp-2* is located on mouse Chr 11 (Table 1). This circumstantial evidence for synteny was confirmed through linkage analysis (see below). For *Zfp-1*, five of the hybrids had the 1.5-kb *Stu*I

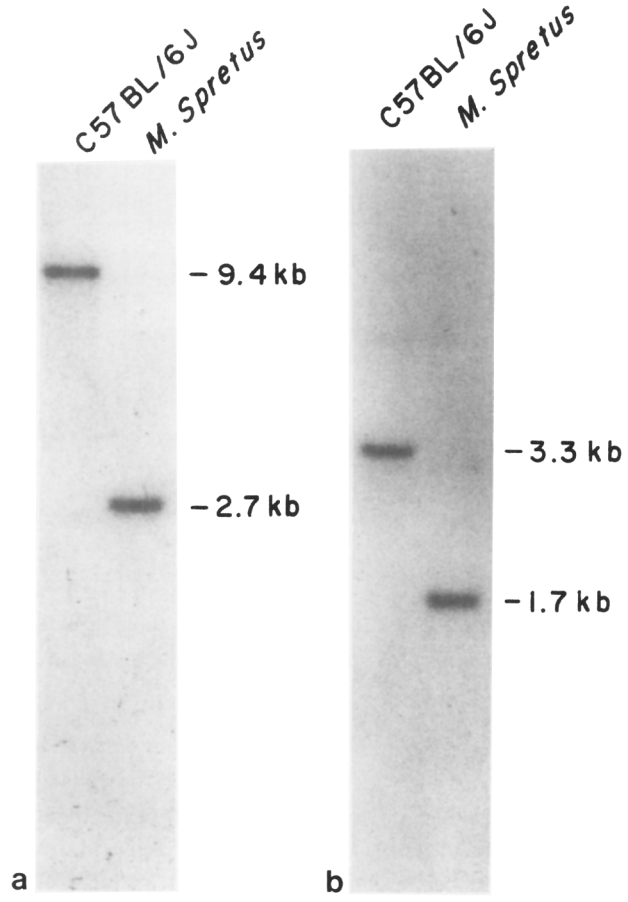

FIG. 2. Restriction fragments used in the segregation analysis of progeny of the backcross (C57BL/6J-*Tr*^{J} *Re*/++) F_1 females × C57BL/6J males. (**a**) Gene, *Zfp-2*; probe pKC6; restriction enzyme, *Stu*I. (**b**) Gene, *Zfp-1*; probe, pKC7; restriction enzyme, *Pvu*II.

fragment characteristic of BALB/cJ (Fig. 1b). There was complete concordancy between occurrence of this fragment among the hybrid lines and presence of mouse Chrs 4 and 8 (Table 1). This result is consistent with the previous linkage assignment of *Zfp-1* to Chr 8 (Chowdhury *et al.*, 1989).

Linkage and Gene Order

To establish linkage of *Zfp-1* and *Zfp-2* to genetic markers on Chrs 8 and 11, respectively, progeny of an interspecific backcross (Robert *et al.*, 1985; Guenet, 1986; Avner *et al.*, 1988) were studied. Genomic DNAs from 44 backcross progeny of the backcross (C57BL/6J-*Tr*^{J} *Re*/++ × *M. spretus* [Spain])F_1 females × C57BL/6J males were digested with *Pvu*II and Southern blots probed with pKC7 for *Zfp-1*. Segregation of the 1.7-kb *Pvu*II restriction fragment characteristic of *M. spretus* was scored (Fig. 2b) and compared to the segregation patterns for six Chr 8 markers *Ank-1, Es-1, Gr-1, Plat, Ucp,* and *Zfp-4* (Table 2). The most likely gene order and recombination frequencies were *Ank-1*/*Plat*–0.045 ± 0.031–*Gr-1*–0.159 ± 0.031–*Ucp*–0.091 ± 0.043–*Es-1*–0.023 ± 0.023–*Zfp-1*/*Zfp-4*. An additional 12 backcross progeny, for a total of 60 progeny (44 + 12 = 60), were typed for both *Zfp-1* and *Zfp-4*. No crossovers were detected between *Zfp-1* and *Zfp-4*, yielding an upper 95% confidence limit on the recombination frequency of 0.049.

To verify evidence for synteny of *Zfp-2* on Chr 11 and to establish linkage, genomic DNAs from 62 progeny of the backcross (C57BL/6J-*Tr*^{J} *Re*/++ × *M. spretus* [Spain]) F_1 females × C57BL/6J males were digested with *Stu*I and Southern blots were probed with pKC6 for *Zfp-2*. Segregation of the 2.7-kb *Stu*I fragment characteristic of *M. spretus* was scored (Fig. 2a) and compared to the segregation pattern for *Trp53*, whose location on Chr 11 has recently been established (Buchberg *et al.*, 1989), and to the previously published patterns for trembler (*Tr*^{J}), rex (*Re*), homeobox-2 (*Hox-2*), and keratin type I (*Krt-1*) (Table 3; Nadeau *et al.*, 1989). Nineteen progeny were not typed for *Tr*^{J}.

An unexpectedly large number of apparent double crossovers were observed. Six of the 62 progeny appeared to have two crossovers between the flanking loci *Tr*^{J} and *Hox-2*, 4 involved *Zfp-2* alone, 1 involved *Trp53* and *Zfp-3*, and 1 involved *Trp53, Zfp-3,* and *Zfp-2* (Table 3). Although we originally suspected that typing errors were responsible for these six progeny, we were unable to identify the source of the error. These progeny were retyped for *Zfp-2, Zfp-3,* and *Trp53,* and results agreed with the original typing. Another explanation is that typing of *Tr*^{J} was not entirely reliable because genetic background modified the trembler phenotype. Some backcross progeny were more severely affected on the hybrid C57BL/6J-"*spretus*" backcross background than on the C57BL/6J background (J. Nadeau and D. Varnum, unpublished data). If only affected progeny are considered, however, four apparent double crossovers remain. Thus, possible *Tr*^{J} mistyping does not fully account for the anomalous doubly recombinant progeny. Further work will be required to understand the origin of these anomalous progeny. These six progeny did not affect the inferred gene order, but clearly affected recombination frequencies. The most likely gene order and recombination frequencies were *Tr*^{J}–0.047 ± 0.032–*Trp53*–0.016 ± 0.016–*Zfp-3*–0.081 ± 0.035–*Zfp-2*–0.302 ± 0.058–*Hox-2*/*Krt-1*/*Re*.

Zfp-1 provides a valuable marker for the central portion of Chr 8 in the CXB set of recombinant inbred strains. An *Msp*I restriction fragment variant was used to map *Zfp-1* in the CXB strains. The progenitors of these strains are BALB/cBy and C57BL/6By. The CXB-E, -G, and -I strains had the 3.5-kb

TABLE 2

Recombination between *Ank-1, Es-1, Gr-1, Plat, Ucp, Zfp-1,* and *Zfp-4* in an Interspecific Linkage Cross between (C57BL/6J-*Tr^{J}Re*/+ + × *Mus spretus*[Spain])F_1 × C57BL/6J

	Allelic combination												
	Ank-1/Plat		*Gr-1*		*Ucp*		*Es-1*		*Zfp-1/Zfp-4*		No. observed
Parental											
	b		*b*		*b*		*b*		*b*		14
	s		*s*		*s*		*s*		*s*		16
Recombinants											
	b	x	*s*		*s*		*s*		*s*		1
	s	x	*b*		*b*		*b*		*b*		1
	b		*b*	x	*s*		*s*		*s*		2
	s		*s*	x	*b*		*b*		*b*		5
	b		*b*		*b*	x	*s*		*s*		2
	s		*s*		*s*	x	*b*		*b*		2
	b		*b*		*b*		*b*	x	*s*		0
	s		*s*		*s*		*s*	x	*b*		1
											44
Recombination frequencies											
Ank-1/Plat–Gr-1:	2/44 = 0.045 ± 0.031										
Gr-1–Ucp:	7/44 = 0.159 ± 0.055										
Ucp–Es-1:	4/44 = 0.091 ± 0.043										
Es-1–Zfp-1/Zfp-4:	1/44 = 0.023 ± 0.023										

Note. An x indicates the position of a crossover. Lod score for order: 0.94. The order given is more than eight times more likely than any other order. The next most likely order reverses the position of *Zfp-1/Zfp-4* and *Es-1*. The combined data of Chowdhury *et al.* (11) and the present study provide compelling evidence that *Zfp-1/Zfp-4* are distal to *Es-1*.

fragment characteristic of BALB/cBy, whereas the CXB-D, -H, -J, and -K strains had the 6.0-kb fragment characteristic of C57BL/6J.

DISCUSSION

One of the principal reasons for mapping genes such as *Zfp-1* and *Zfp-2* is to compare their chromosomal location with that of previously mapped genes to determine whether any existing mutations might involve these genes. Only two mutations, hydrocephalus-3 (*hy-3*) and scabby (*scb*), are located near the likely location of *Zfp-1* on Chr 8, and three mutations, cocked (*co*), nude (*nu*), and open eyelids (*oe*), are located near *Zfp-2* on mouse Chr 11. In *Drosophila,* lack-of-function mutants fail to produce thoracic segments, most abdominal segments, and malpighian tubules (Wieschaus *et al.*, 1984; Jäckle *et al.*, 1985). On the basis of phenotypic effects, it is unlikely that any are candidates for mutations in either of these zinc finger protein genes.

Sequence and restriction fragment analysis demonstrates that the two Krüppel-related genes isolated by Ashworth *et al.* (1989) are distinct from those isolated by Chowdhury *et al.* (1989). Ashworth and colleagues (1989) isolated two Krüppel-related mouse cDNA clones by screening a λ gt10 library of 8.5-day postcoitum embryos and testis cDNA. DNA sequence analysis of these two clones showed that they were different from *Zfp-1* and *Zfp-2* (Ashworth *et al.*, 1989; Chowdhury *et al.*, 1989). Restriction fragment differences confirm that these are different genes. For example, comparison of *Eco*RI digests of C57BL/6J genomic DNA typed with probes that were used to define *Zfp-2* (present study, results not shown) and *Zfp-3* (Ashworth *et al.*, 1989) revealed fragments of different sizes. Similar differences were found in *Hin*dIII digests of C57BL/6J DNA typed with probes that were used to define *Zfp-1* (present study, results not shown) and *Zfp-4* (Ashworth *et al.*, 1989).

The present study, together with those of Ashworth *et al.* (1989) and Chowdhury *et al.*, (1989), provides evidence for two complexes of Krüppel-related zinc finger protein genes, one on Chr 8 between *Um* and *Tat* (see Chowdhury *et al.*, 1989, for evidence that *Zfp-1* located between these two genes) and the other on Chr 11 between *Trp53* and the *Hox-2* and *Krt-2* complexes. The absence of recombination (Table 2) clearly demonstrates that *Zfp-1* and *Zfp-4* define a gene complex.

The evidence for a *Zfp* complex on Chr 11 is more ambiguous. The proximity of *Zfp-2* and *Zfp-3* is difficult to estimate because of the high frequency of apparent double crossovers. These crossovers are unusual because there are few if any precedents in the mouse. Buchberg *et al.* (1989) did not find anomalies

TABLE 3

Recombination between *Tr^J*, *Zfp-2*, *Zfp-3*, *Trp53*, *Hox-2*, *Krt-1*, *and Re* in an Interspecific Linkage Cross between (C57BL/6J–*Tr^J Re*/+ + × *Mus spretus* [Spain])F_1 × C57BL/6J

	Allelic combination												
	Tr^J			*Trp53*			*Zfp-3*			*Zfp-2*		*Hox-2/Krt-1/Re*	No. observed
Parental													
	Tr^J			*b*			*b*			*b*		*Re*	15
	s			*s*			*s*			*s*		*s*	15
Recombinants													
	Tr^J			*b*			*b*			*b*	x	*s*	5
	s			*s*			*s*			*s*	x	*Re*	3
	Tr^J	x		*s*			*s*	x		*b*		*Re*	1
	s			*s*			*s*	x		*b*	x	*s*	1
	Tr^J			*b*			*b*	x		*s*	x	*Re*	2
	s	x		*b*			*b*			*b*	x	*s*	1
													43
				b			*b*			*b*		*Re*	4
				s			*s*			*s*		*s*	7
				s	x		*b*			*b*		*Re*	1
				b			*b*			*b*	x	*s*	3
				s			*s*			*s*	x	*Re*	3
				s			*s*	x		*b*	x	*s*	1
													19

Recombination frequencies

Tr–Trp53:	2/43 = 0.047 ± 0.032
Trp53–Zfp-3:	1/62 = 0.016 ± 0.016
Zfp-3–Zfp-2:	5/62 = 0.081 ± 0.035
Zfp-2–Re:	19/63 = 0.302 ± 0.058
Tr–Re:	13/43 = 0.302 ± 0.070 (including double crossovers)
	8/43 = 0.186 ± 0.059 (only single crossovers)

Note. An x indicates the position of a crossover.

in their comparable interspecific linkage study of loci on mouse Chr 11. In fact, most interspecific crosses show a normal or reduced frequency of multiple crossovers (Mullins *et al.*, 1988; Ceci *et al.*, 1989; Mosely and Seldin, 1989; Siracusa *et al.*, 1989; Crosby *et al.*, 1990; Nadeau *et al.*, 1990). The reason that Buchberg *et al.* (1989) failed to find this anomaly may be that the effect is very localized and that by chance they did not include a locus within this interval. Despite these crossovers, however, we hypothesize that these genes are physically close together as part of a gene complex and that a recombination anomaly is responsible for the double crossovers. Physical mapping of these complexes is under way (K. Willison, personal communication).

Genes involved in development have provided considerable evidence for duplicated chromosomal segments in the mouse genome. Perhaps the strongest example involves genes located on mouse Chrs 11 and 15. Small segments of each chromosome include a family of homeobox genes (Kappen *et al.*, 1989; Schughart *et al.*, 1989) and a family of keratin genes (Nadeau *et al.*, 1989, and in preparation), and preliminary evidence suggests retinoic acid receptor genes (J. Nadeau and colleagues, in preparation). Another excellent example involves the engrailed genes, *En-1* on Chr 1 and *En-2* on Chr 5 (Martin *et al.*, 1990). Moreover, as many as 30% of gene families whose members are located on more than one chromosome belong to duplicated segments (Nadeau, 1990). It is very likely therefore that *Zfp* complexes on Chrs 8 and 11 mark duplicated chromosome segments.

These four genes belong to a large gene family that encode proteins with zinc fingers. In addition to *Zfp-1*, *Zfp-2*, *Zfp-3*, and *Zfp-4*, several zinc finger genes have been identified in mice, including ecotropic viral integration site-1 (*Evi-1*) on Chr 3 (Morishita *et al.*, 1988), testis determining factor (*Tdf*) on the Y chromosome (Page *et al.*, 1987), and the *Krox* gene family (Chavier *et al.*, 1988). In man, at least five genes have been described: glioblastoma (*GL1*) on Chr 12q134–q14.3 (Kinzler *et al.*, 1988), early growth response gene-1 (*EGR1*) on Chr 5q23–31 (Sukhatme *et al.*, 1988), zinc finger protein 3 (*ZFP3*) on Chr 17p12–17 (Ashworth *et al.*, 1989), testis determining factor (*TDF*) on the Y chromosome (Page *et al.*, 1987), and

the candidate gene at the Wilms tumor locus (Call *et al.*, 1990; Gessler *et al.*, 1990). Gene mapping and structural analysis of each of these genes within and between species will provide considerable insight into the origin and evolution of this gene family.

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