Zinc Finger Protein Gene Complexes on Mouse Chromosomes 8 and 11

JOSEPH H. NADEAU,*† CONNIE S. BIRKENMEIER,* KAMAL CHOWDHURY,†
JEFFERY L. CROSBY,*‡ AND PETER A. LALLEY§

*The Jackson Laboratory, Bar Harbor, Maine 04609; †Department of Molecular Cell Biology, Max Planck Institute of Biophysical Chemistry, D-3400 Göttingen-Nikolausberg, West Germany; ‡Biochemistry Department, University of Maine, Orono, Maine 04669; and §Center for Molecular Biology, Wayne State University, Detroit, Michigan 48202

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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 prokaryotic 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).
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, StuI). 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, StuI).

Isozyme Analysis

ES-1 (esterase-1). Kidney samples were diluted with an equal volume of distilled H₂O, 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 mM KH₂PO₄ and 24 mM Na₂HPO₄, 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 EcoRI fragment of λ mkr2 inserted into the EcoRI site in pTZ19R. The subclone pKC7 contains a 1045-bp PstI fragment from λ mkr1 inserted into the PstI 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 EcoRI and pKC7 with PstI. These digests released insert fragments whose sizes were approximately 350 bp and 1 kb, respectively. pKC6 has an RsaI site at bp 61
TABLE 1
Segregation of pKC6 and pKC7 Restriction Fragments in a Panel of Mouse–Chinese Hamster Somatic Cell Hybrids

<table>
<thead>
<tr>
<th>Mouse chromosome</th>
<th>pKC6</th>
<th>pKC7</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBS-1</td>
<td>+</td>
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</tr>
<tr>
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<td>+</td>
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</tr>
<tr>
<td>EBS-74</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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 EcoRI and RsaI produced the fragments of the expected size. pKC7 has a BamHI site at bp 418 and an EcoRI site at position 502 within the 1045-bp insert. Double digests of pKC7 with PstI and either BamHI or EcoRI 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 XbaI fragment was used to follow inheritance of the Zfp-3 allele from M. spretus and an 8.2-kb BglII fragment to follow inheritance of the Zfp-4 allele from M. spretus.

Trp53 Probe

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

Probes for Anklyrin-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 (TrJ) are dominant mutations that were scored visually. Rex heterozygotes have curly whiskers and a wavy coat (Crew and Aubach 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 Stul 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 Stul
FIG. 2. Restriction fragments used in the segregation analysis of progeny of the backcross (C57BL/6J-T'rJ Re/++ X M. spretus) F₁ females X C57BL/6J males. (a) Gene, Zfp-2; probe pKC6; restriction enzyme, StuI. (b) Gene, Zfp-1; probe, pKC7; restriction enzyme, PvuII.

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-T'rJ Re/++ X M. spretus [Spain]) F₁ females X C57BL/6J males were digested with StuI and Southern blots were probed with pKC6 for Zfp-2. Segregation of the 2.7-kb StuI 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'), rex (Re), homeobox-2 (Hox-2), and keratin type 1 (Krt-1) (Table 3; Nadeau et al., 1989). Nineteen progeny were not typed for Tr'.

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' 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' 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' 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' -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 MspI 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 -1 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^Re/+ × Mus spretus[Spain])F, × C57BL/6J

<table>
<thead>
<tr>
<th>Allelic combination</th>
<th>Ank-1/Plat</th>
<th>Gr-1</th>
<th>Ucp</th>
<th>Es-1</th>
<th>Zfp-1/Zfp-4</th>
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<tr>
<td></td>
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<td>s</td>
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<tr>
<td></td>
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<td>b</td>
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<td>s s s x b</td>
<td>b</td>
<td>b</td>
<td>b</td>
<td>s</td>
<td>1</td>
</tr>
</tbody>
</table>

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.

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; Jackle 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 EcoRI 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 HindIII 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 Urn 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, Zfp-2, Zfp-3, Trp53, Hox-2, Krt-1, and Re in an Interspecific Linkage Cross between (C57BL/6J-TrJ Re/+ × Mus spretas [Spain])F₁ × C57BL/6J

<table>
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<th>Allelic combination</th>
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<tr>
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<tr>
<td><strong>No. observed</strong></td>
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<td>15</td>
</tr>
</tbody>
</table>

Recombination frequencies

- **Trs-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 (Chavrier et al., 1988). In man, at least five genes have been described: glioblastoma (GL1) on Chr 12q13-14.3 (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 origin and evolution of this gene family.

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