GENOMICS 20, 203–209 (1994)

Genomic Structure and Chromosomal Location of the Rat Gene Encoding the Zinc Finger Transcription Factor Kid-1

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Received October 27, 1993; revised November 21, 1993

We have previously cloned and sequenced a novel zinc finger cDNA, *Kid-1*, from the rat. Because of its developmentally regulated expression pattern and its suppression after renal injury, as well as its kidney-predominant expression, we propose that *Kid-1* is likely to play an important role in renal gene regulation. *Kid-1* encodes a predicted protein with 13 zinc fingers at the carboxy end and Krüppel-associated box (KRAB) A and B regions at the amino terminus. Expression of a Kid-1–GAL4 chimeric protein results in strong transcriptional repression of cotransfected constructs containing GAL4 binding sites and a chloramphenicol acetyl transferase gene driven by either a minimal promoter or a SV40 enhancer. We now report the cloning, structural organization, and chromosomal localization of the *Kid-1* gene. The *Kid-1* gene is composed of four exons and three introns, closely reflecting the organization of the Kid-1 protein. The KRAB A and B regions are encoded by the second and third exons, respectively. The entire zinc finger region is encoded by the fourth exon. Using a combination of linkage analysis and somatic cell hybrid analysis, *Kid-1* was mapped to rat chromosome (RNO) 10. *Kid-1*, *Il3*, and *Sparc* form a tight linkage group on RNO10. Regional sublocalization to RNO10q21.3–q22 was established by fluorescence *in situ* hybridization. © 1994 Academic Press, Inc.

INTRODUCTION

Zinc finger proteins constitute a major class of transcriptional regulators (Klug and Rhodes, 1987). Among the best studied of this class of transcription factors are the RNA polymerase III-associated transcription factor, TFIIIA, from *Xenopus laevis* (Miller *et al.*, 1985) and the products of *Drosophila* segmentation genes, such as *Krüppel* (Rosenberg *et al.*, 1986) and *hunchback* (Tautz *et al.*, 1987). A subgroup of zinc finger proteins, the C_2H_2 family, is characterized by repeated motifs in which two cysteine and two histidine residues bind to one zinc ion to form a finger-like structure. It is estimated that this superfamily of zinc finger genes in the mammalian genome has more than 100 members (Bellefroid *et al.*, 1989). These genes encode proteins containing as few as 1 to more than 30 fingers. Understanding the genomic structure of genes encoding proteins with zinc finger motifs can lead to predictions of alternatively spliced isoforms that may be differentially expressed in various developmental stages and that may interact with different binding sites on DNA (Hsu *et al.*, 1992) or with different proteins. In addition, the exon–intron structure can suggest functional domains in the protein (Smith *et al.*, 1989).

We have previously cloned and sequenced a novel zinc finger cDNA, *Kid-1*, from the rat (Witzgall *et al.*, 1993). *Kid-1* mRNA accumulates in postnatal renal development and is detected predominantly in the kidney. This gene gives rise to a 2.8-kb mRNA whose open reading frame encodes a protein with 13 zinc fingers at its carboxy terminus. The zinc fingers are divided into groups of 4 and 9. *Kid-1* mRNA levels decline after renal injury secondary to ischemia or folic acid administration, two insults that result in epithelial cell dedifferentiation, followed by regenerative cell proliferation and differentiation. The decreased expression of *Kid-1* early in postnatal development, and when renal tissue is recovering after injury, suggests that the gene product may be involved in the establishment of a differentiated phenotype and/or regulation of the regenerative response in the kidney. *Kid-1* mRNA levels are also reduced in renal cell carcinomas that develop in the Eker rat (Eker and Mossige, 1961) (unpublished observations). Furthermore, Kid-1 is a potent suppressor of transcription. Expression of a Kid-1–GAL4 chimeric protein results in strong suppression of transcription of cotransfected chloramphenicol acetyl transferase reporter constructs containing GAL4 binding sites and either a minimal promoter or a SV40 enhancer (Witzgall *et al.*, 1993). In this report, we describe the genomic structure and chromosomal localization of *Kid-1* in the rat. Chromosomal localization of the gene provides information that may prove useful in the identification of potential develop-

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mental abnormalities or other aberrant phenotypes that may result from abnormal expression of *Kid-1*.

MATERIALS AND METHODS

Screening of Rat Genomic DNA Library

A rat genomic library, prepared from liver and constructed in Charon 4A from a partial *Hae*III digestion, was obtained from Clontech (Palo Alto, CA). The library was plated at a density of 50,000 pfu per 150-mm plate and screened using standard protocols (Sambrook *et al.*, 1989) with a random-primed (Feinberg and Vogelstein, 1983, 1985) 346-bp fragment (nucleotides 552–897 in the cDNA, Z5.9zf- (Witzgall *et al.*, 1993)) from the non-zinc finger region of the *Kid-1* cDNA.

Characterization of Genomic Clones

Positive clones were plaque-purified to homogeneity and phage DNA was prepared from plate lysates according to standard protocols. Phage DNA was cut with *Eco*RI and subcloned into pBluescript (Stratagene, La Jolla, CA). Sequencing was performed on double-stranded DNA by the chain termination method (Sanger *et al.*, 1977) using a kit from U.S. Biochemical Co. (Cleveland, OH). Polymerase chain reaction (PCR) amplifications of genomic clones and genomic DNA were performed with 2.0 U AmpliTaq DNA polymerase (Perkin–Elmer–Cetus) in 100 μl buffer consisting of 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 10 m*M* Tris at pH 8.4. The denaturing, reannealing, and synthesizing temperatures were 95, 50 or 60, and 72°C, with incubation times of 30, 60, and 120 s, respectively. Amplified products were analyzed by agarose gel electrophoresis. The products amplified from the genomic clones were compared in size to PCR fragments obtained using the same primers with the cDNA as the template. Genomic DNA was prepared from rat liver using standard protocols (Ausubel *et al.*, 1987).

Genomic Localization

Somatic cell hybrid analysis. A panel of 16 mouse × rat somatic hybrids was derived from fusion of mouse hepatoma cells (BWTG3) with adult rat hepatocytes as described previously (Szpirer *et al.*, 1984). They were used to segregate all 22 pairs of rat chromosomes. Karyotyping was performed on 15–20 G-banded metaphase spreads for each hybrid, in accordance with the recommendation of the Committee for a Standardized Karyotype of *Rattus norvegicus* (1973). Chromosomal assignment was based on the highest degree of concordance with the pattern of rat chromosome retention in these cell lines. Southern blotting and hybridization with Z5.9zf- were carried out using standard techniques (Yeung *et al.*, 1993b). Filters were hybridized in 10% SDS/7% PEG with 100 μg/ml of sonicated salmon sperm DNA at 65°C and washed in 0.1% SDS/2× SSC (1× SSC is 0.15 *M* NaCl plus 0.015 *M* sodium citrate) at 55°C.

Linkage analysis. Forty backcross animals from (Long Evans × Brown Norway) × Brown Norway matings were obtained from the Laboratory Animal Facility at Fox Chase Cancer Center (Hino *et al.*, 1993). RFLPs were screened using a panel of 20 restriction enzymes and the Z5.9zf- *Kid-1* probe. Linkage of the informative probes was determined from the segregation patterns of the unique Long Evans allele in the progeny. The numbers of rats showing parental and recombinant ditypes were recorded and the recombination frequencies were calculated.

Fluorescence in situ hybridization. Fluorescence *in situ* hybridization of *Kid-1* was carried out using biotin-labeled probe and a fluorescein isothiocyanate–avidin detection and amplification method (Yeung *et al.*, 1993b). Chromosome preparations from rat embryo fibroblasts were aged for 2 weeks and baked for 4 h at 55°C before use. A 2.3-kb genomic fragment containing the 5′ region of the *Kid-1* gene, not containing the zinc finger domain, was purified using a CsCl gradient and biotinylated using nick-translation (Oncor). Twenty nanograms of plasmid probe was hybridized to metaphase chromosomes under competitive suppression conditions in the presence of sonicated fish and rat DNA. Washing conditions consisted of 50% formamide/2× SSC at 42°C (2× 10 min) and 0.5× SSC at 42°C (2 × 4 min). Chromosomes were counterstained with diamidino-2-phenylindole (DAPI) and propidium iodide. Images were captured and merged using a CCD camera (Photometrics, Tucson, AZ) and a computer workstation equipped with a TCL software package (BDS, Pittsburgh, PA).

RESULTS

Cloning of the Kid-1 Gene

To clone the *Kid-1* gene, 250,000 pfu of a genomic library from rat liver were screened at modest stringency (1× SSC at 65°C) with a 346-bp fragment from the non-zinc-finger region of the *Kid-1* cDNA (nucleotides 552–897 (Witzgall *et al.*, 1993)). Six positive clones that hybridized to this probe were identified, each of which was purified to homogeneity. Restriction and hybridization analyses of these six clones showed that they all contained the same 13.5-kb genomic fragment. One clone was chosen for further analysis. *Eco*RI fragments of 1.5, 2.3, and approximately 12 kb were subcloned into pBluescript. The 12-kb fragment was incompletely digested and contained the already-mentioned 2.3-kb fragment at its 5′-end, a 9-kb fragment in the middle, and a 0.4-kb fragment on its 3′-end. To establish that the clone corresponded to the *Kid-1* gene and to determine how much of the cDNA was covered by this genomic clone, hybridization studies were performed with oligonucleotides from various regions of the cDNA to map individual fragments. In addition, regions of the largest subclone (12 kb) were sequenced using oligonucleotides previously used for sequencing the *Kid-1* cDNA. Reactions with both the 5′- and 3′-most oligonucleotides provided sequence present in the cDNA, confirming the identity of the clone as the *Kid-1* gene. Analysis of the 1.5-kb subclone resulted in sequence that was identical to the 3′-end of the *Kid-1* cDNA, immediately downstream of the second *Eco*RI site in the *Kid-1* cDNA. This permitted the construction of a map in which the 1.5-kb fragment constitutes the 3′-end of the genomic clone.

Overlapping primer sets (180–780 bp apart in the cDNA sequence) were used for PCR amplification of the genomic clone and rat liver genomic DNA to confirm that there were no rearrangements in the genomic clone.

Structural Features of the Kid-1 Gene

Sequencing of genomic fragments of the *Kid-1* gene, subcloned into pBluescript, was performed with oligonucleotides derived from the cDNA. The *Kid-1* gene contains four exons and three introns (Fig. 1). This was verified by PCR amplification of the genomic clone and rat genomic DNA. The first exon contains the first 344 bp of the *Kid-1* cDNA. Exon II extends from position 345 to 471 bp, exon III from 472 to 570 bp, and exon IV from 571 to the 3′-end of the cDNA. The exons define structural domains that we previously described for the Kid-1 protein (Witzgall *et al.*, 1993). The 5′-untranslated region and codons for the first 11 amino acids are contained in exon I. The NH_2-terminus of the Kid-1 protein contains a Krüppel-associated box (KRAB), consisting

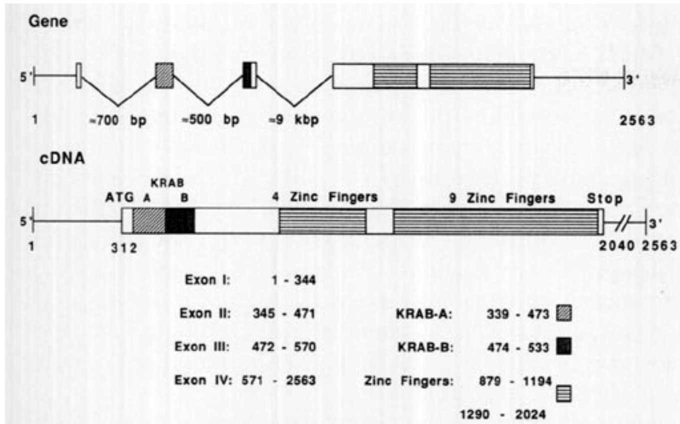

FIG. 1. Genomic structure of *Kid-1*. The intron–exon organization of the *Kid-1* gene is presented in the upper panel, and the structural motifs of the *Kid-1* cDNA in the lower panel. Straight lines represent the noncoding regions in exons I and IV. The second exon encodes the entire KRAB A region (cross-hatched). The KRAB B region (stippled) is encoded by part of exon III. The entire zinc finger region (striped) is encoded by exon IV. The numbers corresponding to each exon and each structural motif in the cDNA correspond to the numbered nucleotides of the cDNA (Witzgall *et al.*, 1993). Intron sizes (700 bp, 500 bp, and 9 kb) are presented.

of A and B domains. These non-zinc-finger regions are conserved in about one-third of all C_2H_2 zinc finger proteins (Bellefroid *et al.*, 1991). The KRAB A domain in *Kid-1* extends from nucleotide 339 to 473 in the cDNA and therefore constitutes the entire second exon. The KRAB B domain is encoded by nucleotides 474 to 533, composing the major part of exon III. The entire zinc finger region, with 13 zinc fingers clustered in groups of 4 and 9 zinc fingers, respectively, lies in exon IV, without being interrupted by an intron. All exon–intron boundaries have typical splice-donor and splice-acceptor sites (Shapiro and Senapathy, 1987) (Fig. 2). Interestingly, intron III contains an incomplete CA repeat [$(CA)_3$-$CC(CA)_4CT(CA)_2TAG(AC)_2GCG(CA)_3$], suggesting the possibility of polymorphisms (Weber, 1990).

Chromosome Localization

Using a combination of linkage analysis and somatic cell hybrid analysis, *Kid-1* was mapped to rat chromosome (RNO) 10. Regional sublocalization to RNO10q21.3–q22 was determined by fluorescence *in situ* hybridization. The pattern of *Kid-1* hybridization was in concordance with the presence or absence of RNO10 in the 16 somatic hybrid cell lines (Table 1). Two additional probes (*Interleukin 3* (*Il3*) and *Sparc*) used in our linkage analysis and one previously mapped RNO10 probe, *Syb2*, also showed the same hybridization pattern, thus mapping all these probes to RNO10.

Polymorphisms between Long Evans and Brown Norway strains were identified for *Kid-1* (*Taq*I), *Il3* (*Pst*I), and *Sparc* (*Pst*I). Among 40 backcross progeny, there was no recombinant among these three probes ($\theta = 0.0$, $Z_{max} = 12.04$); thus, they form a tight linkage group on RNO10.

The fluorescence *in situ* hybridization analysis revealed discrete signals on chromosome 10. Sublocalization based on the DAPI-stained metaphases with a characteristic Q-banding pattern mapped fluorescent signals to RNO10q21.3–q22 (Fig. 3). Seventy-four percent of the metaphases and 32% of RNO10 chromatids showed signals at this band.

DISCUSSION

Comparison of the Zinc Finger-Encoding Region of the *Kid-1* Gene with That of Other Zinc Finger Genes

A zinc finger protein-encoding gene may have introns located within the zinc finger repeat region, so that each finger is coded for by a separate exon, as in the *Xenopus* transcription factor TFIIIA (Tso *et al.*, 1986). Alternatively, introns can divide the gene into regions encoding groups of zinc fingers, such as in *tra-1* (*Caenorhabditis elegans*) (Zarkower and Hodgkin, 1992), *zfh-1* (*Drosophila*) (Fortina *et al.*, 1991), *CF2* (*Drosophila*) (Hsu *et al.*, 1992), and *ovo* (*Drosophila*) (Mével-Ninio *et al.*, 1991). In *CF2*, as in *tra-1*, there are differentially spliced

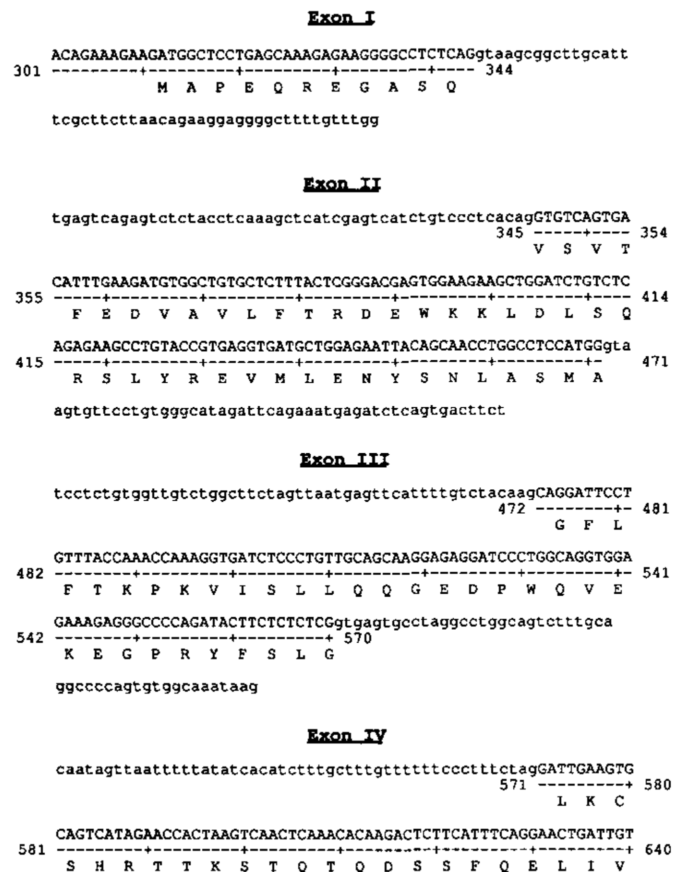

FIG. 2. Exon–intron boundaries of the *Kid-1* gene. Each of the exon–intron boundaries is presented. The numbers correspond to the numbered basepairs of the cDNA (Witzgall *et al.*, 1993). Nucleotides in the exons are in uppercase, whereas those in the introns are in lowercase. Encoded amino acids (in single-letter code) are shown below the coding region.

TABLE 1

Discordance between *Kid-1* and Rat Chromosomes in Somatic Hybrid Cell Lines

	Number of hybrid clones with *Kid-1*/chromosome retention				
Chromosome	+/+	−/−	+/−	−/+	Discordance (%)[a]
1	0	5	10	1	69
2	7	3	3	3	37.5
3	5	4	5	2	44
4	8	1	2	5	44
5	2	6	8	0	50
6	5	5	5	1	37.5
7	7	3	3	3	37.5
8	1	5	9	1	62.5
9	4	5	6	1	44
10	10	6	0	0	0
11	7	4	3	2	31
12	6	4	4	2	37.5
13	7	1	3	5	50
14	4	2	7	3	62.5
15	4	2	6	4	62.5
16	6	1	4	5	56
17	7	2	3	4	44
18	6	2	4	4	50
19	6	3	4	3	44
20	2	6	8	0	50
X	9	1	1	5	37.5
Y	0	6	10	0	62.5

[a] Discordance = [(+/−) + (−/+)]/total number of clones.

mRNAs that contain different numbers of zinc fingers (Hsu *et al.*, 1992; Zarkower and Hodgkin, 1992).

In a number of other zinc finger genes, all zinc fingers are encoded by one exon. The members of the *Egr-1* family of zinc finger genes (Crosby *et al.*, 1992; Rangnekar *et al.*, 1990; Tsai-Morris *et al.*, 1988) belong to this group as do *Krüppel* (Rosenberg *et al.*, 1986), XFG 5-1 (Köster *et al.*, 1991), Zfp-35 (Cunliffe *et al.*, 1990), ZFX (Schneider-Gädicke *et al.*, 1989), ZFY (North *et al.*, 1991), ZNF2 (Rosati *et al.*, 1991), *serendipity* beta (Vincent *et al.*, 1985), and CMPX1/ZNF6 (Lloyd *et al.*, 1991).

In some zinc finger proteins, the fingers are clustered into two groups. Kid-1 (4 and 9 fingers) is such a protein, as are the products of the *hunchback* (4 and 2 fingers) (Tautz *et al.*, 1987) and *serendipity* delta (6 and 1 fingers) (Vincent *et al.*, 1985) genes. In each case, this separation is not mirrored on the genomic level, at which finger-encoding regions are not separated by an intron.

Comparison of the Non-Zinc-Finger Region of the Kid-1 Gene with That of Other Zinc Finger Genes

Specificity of zinc finger proteins may be conferred by their zinc finger domains, which may interact with different binding sites, or by their non-zinc-finger regions, which may bind to specific proteins. It is estimated that approximately one-third of all zinc finger proteins contain a Krüppel-associated box consisting of an A and/or B domain (Bellefroid *et al.*, 1991). Kid-1 contains both of these regions, which are encoded by separate exons. Exon II of the *Kid-1* gene consists exclusively of the entire KRAB A encoding sequence, whereas the KRAB B encoding sequence lies on exon III together with additional non-zinc-finger sequence. A strikingly similar arrangement can be found in the human *ZNF2* gene (Rosati *et al.*, 1991) and in the human clone LDR152 (Constantinou-Deltas *et al.*, 1992). In each case, one exon contains all the sequence information for the KRAB A domain. A corresponding arrangement can be assumed for the human *ZNF43* gene, for which one mRNA species that is lacking the KRAB A region exists (Lovering and Trowsdale, 1991).

Thus, it appears that there are several subfamilies of zinc finger proteins, differing in the arrangement of their zinc fingers and/or in the organization of their non-zinc-finger regions. The zinc fingers may be tandemly repeated in one contiguous stretch or clustered in units of one or more fingers. These patterns are not necessarily mirrored on a genomic level. Individual zinc fingers may be encoded by separate exons (one exon–one finger), as is the case for TFIIIA, or whole zinc finger regions may lie on one exon only, e.g., as in the *Kid-1* gene. How these patterns emerged can only be a subject of speculation at present. In the process of evolution, an "ancestral zinc finger" exon may have been duplicated with or without a neighboring intron, thus giving rise to zinc finger-containing regions encoded by multiple exons or by one single exon.

Differential splicing may result in proteins with varied numbers of zinc fingers and/or differences in the non-zinc-finger region. In the former, exemplified by *CF2* (Hsu *et al.*, 1992), the DNA binding specificity of the protein would differ among the various forms. In the latter, the DNA binding specificity might be the same but the biological effects would be changed because of differences in the non-zinc-finger region. Potential candidates for transacting elements in the non-zinc-finger region are the KRAB domains. Since the KRAB A and B domains on the protein level are encoded by different exons on the genomic level, differentially spliced mRNAs would encode proteins with or without these domains. Whether proteins lacking these domains have modified function and whether there are differentially spliced forms of *Kid-1* with and without the KRAB domains have yet to be determined.

Chromosomal Localization of Kid-1

Three independent methods of genetic and physical mapping have resulted in the localization of *Kid-1* to rat chromosome 10. The gene was sublocalized to 10q21.3–q22 using fluorescence *in situ* hybridization. Until recently, this region has been devoid of markers. In two current publications of the rat gene map, however, 16 structural genes have been assigned to RNO10 (Levan *et al.*, 1991; Serikawa *et al.*, 1992). Of these, 15 belong to one large syntenic group homologous to mouse chromo-

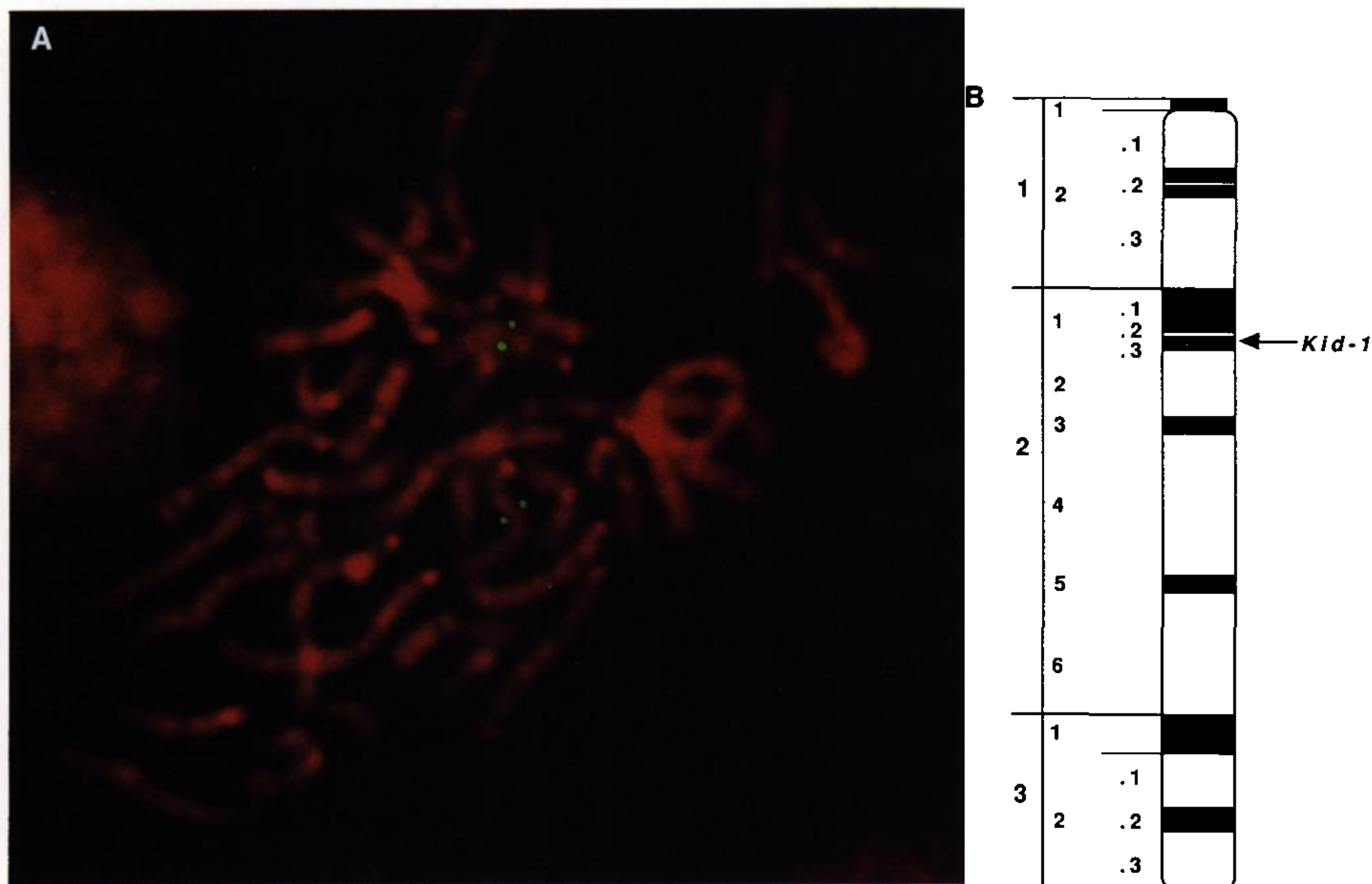

FIG. 3. (**A**) Chromosomal localization of *Kid-1* using fluorescence *in situ* hybridization. Signals were captured with a CCD camera and merged with the DAPI-stained image of the same metaphase using a computer workstation. Symmetrical fluorescent intensities on both homologs of RNO10 indicate the location of *Kid-1,* mapping it to band 10q21.3–q22. (**B**) Diagram of rat chromosome 10 illustrating the location of *Kid-1.*

some (MMU) 11 and human chromosome (HSA) 17. To our knowledge, all of the HSA17 loci currently mapped in the rat are located on RNO10. However, the centromeric third of this chromosome to which *Kid-1* has been mapped is not homologous to HSA17. Although in this study we did not directly map *Kid-1* in the human genome, its location is predicted from comparative analysis with the mouse map in which data are much more abundant. RNO10 and MMU11 share extensive homology, including a region proximal to the "HSA17" segment. Our data indicate that *Il3, Sparc,* and *Kid-1* are tightly linked on RNO10. These genes map >20 cM away from the Eker locus on RNO10 (Yeung *et al.,* 1993a).

In the mouse, *Il3* and *Sparc* map to a region 33 cM from the centromere of MMU11, separated by 1 cM, and this region is homologous to HSA5q (Buchberg *et al.,* 1992). In the human, *IL3* and *SPARC* map to 5q23–q31 and 5q31–q33, respectively (McAlpine *et al.,* 1989). Therefore, we infer that *Kid-1,* if belonging to this syntenic group, would localize near *Il3* and *Sparc* to MMU11 and to HSA5q. There is another gene, *Adra1b,* that maps to RNO10, MMU11, and HSA5q23–q32 (Levan *et al.,* 1991; Serikawa *et al.,* 1992). It is interesting that the distal end of HSA 5q includes genes encoding many growth factors such as colony stimulating factors 1 and 2, acidic fibroblast growth factor, and interleukins 4 and 5, as well as the genes encoding receptors for platelet-derived growth factor, colony stimulating factor 1, and the β-adrenergic and glucocorticoid receptors. In addition, it is of interest that allelic losses at chromosome 5q21 have been identified in human renal cell carcinomas (Morita *et al.,* 1991), raising the possibility that *Kid-1* may play a role in these tumors and act as a tumor-suppressor gene.

ACKNOWLEDGMENTS

The work was supported by National Institutes of Health Grants DK 39773, DK38452, and CA43211. Ralph Witzgall was supported in part by the Deutsche Forschungsgemeinschaft. We thank the following investigators for providing the DNA probes: Dr. M. Young, NIH (Bethesda, MD) (*Sparc*) and Dr. R. Scheller, Stanford (*Syb2*). The probe for *Il3* was obtained from the American Type Culture Collection.

REFERENCES

Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. (1987). "Current Protocols in Molecular Biology," Wiley, New York.

Bellefroid, E. J., Lecocq, P. J., Benhida, A., Pondelet, D. A., Belayew, A., and Martial, J. A. (1989). The human genome contains hundreds of genes coding for finger proteins of the *Krüppel* type. *DNA* **8:** 377–387.

Bellefroid, E. J., Poncelet, D. A., Lecocq, P. J., Revelant, O., and Martial, J. A. (1991). The evolutionarily conserved Krüppel-associated box domain defines a subfamily of eukaryotic multifingered proteins. *Proc. Natl. Acad. Sci. USA* **88:** 3608–3612.

Buchberg, A. M., Buckwalter, M. S., and Camper, S. A. (1992). Mouse chromosome 11. *Mamm. Genome.* **3:** S162–S181.

Constantinou-Deltas, C. D., Gilbert, J., Bartlett, R. J., Herbstreith, M., Roses, A. D., and Lee, J. E. (1992). The identification and characterization of KRAB-domain-containing zinc finger proteins. *Genomics* **12:** 581–589.

Crosby, S. D., Veile, R. A., Donis-Keller, H., Baraban, J. M., Bhat, R. V., Simburger, K. S., and Milbrandt, J. (1992). Neural-specific expression, genomic structure, and chromosomal localization of the gene encoding the zinc-finger transcription factor NGFI-C. *Proc. Natl. Acad. Sci. USA* **89:** 4739–4743.

Cunliffe, V., Williams, S., and Trowsdale, J. (1990). Genomic analysis of a mouse zinc finger gene, *Zfp-35*, that is upregulated during spermatogenesis. *Genomics* **8:** 331–339.

Eker, R., and Mossige, J. (1961). A dominant gene for renal adenomas in the rat. *Nature* **189:** 858–859.

Feinberg, A. P., and Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6–13.

Feinberg, A. P., and Vogelstein, B. (1985). Addendum: A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137:** 266–267.

Fortina, M. E., Lai, Z., and Rubin, G. M. (1991). The *Drosophila zfh-1* and *zfh-2* genes encode novel proteins containing both zinc-finger and homeodomain motifs. *Mech. Dev.* **34:** 113–122.

Hino, O., Klein-Szanto, A. J. P., Freed, J. J., Testa, J. R., Brown, D. Q., Vilensky, M., Yeung, R. S., Tartof, K. D., and Knudson, A. G. (1993). Spontaneous and radiation-induced renal tumors in the Eker rat model of dominantly inherited cancer. *Proc. Natl. Acad. Sci. USA* **90:** 327–331.

Hsu, T., Gogos, J. A., Kirsh, S. A., and Kafatos, F. C. (1992). Multiple zinc finger forms resulting from developmentally regulated alternative splicing of a transcription factor gene. *Science* **257:** 1946–1950.

Klug, A., and Rhodes, D. (1987). 'Zinc fingers': A novel protein motif for nucleic acid recognition. *Trends Biochem. Sci.* **12:** 464–469.

Köster, M., Kühn, U., Bouwmeester, T., Nietfeld, W., El-Baradi, T., Knöchel, W., and Pieler, T. (1991). Structure, expression and *in vitro* functional characterization of a novel RNA binding zinc finger protein from *Xenopus*. *EMBO J.* **10:** 3087–3093.

Levan, G., Szpirer, J., Szpirer, C., Klinga, K., Hanson, C., and Islam, M. Q. (1991). The gene map of the Norway rat (*Rattus norvegicus*) and comparative mapping with mouse and man. *Genomics* **10:** 699–718.

Lloyd, S. L., Sargent, C. A., Chalmers, J., Lim, E., Habeebu, S. S. M., and Affara, N. A. (1991). An X-linked zinc finger gene mapping to Xq21.1–q21.3 closely related to ZFX and ZFY: Possible origins from a common ancestral gene. *Nucleic Acids Res.* **19:** 4835–4841.

Lovering, R., and Trowsdale, J. (1991). A gene encoding 22 highly related zinc fingers is expressed in lymphoid cell lines. *Nucleic Acids Res.* **19:** 2921–2928.

McAlpine, P. J., Shows, T. B., Boucheix, C., Stranc, L. C., Berent, T. G., Pakstis, A. J., and Doute, R. C. (1989). Report of the nomenclature committee and the 1989 catalog of mapped genes, Human Gene Mapping 10. *Cytogenet. Cell Genet.* **51:** 13–66.

Mével-Ninio, M., Terracol, R., and Kafatos, F. C. (1991). The ovo gene of *Drosophila* encodes a zinc finger protein required for female germ development. *EMBO J.* **10:** 2259–2266.

Miller, J., McLachlan, A. D., and Klug, A. (1985). Repetitive zinc-binding domains in the protein transcription factor IIIA. *EMBO J.* **4:** 1609–1614.

Morita, R., Saito, S., Ishikawa, J., Ogawa, O., Yoshida, O., Yamakawa, K., and Nakamura, Y. (1991). Common regions of deletion of chromosomes 5q, 6q, and 10q in renal cell carcinoma. *Cancer Res.* **51:** 5817–5820.

North, M., Sargent, C., O'Brien, J., Taylor, K., Wolfe, J., Affara, N. A., and Ferguson-Smith, M. A. (1991). Comparison of ZFY and ZFX gene structure and analysis of alternative 3′ untranslated regions of ZFY. *Nucleic Acids Res.* **19:** 2579–2586.

Rangnekar, V. M., Aplin, A. C., and Sukhatme, V. P. (1990). The serum and TPA responsive promoter and intron–exon structure of *EGR2*, a human early growth response gene encoding a zinc finger protein. *Nucleic Acids Res.* **18:** 2749–2757.

Rosati, M., Marino, M., Franzé, A., Tramontano, A., and Grimaldi, G. (1991). Members of the zinc finger protein gene family sharing a conserved N-terminal module. *Nucleic Acids Res.* **19:** 5661–5667.

Rosenberg, U. B., Schröder, C., Preiss, A., Kienlin, A., Coté, S., Riede, I., and Jäckle, H. (1986). Structural homology of the product of the *Drosophila Krüppel* gene with *Xenopus* transcription factor IIIA. *Nature* **319:** 336–339.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). "Molecular Cloning: A Laboratory Manual," 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463–5467.

Schneider-Gädicke, A., Beer-Romero, P., Brown, L., Mardon, F., Luoh, S.-W., and Page, D. C. (1989). Putative transcription activator with alternative isoforms encoded by human ZFX gene. *Nature* **342:** 708–711.

Serikawa, T., Kuramoto, T., Hilbert, P., Mori, M., Yamada, J., Dubay, C. J., Lindpainter, K., Ganten, D., Guénet, J.-L., Lathrop, G. M., and Beckmann, J. S. (1992). Rat gene mapping using PCR-analyzed microsatellites. *Genetics* **131:** 701–721.

Shapiro, M. B., and Senapathy, P. (1987). RNA splice junctions of different classes of eukaryotes: Sequence statistics and functional implications in gene expression. *Nucleic Acids Res.* **15:** 7155–7174.

Smith, C. W. J., Patton, J. G., and Nadal-Gerard, B. (1989). Alternative splicing in the control of gene expression. *Annu. Rev. Genet.* **23:** 527–577.

Szpirer, J., Levan, G., Thörn, M., and Szpirer, C. (1984). Gene mapping in the rat by mouse-rat cell hybridization: Synteny of the albumin and α-fetoprotein genes and assignment to chromosome 14. *Cytogenet. Cell Genet.* **38:** 142–149.

Tautz, D., Lehmann, R., Schnürch, H., Schuh, R., Seifert, E., Kienlin, A., Jones, K., and Jäckle, H. (1987). Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes. *Nature* **327:** 383–389.

Tsai-Morris, C.-H., Cao, X., and Sukhatme, V. P. (1988). 5′ flanking sequence and genomic structure of Egr-1, a murine mitogen inducible zinc finger encoding gene. *Nucleic Acids Res.* **16:** 8835–8846.

Tso, J. Y., Van Den Berg, D. J., and Korn, L. J. (1986). Structure of the gene for *Xenopus* transcription factor TFIIIA. *Nucleic Acids Res.* **14:** 2187–2200.

Vincent, A., Colot, H. V., and Rosbash, M. (1985). Sequence and structure of the Serendipity locus of Drosophila melanogaster. *J. Mol. Biol.* **186:** 149–166.

Weber, J. L. (1990). Informativeness of human $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ polymorphisms. *Genomics* **7:** 524–530.

Witzgall, R., O'Leary, E., Gessner, R., Ouellette, A. J., and Bonventre, J. V. (1993). Kid-1, a putative renal transcription factor: Regulation during ontogeny, and in response to ischemia and toxic injury. *Mol. Cell. Biol.* **13:** 1933–1942.

Yeung, R. S., Buetow, K. H., Testa, J. R., and Knudson, A. G., Jr. (1993a). Susceptibility to renal carcinoma in the Eker rat involves a tumor suppressor gene on chromosome 10. *Proc. Natl. Acad. Sci. USA* **90:** 8038–8042.

Yeung, R. S., Taguchi, T., Patriotis, C., Markis, A., Tsichlis, P. N., Levan, K. K., Levan, G., Tartof, K., Hino, O., Knudson, A. G., and Testa, J. R. (1993b). New markers, D16FC1 and Tp12, differentiate between rat chromosomes 16 and 17. *Cytogenet. Cell Genet.* **62:** 149–152.

Zarkower, D., and Hodgkin, J. (1992). Molecular analysis of the C. elegans sex-determining gene *tra-1:* A gene encoding two zinc finger proteins. *Cell* **70:** 237–249.