ELSEVIER

Gene 240 (1999) 13–22

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

www.elsevier.com/locate/gene

Characterization of the mouse *Kid1* gene and identification of a highly related gene, *Kid2* ☆

Nicoletta Tekki-Kessaris [a], Joseph V. Bonventre [b], Catherine A. Boulter [a,*]

[a] *University of Cambridge, Department of Genetics, Downing Street, Cambridge CB2 3EH, UK*
[b] *Medical Services, Massachusetts General Hospital and Department of Medicine, Harvard Medical School, Charlestown, MA 02129, USA*

Received 28 June 1999; received in revised form 15 September 1999; accepted 29 September 1999
Received by R. Di Lauro

Abstract

Kid1 encodes a zinc finger protein that has been implicated in renal cell differentiation. Levels of *Kid1* mRNA correlate with maturation of kidney tubule epithelia in rat post-natal kidney development and during kidney regeneration following injury. KID1 is a putative transcriptional repressor, containing a KRAB domain at its amino terminus that mediates transcriptional repression in transient cell transfection assays when fused to a heterologous DNA-binding domain. In this paper, we describe the isolation and characterization of the mouse homologue of *Kid1* and the identification of a novel highly related mouse gene, *Kid2*, *Kid1* and *Kid2* are tightly linked on mouse chromosome 11 and show conservation across mammals. Both genes are expressed predominantly in the mouse adult kidney and brain, but transcripts are also detected in embryonic brain, kidney, gut and lung, suggesting an additional role for these genes during mouse development. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Kidney; KRAB; Transcription factor; Zinc finger

1. Introduction

Kid1 encodes a zinc finger protein that has been implicated in the differentiation of renal epithelial cells (Witzgall et al., 1993, 1998; Bonventre and Witzgall, 1995). It was originally identified as a gene whose expression is regulated during kidney regeneration following injury, a process that is thought to involve de-differentiation, proliferation and subsequent differentiation of surviving renal epithelial cells (Witzgall et al., 1993). *Kid1* transcripts are detected predominantly in the adult rat kidney where they accumulate during post-natal kidney development (Witzgall et al., 1993). Following experimental renal injury in the rat induced by ischemia or folic acid administration, the levels of *Kid1* mRNA decline (Witzgall et al., 1993). The KID1 protein has been localized mainly in the nuclei of proximal tubule epithelial cells (Witzgall et al., 1998). This expression is downregulated in rodent polycystic kidney disease, a condition characterized by proliferating immature epithelia (Witzgall et al., 1998). The correlation of expression of *Kid1* with post-natal maturation of kidney tubule epithelia and the downregulation in immature proliferating cells has led to the hypothesis that KID1 is involved in the differentiation of renal proximal tubule epithelial cells (Witzgall et al., 1998).

The *Kid1* gene encodes a protein that contains at the carboxy terminus 13 zinc fingers of the C_2H_2 class of transcription factors (Witzgall et al., 1993). At the amino terminus is a highly conserved *Krüppel*-associated box (KRAB) domain that has been shown to mediate transcriptional repression in transient transfection assays using cultured cell lines (Margolin et al., 1994; Pengue et al., 1994; Witzgall et al., 1994). Lying between the KRAB domain and the zinc fingers is a spacer region that contains a number of putative phosphorylation sites for casein kinase II. These sites, together with target sites for cAMP-dependent protein kinase and protein kinase C present elsewhere in the KID1 protein,

Abbreviations: CKII, casein kinase II; KRAB, *Krüppel*-associated box; NLS, nuclear localization signal; RT-PCR, reverse transcription-polymerase chain reaction; TYR, tyrosine kinase; UTR, untranslated region; ZF, zinc finger; ZFP, zinc finger protein.

☆ Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos AF184111 and AF184112.

* Corresponding author. Tel.: +44-1223-333932; fax: +44-1223-333992.
E-mail address: cb@mole.bio.cam.ac.uk (C.A. Boulter)

0378-1119/99/$ - see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0378-1119(99)00440-0

suggest that KID1 protein activity may be modulated by phosphorylation (Witzgall et al., 1993). Within the spacer region is found a 12-amino-acid motif shared by members of the *Raf* family of serine/threonine kinases, suggesting that KID1 may exhibit kinase activity (Witzgall et al., 1993). The presence of an mRNA instability sequence in the 3′ untranslated region (UTR) of the *Kid1* mRNA and a predicted PEST protein instability region in KID1 suggest that both mRNA and protein may be subject to rapid degradation (Witzgall et al., 1993).

The KRAB domain is thought to be one of the most widely distributed transcriptional repression domains identified in mammals. It was identified as a 75-amino-acid region of homology present exclusively at the amino terminus of one-third of the *Krüppel*-type zinc finger proteins encoded in the human genome (Bellefroid et al., 1991). The KRAB region can be subdivided into two boxes, KRAB A and KRAB B, based on their separation or their absence from individual proteins (Bellefroid et al., 1991). The ability of the KRAB domain to exert transcriptional repression when fused to a heterologous DNA-binding domain has been demonstrated for several different KRAB regions and has been attributed to the 45-amino-acid KRAB A box (Margolin et al., 1994; Witzgall et al., 1994).

The mechanism by which KRAB-zinc finger proteins effect transcriptional repression in vivo is still unclear. Cell culture experiments have shown that the KRAB A domain can exert transcriptional repression of TATA-box-containing basal or activated promoters in a distance-independent manner when fused to a heterologous DNA-binding domain (Margolin et al., 1994; Pengue et al., 1994; Witzgall et al., 1994; Pengue and Lania, 1996). A ubiquitously expressed effector protein that interacts with the KRAB A domain has been identified as TIF1, or KAP-1 and the mouse homologue KRIP-1 (Friedman et al., 1996; Kim et al., 1996; Moosmann et al., 1996). TIF1 proteins have been shown to interact in vitro with the putative heterochromatin-associated proteins HP1a and MOD1, suggesting that in some cases, KRAB-mediated transcriptional repression may involve remodelling of heterochromatin-like structures (Le Douarin et al., 1996; Moosmann et al., 1996). Although no specific DNA recognition sequence has yet been identified for any KRAB-zinc finger protein, the zinc fingers of KID1 have been shown to recognize heteroduplex DNA and to localize KID1 in the nucleolus (Elser et al., 1997, Huang et al., 1999).

In this paper, we describe the characterization of the mouse *Kid1* gene and the identification of a novel highly related gene, *Kid2*. We compare their predicted protein sequences and their genomic organization, and demonstrate their evolutionary conservation in mammals. Chromosomal mapping has shown that the two genes are closely linked on mouse chromosome 11. *Kid1* and *Kid2* are expressed in the same wide range of adult mouse tissues, with transcripts being found predominantly in adult kidney and brain. Transcripts of both genes are also detected in a number of embryonic tissues, suggesting an additional role for *Kid1* and *Kid2* during mouse development.

2. Materials and methods

2.1. Library screening

For the isolation of the mouse *Kid1* and *Kid2* genes, bacteriophage λ genomic libraries were screened according to standard protocols (Sambrook et al., 1989). The probe used to screen the library for the presence of *Kid1* was a 217 bp *DdeI* fragment isolated from the rat *Kid1* cDNA (Accession No. M96548). This fragment contains sequences from the 5′ end of exon V encoding the spacer region of KID1 and excluding any zinc fingers (Witzgall et al., 1993). For the isolation of the murine *Kid2* gene, the library was screened with a 585 bp fragment from the rat *Kid1* cDNA, which spanned the first 195 amino acids of the rat KID1 protein, thereby including the KRAB A-encoding region. Probes were prepared using the Rediprime random prime labelling system (Pharmacia Biotech) and purified through Sephadex G-50 Nick columns (Pharmacia Biotech). Hybridization of the Hybond-N+ filters (Amersham) was carried out at 65°C according to the manufacturer's instructions. Positive clones were characterized by restriction mapping and Southern analysis using exon-specific probes from the rat *Kid1* cDNA.

2.2. Southern and Northern Blot analysis

For Southern analysis of genomic DNA, 20 μg of DNA (2 μg for *Drosophila* and *C. elegans*) were digested and subjected to electrophoresis in 0.8% agarose. Southern blotting was carried out on GeneScreen Plus membranes (Du Pont) (Sambrook et al., 1989), and hybridization was performed at 65°C according to the manufacturer's instructions. The probes were prepared by random priming as described above. Autoradiography was carried out for a period of 7–14 days at −70°C with two intensifying screens.

For Northern blot analysis, total RNA was extracted from mouse embryonic and adult tissues by the single-step acid guanidinium thiocyanate–phenol–chloroform extraction method (Chomczynski and Sacchi, 1987). Poly$(A)^+$ RNA was selected by chromatography through oligo (dT) cellulose columns (Sambrook et al., 1989). For Northern analysis, 15 μg of poly$(A)^+$-selected RNA was subjected to denaturing electrophoresis through 1% agarose gels containing 6% formaldehyde and transferred to GeneScreen Plus membranes accord-

ing to Sambrook et al. (1989). Hybridization was performed at 42°C in a buffer containing 50% formamide, 1% SDS, 5× SSC, 1× Denhardt's solution, 20 mM sodium phosphate, 10% dextran sulphate (Pharmacia) and 100 μg/ml of salmon sperm DNA using random primed ^{32}P-labelled probes.

2.3. DNA sequencing and analysis

DNA sequencing was performed on plasmid subclones using a BigDye Terminator Cycle Sequencing Kit with AmpliTaq DNA polymerase (PE Applied Biosystems) and analysed on an ABI PRISM 377 DNA sequencer. Sequence analysis was performed using the Wisconsin package version 10.0 from the Genetics Computer Group (GCG), Madison, Wisc. The subcellular location of the proteins and the various motifs present were predicted using the PSORT II (http://psort.nibb.ac.jp), NNPSL (http://predict.sanger.ac.uk), MotifFinder (http://www.motif.genome.ad.jp) and PESTfind (http://www.at.embnet.org) on-line computer programs.

3. Results

3.1. Isolation of the mouse Kid1 gene

Mouse genomic clones spanning the *Kid1* gene were isolated from a 129/Sv bacteriophage λ library screened with a 217 bp *DdeI* fragment from the rat *Kid1* cDNA as a probe (Accession No. M96548). This fragment contains sequences from the 5′ end of exon V encoding the spacer region of KID1 and excluding any zinc fingers (Witzgall et al., 1993). Southern analysis of mouse genomic DNA showed that this probe hybridizes to single copy sequences in the mouse genome and is therefore specific for the *Kid1* gene (data not shown). From 1.25×10^6 plaques screened, five λ clones that hybridized to this probe were isolated and purified to homogeneity. Two of these, λ1Kid1 and λ4Kid1, were subcloned and characterized in detail. The positions of conserved exons between the rat and mouse homologues was determined by Southern analysis and hybridization with exon-specific cDNA fragments from the rat *Kid1* gene. This was then confirmed by DNA sequencing of the relevant genomic fragments. The organization of the mouse *Kid1* gene is shown in Fig. 1A. Exons I, II, III and IV are clustered together and are separated from exon V by a 7.5 kb intron. This arrangement is conserved with that of the rat homologue (Witzgall et al., 1994).

On the basis of homology to the rat gene, a 1716 bp mouse *Kid1* open reading frame was assembled from genomic sequences. The coding sequence and the positions of exon/intron boundaries were confirmed by reverse transcription-polymerase chain reaction (RT-PCR) amplification of *Kid1* cDNA fragments from adult and embryonic mouse kidney RNA (strain 129/Sv), using primers made to the 5′ and 3′ ends of the coding sequence (data not shown). The 5′ untranslated region (UTR) of the transcript was identified by characterization of a *Kid1* cDNA isolated from a mouse embryonic day 15.5 cDNA library (GenBank Accession No. AF18111), and its position was determined on the genomic λ clones (see Fig. 1A). The 3′ UTR sequenced to date is 576 bp in length, and a putative polyadenylation signal has been identified 541 bp downstream of the stop codon. This is in agreement with a recently published mouse *Kid1* cDNA isolated from a new-born lens library (Brady et al., 1997).

3.2. Identification of a mouse Kid2 gene

Previous studies had shown that KRAB-containing zinc finger protein (KRAB-ZFP) genes very often occur in clusters in the human genome and that such clusters consist of families of highly related genes (Bellefroid et al., 1993; Shannon et al., 1996; Shannon and Stubbs,

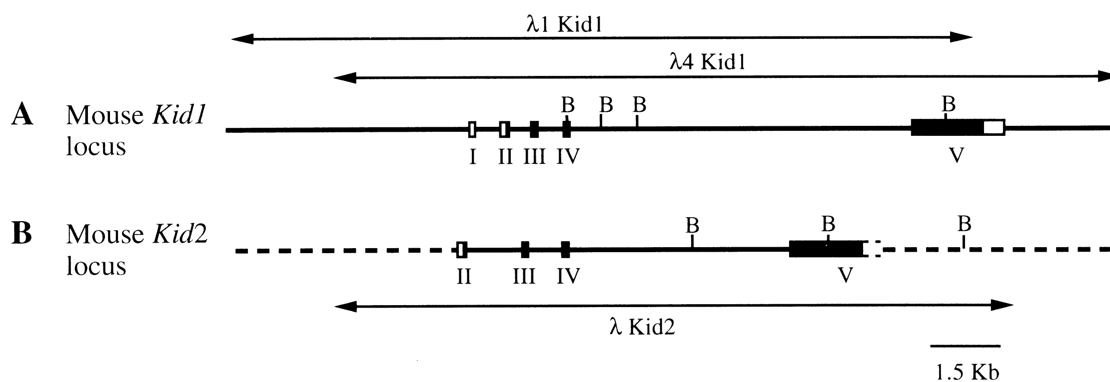

Fig. 1. (**A**) Genomic map of the mouse *Kid1* locus. The positions of exons I–V are shown as boxes. The genomic regions spanned by each of the two bacteriophage λ clones used in the analysis of the locus are indicated above. (**B**) Genomic map of the mouse *Kid2* locus. The positions of exons II–V and of the bacteriophage λ clone used in the analysis are shown. Coding exons are indicated as black boxes. B, *Bam*HI.

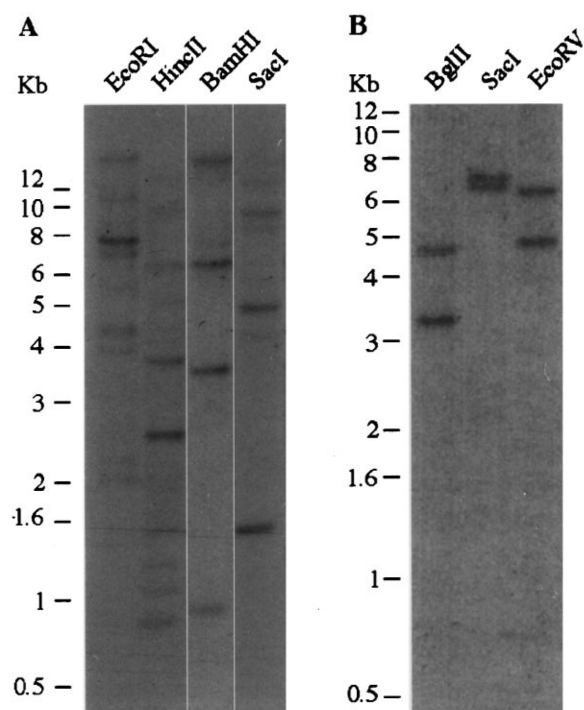

Fig. 2. Southern analysis of mouse genomic DNA digested with several enzymes and hybridized with rat *Kid1* cDNA probes encoding (**A**) the KRAB A region and (**B**) the zinc finger region. The KRAB A-specific probe detects more than one band in the mouse genome, suggesting that there exists a small group of genes highly similar to *Kid1* in this region. The zinc finger probe detects two bands in the mouse genome, indicating that there are only two genes that are highly homologous to *Kid1* in this region.

1998). The extent of similarity between the different members of the family, as well as the extent of conservation of the individual family members across species, is variable. In order to look for genes in the mouse genome that might be related to *Kid1*, cDNA fragments encoding the KRAB A (nucleotides 306–471 from the rat *Kid1* cDNA) and the zinc finger region (nucleotides 1262–1911) of the rat *Kid1* gene were used as probes on Southern blots containing mouse genomic DNA digested with several restriction enzymes. The results are shown in Fig. 2. Under high stringency hybridization conditions, the KRAB A-encoding fragment of the rat *Kid1* cDNA hybridizes to several bands in the mouse genome, indicating the possible existence of a small number of genes highly homologous to *Kid1* in this region (Fig. 2A). The zinc finger-encoding fragment hybridizes to two genomic DNA bands with approximately equal intensity, suggesting that there exists a second gene in the mouse genome that may encode zinc fingers that are very similar to those of *Kid1* (Fig. 2B). In order to isolate this gene, a genomic library from mouse strain 129 was screened with a 585 bp probe spanning the first 195 amino acids of the rat KID1 protein. Ten λ clones hybridizing to this probe were identified, of which one was characterized further. Southern analysis of DNA from this genomic clone using rat *Kid1* exon-specific fragments as probes showed that all four coding exons in *Kid1* hybridized to this clone. However, DNA sequencing of the regions hybridizing to the *Kid1* probes confirmed that this clone corresponded not to *Kid1* but to a novel gene highly homologous to *Kid1*. This was called *Kid2*. Fig. 1B shows the genomic organization of *Kid2*. The KRAB A and KRAB B boxes are encoded by different exons and are separated from the exon encoding the zinc fingers by a large intron, an organization that is very similar to that of *Kid1*.

A 1806 bp ORF encoding a predicted 602 amino acid protein was assembled from genomic regions hybridizing to *Kid1* cDNA fragments and was confirmed by RT-PCR analysis and DNA sequencing of the amplified products (data not shown). The DNA sequence has been deposited in the GenBank database (Accession No. AF18112). Although the 5′ untranslated (UTR) region of *Kid2* has not been identified, for ease of comparison to *Kid1*, we refer to the first identified coding exon as exon II. The position of the translation initiation codon is conserved between *Kid1* and *Kid2* and in both genes is in agreement with the Kozak consensus with a purine residue in position −3 (Kozak, 1984). Exons III and IV encode the KRAB A and KRAB B boxes, respectively. Exon V encodes 13 zinc fingers in two groups of four and nine and a spacer region separating the KRAB domains from the zinc fingers. A translation stop codon has been identified in exon V downstream of the last zinc finger, and a putative polyadenylation signal is located 433 bp downstream of the translation stop codon.

3.3. *Sequence comparisons of the predicted KID1 and KID2 proteins*

Comparison of the mouse KID1 and KID2 predicted amino acid sequences shows a striking conservation between the two (Fig. 3). The results are summarised in Table 1. Both KRAB regions and, in particular, KRAB A, are highly conserved (97.6 and 90.0% amino acid sequence identity in KRAB A and KRAB B, respectively). The zinc finger regions of KID1 and KID2 are very similar, both at the level of amino acid sequence and in the organization of the zinc fingers in two groups of four and nine. The highest homology between the two proteins is observed in the group of nine zinc fingers with the first six of these being 100% identical. The linker region separating the two groups of zinc fingers is also highly conserved in both length and amino acid constitution and shows conservation of the putative cAMP-dependent protein kinase and protein kinase C phosphorylation sites present in KID1 (Witzgall et al., 1993). However, KID1 and KID2 differ markedly in the spacer region between the KRAB B domain and the zinc fingers, showing an amino acid similarity of only

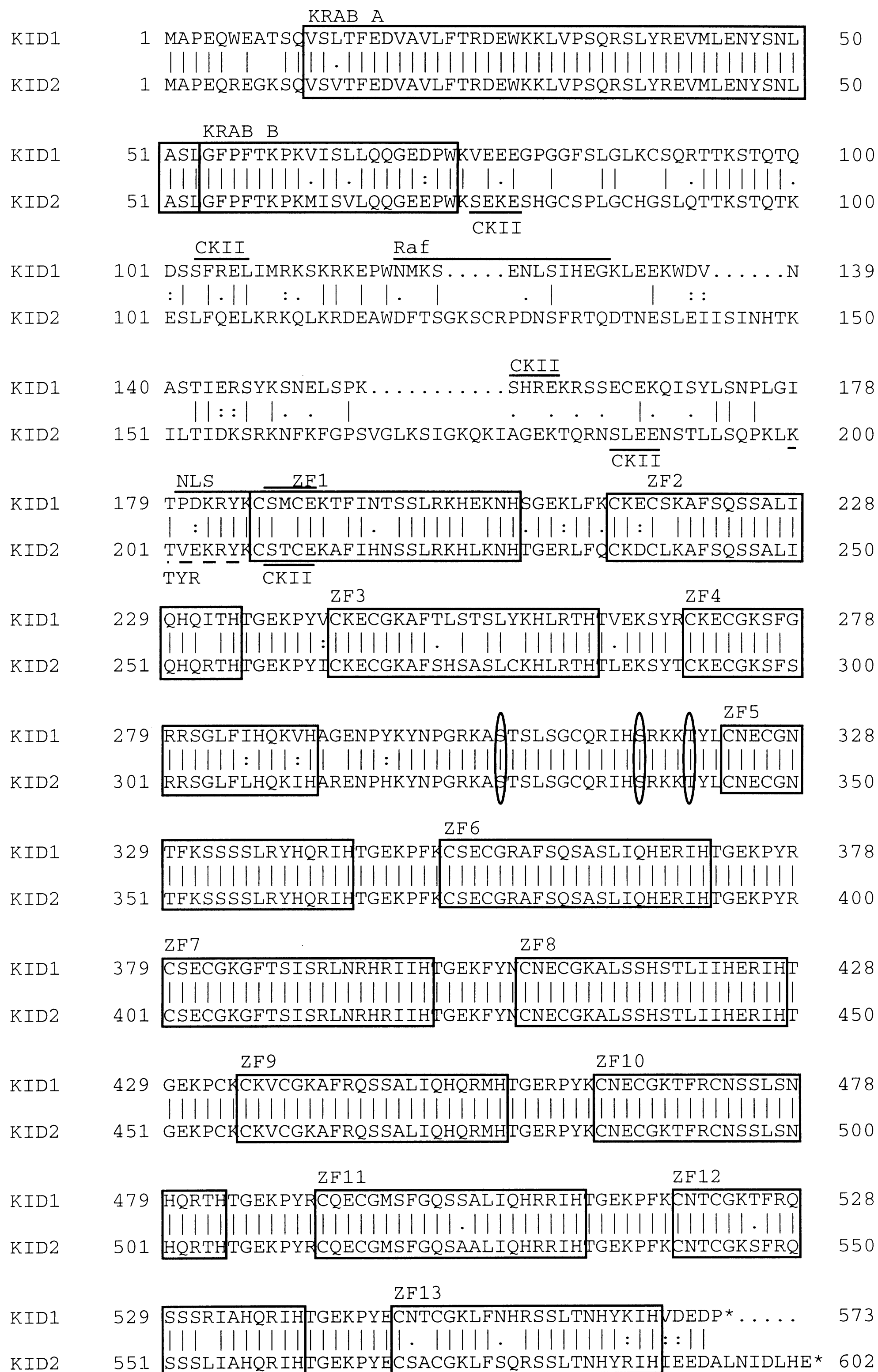

Fig. 3. Comparison of the mouse KID1 and KID2 predicted amino acid sequences. The positions of the KRAB A and B boxes, the zinc fingers (ZF1–ZF13) and *Raf* homology domain, putative phosphorylation sites for Casein kinase II (CKII) and tyrosine kinase (TYR) are indicated. The threonine and two serine residues that may be sites for phosphorylation by cAMP-dependent protein kinase and protein kinase C are shown in circles. The 'spacer' regions span amino acids 74–185 in KID1 and 74–207 in KID2. The 'linker' regions span amino acids 291–322 in KID1 and 313–344 in KID2. KID1 and KID2 are highly homologous in their KRAB zinc finger regions but differ markedly in their spacer region.

Table 1
Amino acid similarities between KID1 and KID2

Amino acid region[a]	KID1 vs. KID2[b] (%)
KRAB A	97.6
KRAB B	90.0
Spacer	32.4
Zinc fingers	93.7

[a] See Fig. 3.
[b] Percentage similarity between KID1 and kID2.

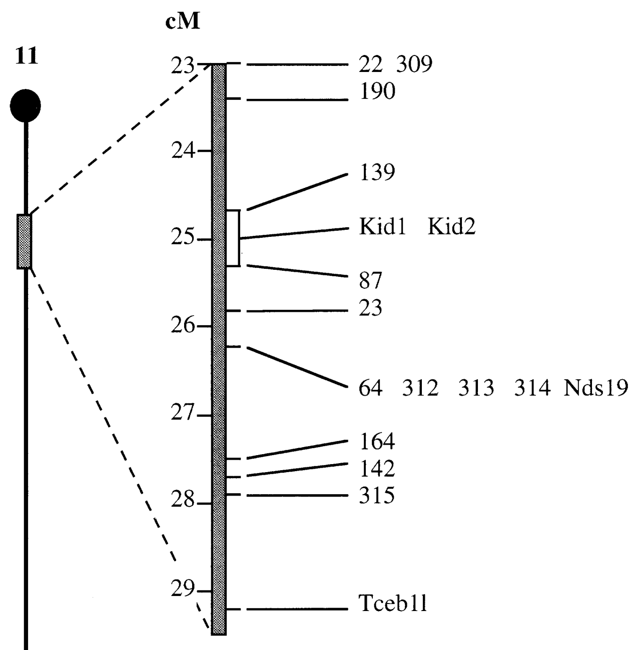

Fig. 4. Localization of the *Kid1* and *Kid2* genes on mouse chromosome 11, between markers D11Mit139 and D11Mit87. The positions of markers correspond to the EUCIB (BSB) mouse chromosome 11 linkage map.

32.4%. In the rat KID1 protein, this region has been reported to encode a putative PEST protein instability sequence and a *Raf* homology domain (Witzgall et al., 1993). Sequence interpretation analysis indicates that, while poor PEST sequences are present in KID1 and KID2, KID2 lacks a putative *Raf* homology domain. Three putative casein kinase II phosphorylation sites are present within the spacer region and the first zinc finger of KID2. In addition, KID2 contains a putative tyrosine kinase phosphorylation site immediately upstream of the first zinc finger. Subcellular localization analysis of the KID1 and KID2 amino acid sequences predicts a nuclear localization for both proteins, with KID1 containing an identifiable SV40-like nuclear localization signal (NLS) upstream of the first zinc finger. For both genes, the DNA fragments encoding the spacer regions of the proteins hybridize to single-copy sequences in the mouse genome, and these fragments can be used as probes to distinguish the *Kid1* and *Kid2* genes.

3.4. Chromosomal mapping of Kid1 and Kid2

To determine the chromosomal positions of *Kid1* and *Kid2*, mapping was performed using the EUCIB *Mus musculus*/*Mus spretus* backcross facility (Breen and the European Backcross Collaborative Group, 1994; Rhodes et al., 1998). The *M. musculus* and *M. spretus* alleles were distinguished by PCR and restriction enzyme digestion of the PCR products. Data are available on http://www.hgmp.mrc.ac.uk/MBx/MBxHomepage.html. A panel of 50 random backcross samples localized *Kid1* and *Kid2* to chromosome 11. A further 63 samples that span the region between the markers D11Mit218 and D11Mit26 were then screened. Both *Kid1* and *Kid2* map between the markers D11Mit139 and D11Mit87 and show 12.5 and 14.29% recombination frequency with the primary anchor locus D11Nds19, respectively (lod scores of 9.89 and 8.6, respectively) (Fig. 4). This is a region of conserved synteny with human chromosome 5 and with rat chromosome 10 to which the rat *Kid1* gene has been mapped (Witzgall et al., 1994). The high degree of similarity of *Kid1* and *Kid2* at the level of genomic organization and exon DNA sequence, together with their tight linkage on mouse chromosome 11, argues that the two genes arose by gene duplication.

3.5. Kid1 and Kid2 are conserved in mammals

To determine whether *Kid1* and *Kid2* have been conserved during evolution, gene-specific probes encoding the spacer regions were used in a zoo-blot analysis, under high stringency conditions. Whilst neither probe hybridized to genomic DNA from chicken, frog, *Drosophila* or *C. elegans* under these conditions, *Kid1* and *Kid2* can be detected in mouse, rat, human, monkey, sheep and cat, with *Kid2* also hybridizing to seal DNA (Fig. 5).

3.6. Kid1 and Kid2 are expressed in the embryo and adult

The tissue and developmental expression of *Kid1* and *Kid2* was analysed by Northern blot analysis of poly(A)$^{+}$ RNA and hybridization with probes specific for each gene (Fig. 6). In adult tissues, the *Kid1* probe detects a major transcript of approximately 3 kb in length and a larger, less abundant transcript of approximately 4 kb in length (Fig. 6A). Brain and kidney appear to be the major sites of expression, although transcripts can also be detected at a low level in liver, lung, muscle, heart, testis, tongue and eye. The *Kid2* probe hybridizes to a single band of approximately 3 kb in length (Fig. 6C) in a pattern identical to that of *Kid1*.

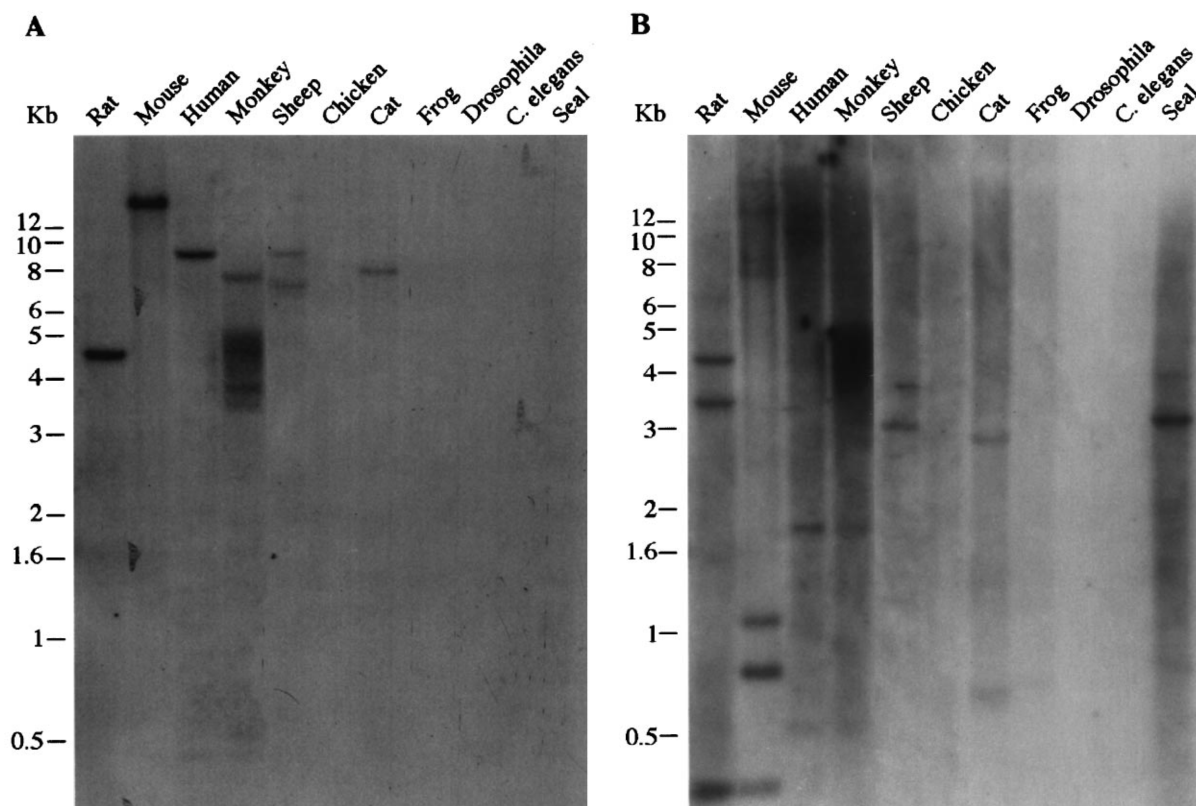

Fig. 5. Zoo-blot analysis of the *Kid1* and *Kid2* genes. A zoo blot was hybridized with the spacer-encoding region of *Kid1* (**A**) and *Kid2* (**B**). The two genes show conservation across a number of mammals.

In adult testis, two bands hybridize to the *Kid2* probe (Fig. 6C).

The expression of *Kid1* and *Kid2* is not confined to adult tissues. Northern blot analysis shows that both genes are expressed in mouse embryonic E16.5 tissues with a similar expression pattern, being detected in E16.5 brain, heart, lung, kidney and gut (Fig. 6E and G). As in adult tissues, two *Kid1* transcripts are found; no major differences are observed in the ratio of these transcripts between different tissues in the embryo or adult.

4. Discussion

In the present study, we report the identification and characterization of *Kid2*, a novel mouse gene that is highly related to the zinc finger gene *Kid1*. The two genes encode typical KRAB-containing zinc finger proteins, which show striking similarities in their KRAB domains and zinc finger regions, but which diverge significantly in their spacer regions. The two genes are conserved in several mammalian species and show overlapping expression patterns in embryonic and adult mouse tissues. The data suggest that *Kid1* and *Kid2* may share common targets inside the cell but may be themselves differentially regulated.

The *Kid2* gene encodes 13 zinc fingers at the carboxy terminus, which are arranged in two groups of four and nine, an organization identical to that of *Kid1*. Comparison of the predicted zinc finger domains of KID1 and KID2 shows a striking conservation in all 13 zinc fingers. Fingers 5–10 show 100% identity, suggesting that putative common cellular targets for the two genes may be recognized through these fingers. High conservation is also observed not only in the arrangement of the zinc fingers in two groups but also in the length and amino acid composition of the linker sequence separating the two groups, arguing for a putative functional significance of this region within the protein.

At the carboxy terminus, KID2 contains the two highly conserved KRAB A and KRAB B boxes. Transient transfection assays using cultured cell lines have shown that KRAB A domains mediate transcriptional repression when fused to a heterologous DNA-binding domain and that this repression may involve protein–protein interactions with other cellular corepressors (Margolin et al., 1994; Pengue et al., 1994; Witzgall et al., 1994; Friedman et al., 1996; Kim et al., 1996; Moosmann et al., 1996; Pengue and Lania, 1996). KID1 and KID2 both contain a KRAB A box that shows a remarkable 97.6% similarity between the two proteins, suggesting that there may exist other cellular factors

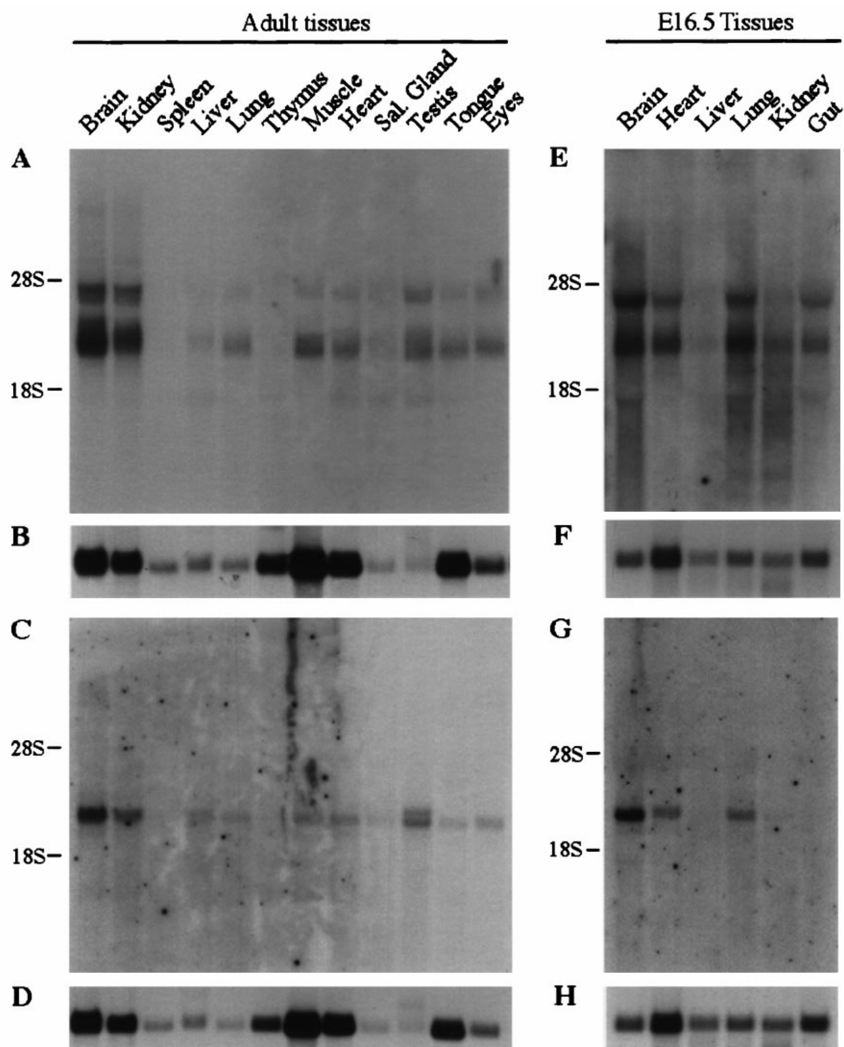

Fig. 6. Northern analysis of RNA isolated from adult and embryonic E16.5 mouse tissues and hybridized with gene-specific probes for *Kid1* (**A**, **E**), *Kid2* (**C**, **G**) and *Gapdh* (**B**, **D**, **F** and **H**). The positions of the 28S and 18S RNAs are indicated. The *Kid1* probe detects a major transcript of approximately 3 kb in length and a larger, less abundant transcript of approximately 4 kb in length. The *Kid2* probe hybridizes to a single band of approximately 3 kb in length. In adult testis, two bands hybridize to the *Kid2* probe. *Kid1* and *Kid2* show overlapping expression patterns.

that may interact specifically with KID-like KRAB domains, thereby exerting additional specificity to the mechanism of KRAB-mediated repression. KID1 and KID2 also show a high conservation in their KRAB B domains, the function of which remains unknown.

The KRAB domains in KID2 are separated from the zinc fingers by a 400 bp spacer region. This region in KID1, as well as the shorter region separating zinc fingers 4 and 5, encode various putative protein regulatory elements, suggesting that the KID1 protein may have a short half-life inside the cell, may show potential kinase activity and may itself be controlled by phosphorylation by casein kinase II, cAMP-dependent protein kinase and protein kinase C (Witzgall et al., 1993). All three types of phosphorylation sites present in KID1 are also present in KID2 with the addition of a phosphorylation site for tyrosine kinase, suggesting that the activity of KID2 may be differentially regulated. However, neither a strong PEST protein instability sequence nor a *Raf* homology domain has been identified in KID2. This suggests that KID2 may be more stable inside the cell, and the absence of a putative kinase domain may indicate that this protein does not have a *Raf*-type kinase activity to phosphorylate and possibly regulate other proteins.

Chromosomal mapping and zoo-blot analysis revealed that *Kid1* and *Kid2* are tightly linked on chromosome 11, and both genes show conservation across mammals including mouse, rat human, monkey, sheep and cat. The tight linkage of the two *Kid* genes on the chromosome, the high degree of identity in the KRAB and zinc finger encoding regions, and the divergence in the spacer region suggest that, following possible duplication of an ancestral gene, the spacer-encoding region

may have diverged within species due to a lack of a functional domain encoded by this region. However, the conservation of this spacer region of each gene across several mammalian species indicates that selection pressure has maintained this sequence during evolution, suggesting that KID1 and KID2 may have acquired and maintained distinct functions inside the cell. Chromosomal clustering of KRAB-ZFP genes is common and has been previously reported for several subgroups of genes, including two subgroups of genes on human chromosome 19 (Bellefroid et al., 1993; Shannon et al., 1996). However, the extent of conservation of the individual members of each group across species and the extent of similarity amongst the members of each group within species appears to be highly variable (Bellefroid et al., 1993; Shannon et al., 1996; Shannon and Stubbs, 1998). In the case of *Kid1*, a human gene homologous to the rat *Kid1* gene has been identified on human chromosome 5 (Omori et al., 1997; Jacob et al., 1998). However, whether this gene corresponds to the human homologue of *Kid1* or to another related gene remains to be determined.

Expression analysis of the mouse *Kid1* and *Kid2* genes has shown that the two have overlapping patterns of expression in the embryo, as well as in the adult. Both genes are expressed very highly in adult brain and kidney and at lower levels in other tissues. In the embryo, the two genes show a broad pattern of expression in all tissues examined except liver, suggesting a role for these genes during embryonic development. In adult rat kidney, KID1 has been localized to proximal tubule epithelial cells, where it has been postulated to be involved in terminal differentiation (Witzgall et al., 1993). Our expression data showing *Kid1* transcripts in mouse embryonic kidney suggest an additional role for this gene during nephrogenesis. The overlapping tissue distribution of *Kid1* and *Kid2* transcripts raises the possibility of functional co-operation between the two proteins. Such co-operation and the functions of KID1 and KID2 in the kidney as well as in other tissues during development and in the adult mouse may be determined by gene targeting and in-vivo inactivation of the two genes.

Acknowledgements

We acknowledge that the mapping was performed using the EUCIB facility run by Dr Michael Rhodes at the Human Genome Mapping Unit, Hinxton Hall, supported by the Medical Research Council. DNA sequencing was carried out by the DNA sequencing facility at the Department of Genetics, University of Cambridge. We also thank P. Lio for useful discussions on sequence interpretation analysis. J.V.B. is supported in part by NIH MERIT Award DK 39773, and C.A.B. is a Lister Institute Research Fellow. We acknowledge the support of the National Kidney Research Fund.

References

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

Bellefroid, E.J., Marine, J.C., Ried, T., Lecocq, P.J., Riviere, M., Amamiya, C., Poncelet, D.A., Coulie, P.G., de Jong, P., Szpirer, C., Ward, D.C., Martial, J.A., 1993. Clustered organisation of homologous KRAB zinc finger genes with enhanced expression in human T lymphoid cells. EMBO J. 12, 1363–1374.

Bonventre, J.V., Witzgall, R., 1995. KID1, a kidney transcription factor. Exp. Nephrol. 3, 159–164.

Brady, J.P., Duncan, M.K., Wawrousek, E.F., Piatigorsky, J., 1997. The transcription factor KID1 is highly expressed in both eye and kidney of the mouse. Exp. Eye Res. 64, 287–290.

Breen, M., European Backcross Collaborative Group 1994. Towards high resolution maps of the mouse and human genomes — A facility for ordering markers to 0.1 cM resolution. Hum. Mol. Genet. 3, 621–627.

Chomczynski, P., Sacchi, N., 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

Elser, B., Kriz, W., Bonventre, J.V., Englert, C., Witzgall, R., 1997. The Krüppel-associated box KRAB)-zinc finger protein KID1 and the Wilms' tumour protein WT1, two transcriptional repressor proteins, bind to heteroduplex DNA. J. Biol. Chem. 272, 27 908–27 912.

Friedman, J.R., Fredericks, W.J., Jensen, D.E., Speicher, D.W., Huang, X.P., Neilso, E.G., Rauscher, F.J., 1996. KAP-1, a novel corepressor for the highly conserved KRAB repression domain. Genes Dev. 10, 2067–2078.

Huang, Z., Philippin, B., O'Leary, E., Bonventre, J.V., Kriz, W., Witzgall, R., 1999. Expression of the transcriptional repressor protein KID1 leads to the disintegration of the nucleolus. J. Biol. Chem. 274, 7640–7648.

Jacob, A.N., Manjunath, N.A., Bra-Ward, P., Kandpal, R.P., 1998. Molecular cloning of a zinc finger gene eZNF from a human inner ear cDNA library, and in situ expression pattern of its mouse homologue in mouse inner ear. Somat. Cell Mol. Genet. 24, 121–129.

Kim, S.S., Chen, Y.M., O'Leary, E., Witzgall, R., Vidal, M., Bonventre, J.V., 1996. A novel member of the RING finger family, KRIP-1, associates with the KRAB-A transcriptional repressor domain of zinc finger proteins. Proc. Natl. Acad. Sci. USA 93, 15 299–15 304.

Kozak, M., 1984. Compilation and analysis of sequences upstream from the translational start site in eukaryotic mRNAs. Nucleic Acids Res. 25, 857–872.

Le Douarin, B., Nielsen, A.N., Garnier, J.M., Ichinose, H., Jeanmougin, F., Losson, R., Chambon, P., 1996. A possible involvement of TIF1α and TIF1β in the epigenetic control of transcription by nuclear receptors. EMBO J. 15, 6701–6715.

Margolin, J.F., Friedman, J.R., Meyer, W.K.H., Vissing, H., Thiesen, H.J., Rauscher, F.J., 1994. Krüppel-associated boxes are potent transcriptional repression domains. Proc. Natl. Acad. Sci. USA 91, 4509–4513.

Moosmann, P., Georgiev, O., Le Douarin, B., Bourquin, J.P., Schaffner, W., 1996. Transcriptional repression by RING finger protein TIF1b that interacts with the KRAB repressor domain of KOX1. Nucleic Acids Res. 24, 4859–4867.

Omori, Y., Kyushiki, H., Takeda, S., Suzuki, M., Kawai, A., Fujiwara,

T., Takahashi, E., Nakamura, Y., 1997. Cloning, expression and mapping of a novel human zinc-finger gene TCF17 homologous to rodent Kid1. Cytogenet. Cell Genet. 78, 285–288.

Pengue, G., Calabro, V., Cannada Bartoli, P., Pagliuca, A., Lania, L., 1994. Repression of transcriptional activity at a distance by the evolutionary conserved KRAB domain present in a subfamily of zinc finger proteins. Nucleic Acids Res. 22, 2908–2914.

Pengue, G., Lania, L., 1996. KRUPPEL-associated box-mediated repression of RNA polymerase II promoters is influenced by the arrangement of basal promoter elements. Proc. Natl. Acad. Sci. USA 93, 1015–1020.

Rhodes, M., et al., 1998. A high resolution map of the mouse genome. Genome Res. 8, 531–542.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, New York.

Shannon, M., Ashworth, L.K., Mucenski, M.L., Lamerdin, J.E., Branscomb, E., Stubbs, L., 1996. Comparative analysis of a conserved zinc finger gene cluster on human chromosome 19q and mouse chromosome 7. Genomics 33, 112–120.

Shannon, M., Stubbs, L., 1998. Analysis of homologous XRCC1-linked zinc finger gene families in human and mouse: evidence for orthologous genes. Genomics 49, 112–121.

Witzgall, R., O'Leary, E., Gessner, R., Ouellette, A.J., Bonventre, J.V., 1993. KID1 a putative renal transcription factor: regulation during ontogeny and in response to ischemia and toxic injury. Mol. Cell. Biol. 13, 1933–1942.

Witzgall, R., Volk, R., Yeung, R.S., Bonventre, J.V., 1994. Genomic structure and chromosomal location of the rat gene encoding the zinc finger transcription factor KID1. Genomics 20, 203–209.

Witzgall, R., Obermuller, N., Bolitz, U., Calvet, J.P., Cowley Jr., B.D., Walker, C., Kriz, W., Gretz, N., Bonventre, J.V., 1998. KID1 expression is high in differentiated renal proximal tubule cells and suppressed in cyst epithelia. Am. J. Physiol. 275, F928–F937.