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Kid-1, a Kidney Transcription Factor

Joseph V. Bonventre
Ralph Witzgall

Medical Services, Massachusetts General Hospital, Charlestown, Mass., and Department of Medicine, Harvard Medical School, Boston, Mass., USA

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The kidney is an organ with heterogeneous populations of cells, each with a specific phenotype. Kidney development and cellular differentiation are likely dependent upon an ordered activation of a number of genes whose encoded proteins determine phenotype. The expression pattern of many of these proteins is likely tissue-specific and regulated to a large degree at the level of transcription. One of the ways to achieve kidney-specific protein expression is to express kidney-specific transcription factors. These proteins may be important, not only for renal development and differentiation, but also for the processes involved in repair of the kidney after an injury. The mechanism of kidney injury and repair recapitulates many aspects of development, since it involves dedifferentiation and regeneration of epithelial cells followed by differentiation and restoration of the mature phenotype [1, 2].

Identification and Expression Pattern of Kid-1

The *Kid-1* cDNA was cloned [3] from rat kidney cDNA libraries screened with a degenerate oligonucleotide, encoding the H/C link, a highly conserved stretch of seven amino acids, present between the individual fingers of many members of the C_2H_2 (cysteine$_2$-histidine$_2$) family of zinc finger transcription factors [4]. Sequencing confirmed that *Kid-1* belonged to the zinc finger family [3].

The name *Kid-1* was given to the cDNA because the *Kid-1* 2.8-kb mRNA is detected predominantly in the kidney, and mRNA levels are developmentally regulated, and modulated by renal ischemia [3]. On Northern blots of RNAs from various organs *Kid-1* mRNA could be found only in the kidney. By reverse transcriptase polymerase chain reaction (RT-PCR), small amounts of *Kid-1* mRNA are found in the spleen, brain, liver and testis. *Kid-1* mRNA is marginally detectable by Northern analysis in rat kidneys taken at the time of birth, at a time when the kidney is not fully developed. mRNA levels become easily seen at 15 days and accumulate to greater levels in the adult rat.

Kid-1 mRNA levels are reduced after renal ischemia and reperfusion, a stimulus which results in dedifferentiation and mitogenesis of surviving cells in order to achieve tissue repair [5]. mRNA levels of *Kid-1* rapidly decline after 30 min of unilateral ischemia and 5 h of reperfusion [3]. There is a marked decrease in the steady-state levels of *Kid-1* mRNA up to 96 h after reperfusion. Normal mRNA levels are restored after 7 days of reperfusion. Similarly, *Kid-1* mRNA levels fall after folic acid-induced acute tubular necrosis. *Kid-1* mRNA levels are also reduced in renal cell carcinomas that develop in the Eker rat [6] (unpublished observations). The decreased expression of *Kid-1* early in postnatal development, and when renal tissue is recovering after injury, suggests that the gene

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Joseph V. Bonventre, MD, PhD
Massachusetts General Hospital East
149-4002 13th Street
Charlestown, MA 02129-2060 (USA)

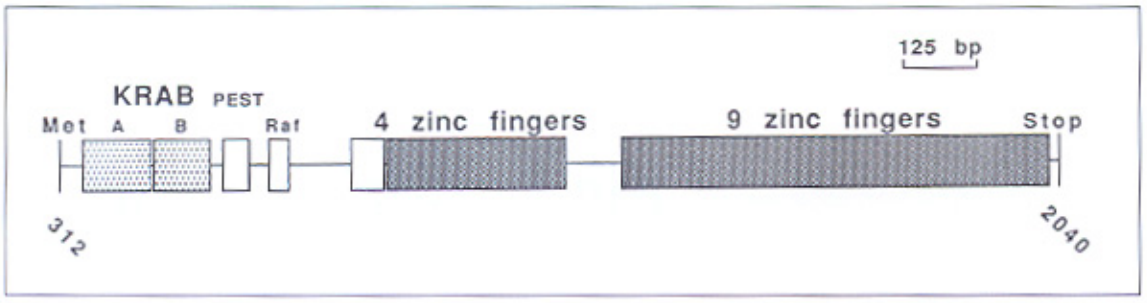

Fig. 1. Structural characteristics of the Kid-1 protein. The protein has 13 zinc fingers in groups of 4 and 9 at the C-terminus. There is a KRAB-A and -B region at the N-terminus. The protein also contains PEST and Raf homology domains.

product may be involved in establishment of a differentiated phenotype and/or regulation of the regenerative response in the kidney.

Characteristics of the Kid-1 Protein (fig. 1)

The open reading frame of the *Kid-1* mRNA encodes a protein with 13 zinc fingers of the C_2H_2 class at its carboxy terminus. The C_2H_2 family of zinc finger proteins is characterized by repeated motifs in which two cysteine and two histidine residues bind to one zinc ion to form a finger-like structure. The zinc fingers of Kid-1 are divided into groups of 4 and 9 with a spacer of 32 amino acids between the two groups of fingers. Zinc finger proteins constitute a major class of transcriptional regulators [4]. Among the best studied of this class of transcription factors are the RNA polymerase-III-associated transcription factor, TFIIIA, from *Xenopus laevis* and the products of Drosophila segmentation genes, such as *Krüppel* and *hunchback* [4]. It is estimated that the C_2H_2 superfamily of zinc finger genes in the mammalian genome has more than one hundred members [7] which encode proteins containing as few as one to more than 30 fingers. It is not clear what is the significance of the grouping of the zinc finger domain of *Kid-1* into clusters of 4 and 9 zinc fingers. While conjectural at this point it has been proposed that this pattern may define a subgroup of zinc finger proteins that regulate tissue-specific gene expression during differentiation [8]. Another transcription factor, MZF1, is expressed in hematopoietic progenitor cells that are committed to myeloid lineage differentiation. Like Kid-1, MZF1 contains 13 zinc fingers grouped into two domains of 4 and 9. Fingers 4 and 5 of MZF1 are separated by 24 amino acids [8].

There are structural features of the *Kid-1* mRNA and deduced protein sequence which suggest that both may be short-lived. An 'AUUUA' sequence at residues 2,137–2,141 may contribute to a short half-life of the message, since this pentanucleotide sequence confers mRNA instability. There also is a consensus region (PEST sequence) in the NH_2 terminus of the deduced Kid-1 protein which is present in proteins with short half-lives. This peptide sequence, rich in proline, acidic, serine, and threonine residues, has been proposed to be the correlate of the 'AUUUA' sequence on the protein level. A short half-life would be desirable for a transcription factor important for regulation of genes whose on-off times have to be brief.

Another potentially important feature of the predicted Kid-1 polypeptide is the presence of a 12-amino-acid motif shared by all members of the Raf family of serine/threonine kinases. This motif lies in the catalytic subdomain VI of protein kinases suggesting that Kid-1 may have kinase activity. The glutamic acid residue in the catalytic domain, together with the aspartic acid residue seven amino acids downstream may support ATP binding. A region somewhat homologous to kinase subdomain VI has been identified in the deduced protein sequence of one of the subunits of TFIIE, a general transcription factor [9].

There are structural aspects of the deduced Kid-1 protein which suggest it may be a substrate for phosphorylation. There are three potential casein kinase II phosphorylation sites in the non-zinc finger region of Kid-1. In addition the 32 amino acid spacer between the 4th and 5th zinc fingers contains four serines and one threonine. Two of the serines and the threonine are preceded by an arginine at position –3, a consensus motif for cAMP-dependent protein kinase and protein kinase C.

The Kid-1 protein, and approximately one-third of all other zinc finger proteins, contain a highly conserved region of approximately 75 amino acids at their NH_2 terminus, named Krüppel-associated box (KRAB), which is subdivided into A and B domains. Similar to regions found in a subset of homeodomain proteins, the paired box and POU domain, the KRAB domain is rich in charged amino acids [10]. Because of the potential α-helical structure of KRAB domains, it has been proposed that this domain mediates protein-protein interactions.

Genomic Structure and Chromosomal Location of Kid-1

We have recently described the genomic structure and chromosomal location of *Kid-1* in the rat. We reported that the *Kid-1* gene contains 4 exons and 3 introns. Recently, we were successful in isolating the 5′ region of the gene and another cDNA with more 5′-sequence. We found that there was an additional small exon corresponding to the non-coding, very 5′-end of this new cDNA. Our original cDNA contained intron sequence at its 5′, non-coding end due to incomplete splicing of the mRNA. The exons define structural domains of the Kid-1 protein [3]. The KRAB-A domain is encoded by an entire exon. The KRAB-B domain comprises the major part of an exon. The entire zinc finger region lies on a single exon, without being interrupted by an intron. All exon-intron boundaries have typical splice donor and splice acceptor sites.

Using a combination of linkage analysis and somatic cell hybrid analysis, *Kid-1* was mapped to rat chromosome (RNO) 10 [11]. Regional sublocalization to RNO10q21.3-q22 was established 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. Polymorphisms between Long Evans and Brown Norway strains were identified for *Kid-1* (*Taq*I), *Il3* (*Pst*I), and *Sparc* (*Pst*I), two other genes that mapped to a similar region of RNO10. Among 40 backcross progeny, there was no recombinant among these 3 probes ($\theta = 0.0$, $Z_{max} = 12.04$); thus, they form a tight linkage group on RNO10.

On Southern blots Kid-1 cDNA hybridizes with human genomic DNA. Although we have not mapped *Kid-1* in the human genome, its location is predicted from comparative analysis with the mouse map, where data are much more abundant. Rat chromosome 10 and mouse chromosome 11 share extensive homology including a region proximal to the 'HSA17' segment. Our data indicate that the tightly linked *Il3*, *Sparc* and *Kid-1* map >20 cM away from the Eker locus on rat chromosome 10 [12]. In the human, *IL3* and *SPARC* map to 5q23-q31 and 5q31-q33, respectively [13]. Therefore, *Kid-1*, if belonging to this syntenic group, would localize near *IL3* and *SPARC* on human chromosome 5q. It is interesting that the distal end of 5q includes genes encoding many growth factors such as colony stimulating factors 1 and 2, acidic fibroblast growth factor, 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 [14], raising the possibility that *Kid-1* may play a role in these tumors and that *Kid-1* may act as a tumor suppressor gene.

The Krüppel Associated Box A (KRAB-A) Motif of Kid-1 and Other Zinc Finger Proteins Mediates Transcriptional Repression

Since the genes to which Kid-1 binds are not known at present, its effect on transcription must be studied in a system which takes advantage of the fact that transcription factors can be treated as having two domains, a DNA binding domain and a *trans*acting domain. The effects of the *trans*acting domain can be studied by creating a fusion protein of this domain to a DNA binding domain of a heterologous protein and coexpressing this fusion protein with a reporter plasmid whose level of transcription can be easily monitored and which has a binding site for the fusion protein. When COS or LLC-PK_1 cells are transfected with fusion constructs encoding the non-zinc finger region of Kid-1 and the GAL4 DNA-binding domain, chloramphenicol acetyl transferase (CAT) activity from cotransfected reporter constructs containing GAL4 binding sites and either a minimal promoter or a SV40 enhancer is strongly repressed [3]. Deletion mutagenesis experiments were performed in which fusion proteins of the GAL4 DNA-binding domain with various segments of the non-zinc finger region of Kid-1 were expressed (fig. 2). These studies revealed that the transcriptional repressor effect of Kid-1 depended upon the presence of the KRAB-A domain. The fusion protein of the GAL4 DNA binding domain and the entire non zinc finger region of Kid-1 as well as the fusion construct including only the KRAB-A domain of Kid-1, repressed transcription in a dose-dependent fashion with similar efficiency. The KRAB-A region of a human zinc finger protein ZNF2

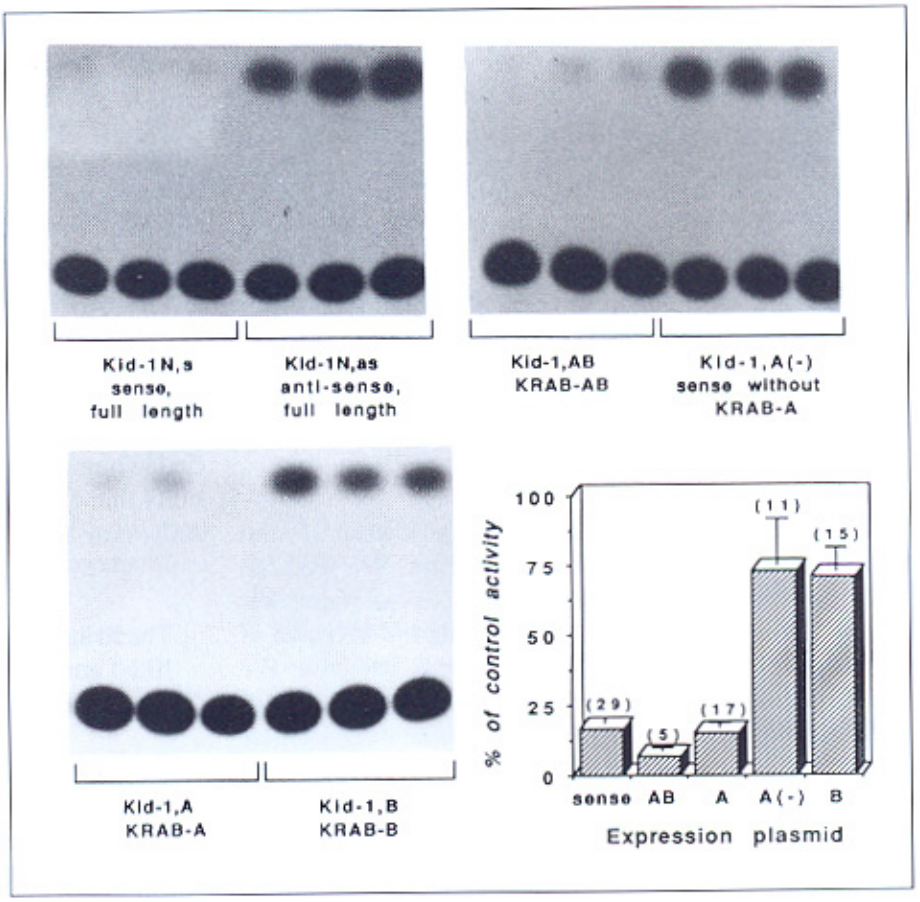

Fig. 2. Thin-layer chromatography assays of CAT activities in cells cotransfected with an SV40 driven CAT-reporter construct containing GAL4 binding regions and expression constructs encoding fusion proteins consisting of the DNA binding region of GAL4 and various parts of the non-zinc finger region of Kid-1. Kid-1N,s encodes the entire non-zinc finger region; Kid-1N,as is the anti-sense control construct; Kid-1,AB and Kid-1,A encode the KRAB-A and B or A alone regions; Kid-1,A(–) encodes all of the non-zinc finger region except for the KRAB-A motif, and Kid-1,B encodes the KRAB-B region only. When compared to the CAT activity observed in cells transfected with the control antisense construct (Kid-1N,as), all constructs including the KRAB-A region resulted in decreased CAT activity and each of those not containing the KRAB-A region lacked repressor activity. The graph in the lower right hand corner provides quantitative CAT activities with activity obtained with the anti-sense Kid-1N,as plasmid taken as 100%. The numbers above the standard error bars indicate the number of independent experiments. Taken from reference 15 with permission.

was as strong a repressor as the KRAB-A domain of Kid-1, indicating that the repressor activity of KRAB-A is not specific to Kid-1. The repressor activity of the KRAB-A region occurred only when it was bound to DNA via the GAL4 DNA binding domain. We found that a number of substitutions in conserved amino acids in the KRAB-A domain inhibited the repressor activity of this motif [15]. This work defining the repressor activity of the KRAB-A domain was published simultaneously with studies from the laboratory of Dr. F. Rauscher [16] in which very similar results were found. Rauscher's group found that the KRAB-A box of KOX1, another C_2H_2 zinc finger protein, was necessary and sufficient for transcriptional repression.

These data provide functional evidence for the importance of the KRAB-A domain, a highly conserved motif found in many zinc finger proteins. With the widespread conservation of the KRAB domain, it is possible that transcriptional repressor activity is associated with many members of this subfamily of C_2H_2 zinc finger proteins. The KRAB-A domain therefore represents the first known widely distributed transcriptional repressor motif.

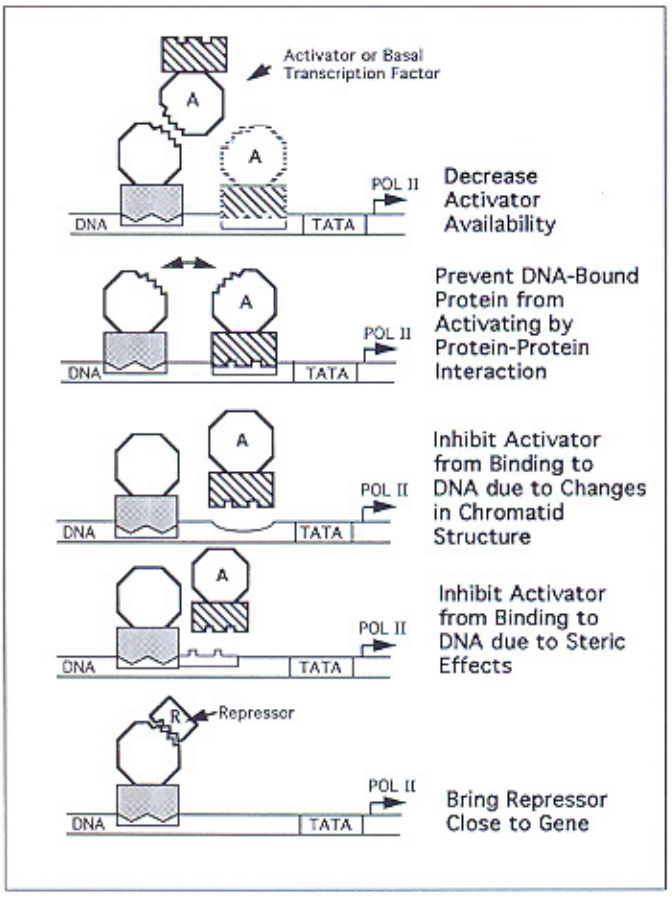

Fig. 3. Possible mechanisms of repression of transcription. This figure illustrates several possible mechanisms of repression of transcription. In the first a repressor protein may bind an activator (A), thus reducing its availability and preventing the activator from binding to DNA and enhancing transcription. The repressor protein may or may not be bound to DNA. As a second mechanism, there may be direct interaction of the repressor protein with a basal transcription factor or an activator, altering its ability to activate but not preventing it from binding to the DNA. In the third depicted mechanism, as a result of repressor protein binding to DNA, there is a change in chromatid structure which prevents the activator from binding to the DNA. A fourth process by which a protein can repress transcription is due to a steric hindrance effect in which binding of the repressor protein physically prevents an activator from binding to DNA. Finally a protein, such as Kid-1, may repress transcription by bringing a repressor protein (R) close to the promoter or enhancer region of a target gene.

At present we do not understand how the KRAB-A domain represses transcription. Some possible mechanisms of transcriptional repression are delineated in figure 3. Our experiments indicate that the KRAB-A domain has to be bound (indirectly through a DNA-binding domain) to DNA to be able to repress transcription. It does not suffice to overexpress a KRAB-A-containing protein like Kid-1 to repress transcription, arguing against a 'squelching effect' by KRAB proteins. This lack of a 'squelching effect' infers specificity, since there are potentially large numbers of zinc finger proteins of the KRAB family expressed in a given cell with the specificity conferred by the DNA-binding characteristics of the specific zinc finger protein. We suggest the following two models for repression by the KRAB-A domain. Binding of a KRAB-A-containing protein may lead to a change in local 'chromatin structure' and thus impair binding of other transcription factors to their binding sites. It is also possible that the KRAB-A domain prevents 'assembly' of the basal transcription factors or 'locks' them in an inactive state, and therefore inhibits transcription.

Similar to the *Kid-1* gene [11], the KRAB-A domain is encoded by a single exon in the human *ZNF2* [17] and *ZNF45* [18] genes. A corresponding arrangement can be assumed for the human *ZNF43* gene, where one alternatively spliced mRNA species has been identified which lacks the KRAB-A region [19]. Hence, by alternative splicing, cells may produce zinc finger proteins with or without the KRAB-A transcriptional repressor domain.

Conclusions

In conclusion, the expression pattern of *Kid-1* suggests that it may play a role in kidney-specific gene expression and possibly in the mitogenic and differentiation responses. Much more needs to be known about the role of this protein, including what *trans*acting factors regulate its expression and what target genes it binds to and regulates. However, the study of this gene has already led to the identification of a region present in a large number of C_2H_2 zinc finger proteins that confers transcriptional repression. Relating physiological and pathophysiological expression patterns to fundamental biology of transcriptional regulation will likely ultimately lead to an understanding of gene expression in the kidney and the regulation of kidney development and repair after injury. The understanding of *Kid-1*'s expression patterns and regulation, as well as DNA-binding characteristics, hopefully will lead to important insight into the regulation of renal development, mitogenesis and differentiation.

Acknowledgments

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References

1 Bacallao R, Fine LG: Molecular events in the organization of renal tubular epithelium: From nephrogenesis to regeneration. Am J Physiol 1989;257:F913–F924.

2 Bonventre JV: Mechanisms of ischemic acute renal failure. Kidney Int 1993;43:1160–1178.

3 Witzgall R, O'Leary E, Gessner R, Ouellette AJ, Bonventre JV: Kid-1, a putative renal transcription factor: Regulation during ontogeny, and in response to ischemia and toxic injury. Mol Cell Biol 1993;13:1933–1942.

4 El-Baradi T, Pieler T: Zinc finger proteins: What we know and what we would like to know. Mech Develop 1991;35:155–169.

5 Witzgall R, Brown D, Schwarz C, Bonventre JV: Localization of proliferating cell nuclear antigen, vimentin, c-fos, and clusterin in the postischemic kidneys. Evidence for a heterogeneous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. J Clin Invest 1994; 93:2175–2188.

6 Eker R, Mossige J: A dominant gene for renal adenomas in the rat. Nature 1961;189:858–859.

7 Bellefroid EJ, Lecocq PJ, Benhida A, Pondelet DA, Belayew A, Martial JA: The human genome contains hundreds of genes coding for finger proteins of the *Krüppel* type. DNA 1989; 8:377–387.

8 Morris JF, Hromas R, Rauscher FJ III: Characterization of the DNA-binding properties of the myeloid zinc finger protein MZF1: Two independent DNA-binding domains recognize two DNA consensus sequences with a common G-rich core. Mol Cell Biol 1994;14:1786–1795.

9 Peterson MG, Inostroza J, Maxon ME, Flores O, Admon A, Reinberg D, Tjian R: Structure and functional properties of human general transcription factor IIE. Nature 1991;354:369–373.

10 Bellefroid EJ, Poncelet DA, Lecocq PJ, Revelant O, Martial JA: The evolutionarily conserved Krüppel-associated box domain defines a subfamily of eukaryotic multifingered proteins. Proc Natl Acad Sci USA 1991;88:3608–3612.

11 Witzgall R, Volk R, Yeung R, Bonventre JV: Genomic structure and chromosomal localization of the rat gene encoding the zinc finger transcription factor Kid-1. Genomics 1994;20: 203–209.

12 Yeung RS, Buetow KH, Testa JR, Knudson AG Jr: Susceptibility to renal carcinoma in the Eker rat involves a tumor suppressor gene on chromosome 10. Proc Natl Acad Sci USA 1993;90:8038–8042.

13 McAlpine PJ, Shows TB, Boucheix C, Stranc LC, Berent TG, Pakstis AJ, Doute RC: Report of the nomenclature committee and the 1989 catalog of mapped genes, Human Gene Mapping 10. Cytogenet Cell Genet 1989;51:13–66.

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

15 Witzgall R, O'Leary E, Leaf A, Önaldi D, Bonventre JV: The Krüppel-associated box-A (KRAB-A) domain of zinc finger proteins mediates transcriptional repression. Proc Natl Acad Sci USA 1994;91:4514–4518.

16 Margolin JF, Friedman JR, Meyer WKH, Vissing H, Thiesen HJ, Rauscher FJ III: Krüppel-associated boxes are potent transcriptional repression domains. Proc Natl Acad Sci 1994;91: 4509–4513.

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

18 Constantinou-Deltas CD, Gilbert J, Bartlett RJ, Herbstreith M, Roses AD, Lee JE: The identification and characterization of KRAB-domain-containing zinc finger proteins. Genomics 1992;12:581–589.

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