**Protein family review**

**KRAB-containing zinc-finger repressor proteins**

Raul Urrutia

Address: Gastroenterology Research Unit, Saint Mary's Hospital and Department of Biochemistry and Molecular Biology and Tumor Biology Program, Mayo Clinic, Rochester, MN 55905, USA. E-mail: urrutia.raul@mayo.edu

Published: 23 September 2003

*Genome Biology* 2003, 4:231

The electronic version of this article is the complete one and can be found online at http://genomebiology.com/2003/4/10/231

© 2003 BioMed Central Ltd

**Summary**

The largest family of zinc-finger transcription factors comprises those containing the Krüppel-associated box (or KRAB domain), which are present only in tetrapod vertebrates. Many genes encoding KRAB-containing proteins are arranged in clusters in the human genome, with one cluster close to chromosome 9q13 and others in centromeric and telomeric regions of other chromosomes, but other genes occur individually throughout the genome. The KRAB domain, which is found in the amino-terminal region of the proteins, behaves as a transcriptional repressor domain by binding to corepressor proteins, whereas the C2H2 zinc-finger motifs bind DNA. The functions currently proposed for members of the KRAB-containing protein family include transcriptional repression of RNA polymerase I, II, and III promoters and binding and splicing of RNA. Members of the family are involved in maintenance of the nucleolus, cell differentiation, cell proliferation, apoptosis, and neoplastic transformation.

**Gene organization and evolutionary history**

Zinc-finger proteins containing the Krüppel-associated box (KRAB-containing proteins) were discovered in 1991 by Bellefroid *et al.* [1]. They make up approximately one third (290) of the 799 different zinc-finger proteins present in the human genome, and as a result, this group of proteins is the largest single family of transcriptional regulators in mammals. Many genes encoding KRAB-containing proteins are arranged in clusters, but others occur individually throughout the genome. The best characterized cluster is on 19q, containing 148 genes (51% of the family) within a region close to 19q13 [2]; other clusters are in centromeric and telomeric regions of other chromosomes. In particular, members of the family containing SCAN domains (see below) are clustered on 3p21-22, 6p21-22, 16p13.3, and 17p12-13. Non-clustered genes encoding KRAB-containing proteins are scattered over the other chromosomes, with about half on autosomes and half on sex chromosomes. Although the expression of genes of other clustered families, such as homeobox genes, is coregulated, it remains to be determined whether a comparable mechanism operates for genes encoding KRAB-containing proteins, and more studies are needed to show how chromosome organization influences the expression patterns of this family.

As shown in Figure 1, KRAB-containing proteins are characterized by the presence of a DNA-binding domain made up of between 4 and over 30 zinc-finger motifs and a KRAB domain. The KRAB domain, located near the amino terminus of the protein, consists of one or both of the KRAB A box and the KRAB B box (see below). Other domains, such as the SCAN domain, are found in a small subset of members of the family [2,3] (Table 1). The two boxes of the KRAB domain are always encoded by individual exons separated by introns of variable sizes. This exon-intron composition allows the generation of different products by alternative splicing. In fact, zinc-finger proteins that contain only a KRAB A domain, for instance, can originate either from a gene that lacks the KRAB B domain or from one with both KRAB A and B that generates a ‘KRAB A-only’ transcript by alternative splicing. In contrast, the zinc-finger domain (including all the zinc-finger motifs) is often encoded by a single exon. This is remarkable given that other families of zinc-finger proteins containing fewer zinc fingers (such as the Sp1-like proteins, which have three) have more than one exon to encode the DNA-binding domain. Multi-zinc-finger proteins of the KRAB-containing protein family may have been subjected to different selective pressures from proteins with
fewer zinc fingers; this idea is supported by other evolutionary features, discussed below.

Perhaps the most remarkable feature of the KRAB-containing proteins is the fact that they are present only in tetrapod vertebrate genomes. The KRAB domain is absent from the sequences of zinc-finger proteins from fish, Drosophila, plants, yeast, and other fungi, but it has been identified in the human, mouse, rat, chicken and frog genomes [3]. Although the name ‘Krüppel-associated box’ implies that the KRAB domain is present in proteins that have zinc fingers similar to the ones found in Drosophila Krüppel, Krüppel itself does not have a KRAB box. This distribution suggests that the emergence of the KRAB domain is a relatively recent event in evolution, even though a large part of each KRAB-containing protein is composed of zinc-finger motifs, which are present in organisms ranging from unicellular eukaryotes to humans. Currently, the reason for the expansion of the family in tetrapods remains unknown, although clues may come from a better understanding of their transcriptional-regulatory functions. It is likely, however, that they evolved to provide vertebrates with a key function that underlies their development, such as aspects of the immune system or the nervous system.

**Characteristic structural features**

Members of the KRAB-containing protein family bind DNA through their C₂H₂ zinc-finger domains [3], and the KRAB domain functions as a strong transcriptional repressor.
### Table 1

Summary of the functional features of KRAB-containing zinc-finger repressor proteins

| Subfamily       | Protein | Species | Chromosomal localization | Number of zinc fingers | Expression pattern                  | Proposed function                                                                 |
|-----------------|---------|---------|--------------------------|------------------------|-------------------------------------|-----------------------------------------------------------------------------------|
| **A + B subfamily** | HKr18   | Human   | 19                       | 20                     | Ubiquitous                          | Repressor or RNA pol II                                                           |
|                 | HKr19   | Human   | 7                        | 11                     | Testis                              | Cell differentiation                                                              |
|                 | KID-1   | Human   | 5q35.3                   | 13                     | Ubiquitous                          | Nucleolar integrity                                                               |
|                 | Kid2    | Mouse   | 11                       | 13                     | Embryonic brain, kidney, gut and lung | Mouse development                                                                 |
|                 | Kid3    | Mouse   | 11                       | 11                     | Embryonic (E16.5) kidney, gut, lung and heart | Kidney development                                                               |
|                 | KOXI    | Human   | 12q24.33                 | 9                      | Ubiquitous                          | Repressor of RNA pol I, II, and II promoters                                       |
|                 | KRAZ1   | Mouse   | 17                       | 15                     | Ubiquitous                          | Repressor or RNA pol II                                                           |
|                 | KRAZ2   | Mouse   | 5                        | 9                      | Ubiquitous                          | Repressor or RNA pol II                                                           |
|                 | KS1     | Rat     | 10                       | 10                     | Ubiquitous                          | Tumor suppressor                                                                  |
|                 | KZF-1   | Rat     | 6                        | 9                      | Testis                              | Spermatogenesis                                                                   |
|                 | RbaK    | Human   | 7                        | 16                     | Ubiquitous                          | Cell cycle arrest                                                                 |
|                 | RITA    | Human   | 9q13                     | 12                     | Ubiquitous                          | Thyroid carcinoma                                                                 |
|                 | ZBRK1   | Human   | 19q13.41                 | 8                      | Skeletal muscle                     | Interaction with Brca I                                                            |
|                 | ZF5128  | Human   | 19                       | 9                      | Ubiquitous                          | T cell activation                                                                 |
|                 | ZNF41   | Human   | Xp11.2                   | 18                     | Ubiquitous                          | Flanking a translocation breakpoint in synovial sarcoma                            |
|                 | ZNF43   | Human   | 19p13.1-p12              | 22                     | T cell, B cell, and Ewing cells     | Differentiation and growth arrest in Ewing cells                                  |
|                 | ZNF85   | Human   | 19p13.1-p12              | 15                     | Ubiquitous                          | Repressor RNA pol II                                                              |
|                 | ZNF91   | Human   | 19p12                    | 27                     | Seminoma and lymphoid cells         | Repression of the human Fc gamma RIIB gene                                         |
|                 | ZNF133  | Human   | 20p11.23                 | 15                     | Ubiquitous                          | Repressor RNA pol II                                                              |
|                 | ZNF140  | Human   | 12q24                    | 9                      | Lymphoid cells                      | Repression of the human Fc gamma RIIB gene                                         |
|                 | ZNF141  | Human   | 4p16.3                   | 10                     | Ubiquitous                          | Candidate for the Wolf-Hirschhorn syndrome                                         |
|                 | ZNF157  | Human   | Xp11.2                   | 12                     | Blood vessels                       | Potential hotspot for neurogenetic disorders                                       |
| **A subfamily**  | HZF12   | Human   | 19                       | 9                      | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | MZF31   | Mouse   | 2                        | 9                      | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | pMLZ-8  | Mouse   | 4                        | 15                     | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | ZK1     | Human   | 19p13.2                  | 15                     | Hematopoietic and various cancer cells | Radiation-induced apoptotic cell death                                              |
|                 | ZNF136  | Human   | 19p13.1-p12              | 13                     | Ubiquitous                          | Weak repressor of RNA pol II                                                      |
| **A + b subfamily** | HZF4    | Human   | 19p13.32                 | 18                     | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | Zip93   | Human   | 19p13.1-p12              | 15                     | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | rKr2    | Rat     | 1                        | 19                     | Central nervous system and testis   | Maturation of neurons and oligodendrocyte                                        |
|                 | ZNF45   | Human   | 19p13.2                  | 11                     | Ubiquitous                          | Potential hotspot for malignant disorders                                         |
|                 | ZNF155  | Human   | 19q13.2                  | 11                     | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | ZNF221  | Human   | 19q13.2                  | 15                     | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | ZNF222  | Human   | 19                       | 7                      | Ubiquitous                          | Repressor of RNA pol II                                                           |
|                 | ZNF224  | Human   | 19                       | 16                     | Ubiquitous                          | Repressor of RNA pol II                                                           |
domain [4]. Some members of the family also have SCAN domains. No crystal structures of KRAB-containing proteins have yet been solved.

**Zinc-fingers**

The C2H2 zinc finger motifs found in the KRAB-containing proteins and other zinc-finger proteins are defined by the presence of the consensus sequence $\text{His-X-Cys-X(2-4)-Cys-X3-} \text{His}$, where X represents any amino acid and His represents a hydrophobic residue. The two cysteine and two histidine residues coordinate a zinc ion and fold the domain into a finger-like projection that can interact with DNA. Previous studies strongly suggest that each of these motifs can contact three to four nucleotides [5]. KRAB-containing proteins often contain 10 or more zinc fingers, and proteins with up to 34 are known. Until recently, it had not been investigated fully whether these zinc fingers bind DNA in a sequence-specific manner or function in transcriptional regulation outside of an artificial Gal4-based transcriptional assay. During the last two years, however, our laboratory and others have provided evidence that wild-type KRAB-containing proteins are indeed transcriptional repressors that use most of their collection of zinc fingers to bind to DNA [5].

A conserved motif in another family of mammalian proteins, the SSX proteins, has a low degree of similarity with the KRAB domain. Proteins containing the ‘SXX KRAB domain’ sequence do not have zinc fingers and are not grouped into the KRAB-containing protein family [7]. Functional analyses have been important in dissecting the functional differences between the SSX and KRAB domains, which are 39 to 49% similar to each other [7]: SSX-KRAB-related domains poorly repress heterologous promoters and do not interact with Kap1 (see below).

**The KRAB domain**

The KRAB domain spans approximately 50-75 amino acids and is divided into the A and B boxes (Figure 2a); the A box plays a key role in repression by binding to corepressors, and the B box enhances the repression mediated by the A box through as-yet unknown mechanisms [6]. Whether or not the amino-terminal domain contains the A box, the B box, or both, it is always known as the KRAB domain (Figure 2a). The mammalian KRAB-containing zinc-finger proteins can be divided into three subfamilies on the basis of the primary structure of this amino-terminal repressor domain [3]: those that contain an A box alone (the KRAB A subfamily), those with a combination of the A and B boxes (KRAB A + B), and those with an A box combined with a divergent B box, sometimes called the b box (KRAB A + b). Further analysis of the family may reveal other subfamilies.

A conserved motif in another family of mammalian proteins, the SSX proteins, has a low degree of similarity with the KRAB domain. Proteins containing the ‘SXX KRAB domain’ sequence do not have zinc fingers and are not grouped into the KRAB-containing protein family [7]. Functional analyses have been important in dissecting the functional differences between the SSX and KRAB domains, which are 39 to 49% similar to each other [7]: SSX-KRAB-related domains poorly repress heterologous promoters and do not interact with Kap1 (see below).

**The SCAN domain**

A defined subset of KRAB-containing zinc-finger transcription factors contains a SCAN domain, which is named after the first letters of the proteins in which it was originally described (SRE-ZBP, Ctfn51, AW-1, and Number 18 cDNA) [8]; it is also known as LeR because of its leucine-rich primary structure. The SCAN domain is at least 87 amino acids in length (Figure 2b); it is vertebrate-specific, and it is never repeated within a protein. It is not
associated with transcriptional regulation but instead allows homo- and hetero-dimerization with other SCAN-containing zinc-finger proteins [9]; the mechanisms involved in these dimerization phenomena remain poorly understood. Taken together, the reduced number of genes encoding these proteins in mammals, their clustered genomic organization, and their ability to form dimers suggest that KRAB-containing zinc-finger proteins with SCAN domains may either all participate in similar functional processes or all be regulated in a similar manner.

Localization and function

The functions currently known for members of the KRAB-containing protein family include transcriptional repression of RNA polymerase I, II, and III promoters, binding and splicing of RNA, and control of nucleolus function. The functions of most of the family have not been well studied, but a few examples are as follows. The human Kid1 protein can bind to heteroduplex DNA structures and is localized to the nucleolus [10]. Once in the nucleolus, Kid1 induces nucleolar disintegration and greatly reduces the synthesis of ribosomal RNA by RNA polymerase I, which takes place in this subnuclear compartment. Moreover, the KRAB domain of Kid1 is necessary for both of these phenomena, suggesting that the protein may repress transcription by RNA polymerase I in Gal4-based assays [11]. Thus, it is likely that the KRAB domain functions differently in the full-length Kid1 protein than in a chimeric fusion protein (as used in the Gal4 assay) or that the KRAB domains of Kox1 and Kid1 behave differently at RNA polymerase I promoters. More studies are needed to differentiate between these possibilities.

In contrast to Kid-1, human Znf74 is found in discrete granular structures in the nucleus, is tightly associated with the nuclear matrix, binds to RNA, and interacts with RNA polymerase II [12]. This KRAB-containing protein contains a truncated KRAB A domain and 12 different C2H2 zinc-finger motifs that are sufficient for targeting the protein to the nuclear matrix as well as for RNA binding. In addition, Znf74 interacts with the hyperphosphorylated form of RNA polymerase II and colocalizes with it in nuclear domains that are enriched in splicing factors. These findings suggest that Znf74 may regulate gene expression through both transcriptional and post-transcriptional mechanisms. KS1, which has ten zinc-finger domains and both KRAB A and B boxes, is a strong repressor of RNA polymerase activity by the Kap1-mediated mechanism described below [5]. KS1 is also a suppressor of the neoplastic transformation that is mediated by several oncogenes [13].

The biochemical functions of KRAB-containing proteins described above are thought to be critical to their cellular roles, which include cell differentiation, cell proliferation, apoptosis, and neoplastic transformation. Krim-1B, a KRAB-containing protein with nine zinc-finger motifs, antagonizes the growth regulatory properties of the oncogene product c-Myc by binding to it via the second zinc finger [14]. The
interaction between Krim-1B and c-Myc decreases the transcriptional transactivation of c-Myc that is dependent on c-Myc binding to the E-box in the promoters of its target genes. Other KRAB-containing proteins are involved in the regulation of cell proliferation. The leucine zipper and sterile-alpha motif protein kinase (ZAK) has been implicated in the regulation of cell-cycle arrest by decreasing cyclin-E expression, and a KRAB-containing protein has been shown to be associated with ZAK, playing a role in this phenomenon [15]. The expression of the KRAB-containing protein AJ8, for instance, is developmentally regulated in embryonic tibiae and calvariae, suggesting a role in the maturation of bone cells, and the overexpression of AJ8 in osteoblastic cells represses known markers of osteoblast differentiation [16]. Some KRAB proteins also appear to be involved in the regulation of apoptosis. Myeloid cells transfected with the cDNA of the KRAB-containing protein ZK1 are more sensitive to cell death induced by ionizing radiation than non-transfected cells [17]. Together, these examples support a role for KRAB-containing proteins in the regulation of morphogenesis. Consequently, several laboratories, including mine, have been investigating the functional association of these proteins with pathophysiological processes. Although there has not been any definitive proof on the causal role of KRAB-containing proteins in human diseases, using gene-mapping techniques, some KRAB-containing proteins have been proposed to be candidate genes for developmental and neoplastic disorders, as well as for schizophrenia [18,19]. The lack of functional evidence at this point makes this association tenuous, however. A better understanding of the molecular mechanisms underlying the functions of KRAB-containing proteins will have important biological implications.

Mechanism of function
Studies by three laboratories have identified a 100 kDa corepressor protein for KRAB domains, known as Kap1, Tif1β, or Krip1 [20-22]. Binding to a RING-B-box coiled-coil (RBCC) motif of Kap1 is an absolute requirement for KRAB-containing proteins to mediate transcriptional repression. These elegant studies [20-22] demonstrated that Kap1 binds to KRAB domains as an oligomer, functioning as a scaffold to recruit heterochromatin protein 1 isoforms (HP1α, HP1β, and HP1γ), histone deacetylases (HDACs), and Setdb1, a novel SET-domain protein that methylates lysine 9 of histone H3. Interestingly, HP1 proteins bind to Lys9-methylated histone H3 in order to condense chromatin [23-28]. Together, these findings have recently led to the proposal of the model shown in Figure 3 [27]. The model predicts that KRAB-containing proteins bind to their corresponding DNA sequence, triggering the recruitment of Kap1; subsequently, Kap1 forms a scaffold containing HP1, Setdb1, and an HDAC, and silences gene expression by forming a facultative heterochromatin environment on a target promoter. This model would suggest a KRAB-mediated stepwise assembly of a powerful corepressor complex. Further examination is needed, however, of whether the complex is instead preformed and then recruited by a KRAB-domain on particular promoter. Also, as these proteins can all be regulated by post-translational modifications, it is not clear whether the corepressor complexes predicted by the model always contain Kap1, HP1, and SETDB1. Despite these questions, the building of this model is one of the most significant steps forward in this field of research.

Frontiers
KRAB-containing proteins were discovered in 1991. Today, a significant amount of information is known on both the structural and the basic biochemical properties of these proteins. Many questions remain to be addressed, however, including why there are so many proteins in the family although they are found only in tetrapods; the origin and function of their clustered genomic organization; the distinct cellular functions of each member of the family; how the domains within the proteins cooperate to achieve a specific
cellular function; and how the proteins are regulated by post-translational modification. We anticipate that future studies in this field will be exciting and illuminating.

Acknowledgements

I thank Todd Clark for providing the Figure I and G. Callahan and M. Fernandez-Zapico for critically reading the manuscript. This work was made possible by funding from the National Institutes of Health (DK52913 and DK56620), the Lustgarten Foundation for Pancreatic Cancer Research, and the Mayo Clinic Cancer Center to R.U.

References

1. Bellefroid EJ, Poncelet DA, Leocq PJ, Revelant O, Martial JA: The evolutionarily conserved Kruppel-associated box domain defines a subfamily of eukaryotic multizinc-fingered proteins. Proc Natl Acad Sci USA 1991, 88:3608-3612. The authors show that the KRAB domain is present in about one-third of zinc-finger proteins analyzed and that it is a conserved domain of 75 amino acids located in the amino-terminal portion of these proteins.

2. Rousseau-Fécril MF, Koczan D, Legrand I, Möller S, Auran S, Thiesen HJ: The KOX zinc finger genes: genome wide mapping of 368 ZNF PAC clones with zinc finger gene clusters predominantly in 23 chromosomal loci are confirmed by human sequences annotated in EnsEMBL. Cytogenet Genome Res 2002, 98:17-53. In this article, the authors generated phylogenetic trees of all KRAB-containing human zinc-finger proteins with the goal of documenting their evolution in primates.

3. Looman C, Abrink M, Mark C, Hellman L: KRAB zinc finger proteins: a novel family of the multizinc-finger mechanisms governing their increase in numbers and complexity during evolution. Mol Biol Evol 2002, 19:2118-2130. This article shows that both the KRAB A + B and the KRAB A subfamilies of zinc-finger proteins may have originated from a single member or a few closely related members of the KRAB A + B family. The KRAB A + B family is also the most prevalent among the KRAB zinc-finger genes.

4. Witvill R, O’Leary E, Leaf A, Onalda D, Bonventre JV: The Kruppel-associated box-A (KRAB-A) domain of zinc finger proteins mediates transcriptional repression. Proc Natl Acad Sci USA 1994, 91:4514-4518. KRAB domains can inhibit the activating function of known transcriptional regulators, and the KRAB domain silences both activated and basal promoter activity of TATA-containing promoters.

5. Gebefelin B, Urrutia R: Sequence-specific transcriptional repression by KS1, a multiple-zinc-finger-Kruppel-associated box protein. Mol Cell Biol 2001, 21:928-939. This article demonstrates that KRAB-containing proteins have a sequence-specific repression function and characterizes the manner by which these proteins bind to DNA.

6. Vissing H, Meyer WK, Aagard L, Thiesen HJ: Repression of transcriptional activity by heterologous KRAB domains present in zinc finger proteins. FEBS Lett 1995, 369:153-157. The authors report the characterization of the KRAB domain from ZNF133, which is composed of only the KRAB A box. They show that the A box alone is a weaker suppression domain than the A + B boxes, but when fused to a heterologous KRAB B box, it induces repression as potently as do previously reported KRAB domains.

7. Lim FL, Souleza M, Koczan D, Thiesen HJ, Knight JC: A KRAB-related domain and a novel transcription repression domain in proteins encoded by SSX genes that are disrupted in human sarcomas. Oncogene 1998, 17:2013-2018. The KRAB-related SSX domain, unlike the KRAB domain of Koxl, neither interacts with Kap1 nor represses transcription. The authors propose that the functions of the SSX-KRAB domain and typical KRAB domains are different.

8. Collins T, Stone JR, Williams AJ: All in the family: the BTB/POZ, KRAB, and SCAN domains. Mol Cell Biol 2001, 21:3609-3615. This review article describes the type, structure, and functions of different domains founds in KRAB-containing proteins.

9. Honer C, Chen P, Toth MJ, Schumacher C: Identification of SCAN dimerization domains in four gene families. Biochim Biophys Acta 2001, 1517:441-448. The authors identified several genes that contain both SCAN and KRAB domains.

10. Huang Z, Philippin B, O’Leary E, Bonventre JV, Krich W, Witvill R: Expression of the transcriptional repressor protein Kid-I leads to the disintegration of the nucleolus. J Biol Chem 1999, 274:7607-7614. Kid-I can regulate nucleolar structure.

11. Moosmann P, Georgiev O, Thiesen HJ, Hagmann M, Schaffner W: Silencing of RNA polymerases II and III-dependent transcription by the KRAB protein domain of KOX1, a Kruppel-type zinc finger factor. Biochem J 2001, 361:367-377. Evidence that KRAB domains may inhibit some component(s) of RNA polymerase II and III transcription.

12. Grondin B, Bazinet M, Aubry M: The KRAB zinc finger gene ZNF74 encodes an RNA-binding protein tightly associated with the nuclear matrix. Mol Cell Biol 2001, 21:7557-7567. KRAB-containing proteins that associate with the nuclear matrix can bind RNA.

13. Gebelein B, Fernandez-Zapico M, Imoto M, Urrutia R: KRAB-independent suppression of neoplastic cell growth by the novel zinc finger transcription factor KSI. J Clin Invest 1998, 102:1911-1919. This article shows that KRAB-containing proteins can silence gene expression in a sequence-specific manner by binding to DNA via most of their zinc fingers.

14. Hennemann H, Vassen L, Geisen C, Eilers M, Moroy T: Identification of a novel Kruppel-associated box domain protein, Krmm-I, that interacts with c-Myc and inhibits its oncopgenic activity. J Biol Chem 2003, 278:28799-28811. This article reports that Krmm-I, a KRAB-containing protein, participates in cell-growth regulation. In addition, the authors provide good mechanistic insights into how this protein mediates this function.

15. Yang JJ: A novel zinc finger protein, ZZaPK, interacts with ZAK and stimulates the ZAK-expressing cells re-entering the cell cycle. Biochem Biophys Res Commun 2003, 301:71-77. The author describes the cloning of a cDNA encoding a protein designed as ZZaPK (zinc finger and ZAK associated protein with KRAB domain). ZAK is a protein that participates in cell-cycle arrest via a downregulation of cyclin E expression. ZZaPK is thought to take part in this phenomenon in carcer cell lines.

16. Jheon AH, Ganss B, Cheifetz S, Sodek J: Characterization of a novel KRAB/C2H2 zinc finger transcription factor involved in bone development. J Biol Chem 2001, 276:18282-18289. This article reports the use of differential display PCR to identify a novel zinc-finger transcription factor (A18) that is induced during the differentiation of bone cells in vitro and in vivo. A18 inhibits Runx2-mediated osteogenic differentiation.

17. Katoh O, Oguri T, Takahashi T, Takai S, Fujiwara Y, Watanabe H: Identification of novel zinc finger transcription factors associated with schizophrenia. Brain Res 2004, 1019:73-82. The manifestation of schizophrenic symptoms in individuals with interstitial deletions genes located at 22q11.2 reveals that there are positional candidates for schizophrenia susceptibility. This article reports the occurrence of polymorphisms in the sequence of ZNF74, which is located in this area, and suggest that this gene is one of the modifying factors for schizophrenia.

18. Takase K, Ohtsuki T, Migita O, Toru M, Inada T, Yamakawa-Kobayashi K, Arimani T: Association of ZNF74 gene genotypes with age-at-onset of schizophrenia. Schizophr Res 2001, 52:161-165. The manifestation of schizophrenic symptoms in individuals with interstitial deletions genes located at ZNF133, ZNF136 and ZNF140 contain a KRAB segment. On the basis of their map position, these ZNF genes are putative candidate genes for both developmental and malignant disorders.
20. Friedman JR, Fredericks WJ, Jensen DE, Speicher DW, Huang XP, Neilsen EG, Rauscher F J 3rd: KAP-1, a novel corepressor for the highly conserved KRAB repression domain. Genes Dev 1996, 10:2067-2078. This paper and [21,22] report the identification of Kap1/Tif1b/Krip1 and its characterization as a corepressor for KRAB-containing proteins.

21. Kim SS, Chen YM, O'Leary E, Witzgall R, Vidal M, Bonventre JV: 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 1996, 93:15299-15304. See [20].

22. Moosmann P, Georgiev O, Le Douarin B, Bourquin JP, Schaffner W: Transcriptional repression by RING finger protein TIF1 beta that interacts with the KRAB repressor domain of KOX1. Nucleic Acids Res 1996, 24:4859-4867. See [20].

23. Nielsen AL, Ortiz JA, You J, Oulad-Abdelghani M, Khechumian R, Gansmuller A, Champon P, Losson R: Interaction with members of the heterochromatin protein 1 (HP1) family and histone deacetylation are differentially involved in transcriptional silencing by members of the TIF1 family. EMBO J 1999, 18:6383-6395. This article provides evidence that the silencing activity of Tif1a depends on histone deacetylation, whereas that of the closely related Tif1b may be mediated by both HP1 binding and histone deacetylation.

24. Lechner MS, Begg GE, Speicher DW, Rauscher F J 3rd: Molecular determinants for targeting heterochromatin protein 1-mediated gene silencing: direct chromoshadow domain-KAP-1 corepressor interaction is essential. Mol Cell Biol 2000, 20:6449-6465. This paper and [25] describe the interaction of Kap1 with HP1 isoforms, linking the role of KRAB-containing proteins to chromatin structure and dynamics.

25. Peng H, Begg GE, Schultz DC, Friedman JR, Jensen DE, Speicher DW, Rauscher F J 3rd: Reconstitution of the KRAB-KAP-1 repressor complex: a model system for defining the molecular anatomy of RING-B box-coiled-coil domain-mediated protein-protein interactions. J Mol Biol 2000, 295:1139-1162. See [24].

26. Ryan RF, Schultz DC, Ayyanathan K, Singh PB, Friedman JR, Fredericks WJ, Rauscher F J 3rd: KAP-1 corepressor protein interacts and colocalizes with heterochromatic and euchromatic HP1 proteins: a potential role for Kruppel-associated box-zinc finger proteins in heterochromatin-mediated gene silencing. Mol Cell Biol 1999, 19:4366-4378. The in vitro studies reported in this article confirm that Kap1 is capable of directly interacting with the human M31 and HP1α, which are normally found in centromeric heterochromatin, as well as M32 and hHP1γ, both of which are found in euchromatin.

27. Schultz DC, Ayyanathan K, Negorev D, Maul GG, Rauscher F J 3rd: SETDB1: a novel KAP-1-associated histone H3, lysine 9-specific methyltransferase that contributes to HP1-mediated silencing of euchromatic genes by KRAB zinc-finger proteins. Genes Dev 2002, 16:919-932. A report that Kap1 interacts with Setdb1, a novel SET-domain protein with methyltransferase activity specific to lysine 9 of histone H3.

28. Schultz DC, Friedman JR, Rauscher F J 3rd: Targeting histone deacetylase complexes via KRAB-zinc finger proteins: the PHD and bromodomains of KAP-1 form a cooperative unit that recruits a novel isoform of the Mi-2alpha subunit of NuRD. Genes Dev 2001, 15:428-443. This article presents evidence supporting the model that the KRAB domain functions via Kap1 to target the histone-deacetylase and chromatin-remodeling activities of the NuRD complex to specific gene promoters in vivo.