Protein family review

KRAB-containing zinc-finger repressor proteins

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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 C_2H_2 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

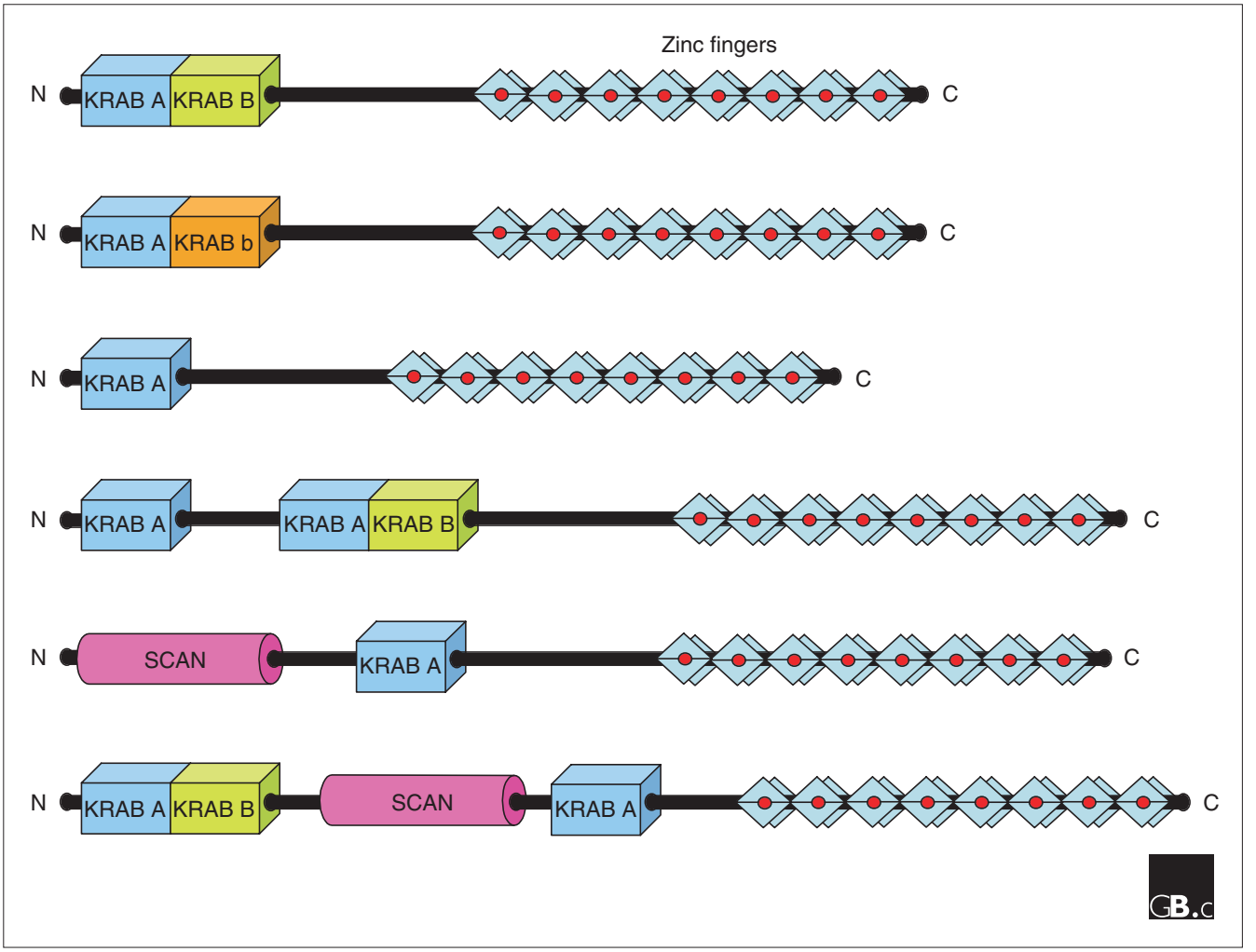

Figure 1
Primary structures of typical KRAB-containing zinc-finger proteins, illustrating the range of domains they contain. Note that the number of zinc fingers among proteins in the family is very variable, ranging from 4 to over 34; only 8 are shown in each structure here, for simplicity. The KRAB domain consists of the A and B boxes; some proteins contain a variant called the b box. Some members of the family have a leucine-rich SCAN domain that allows homo- and hetero-dimerization with other SCAN-containing zinc-finger proteins. Several proteins have been found corresponding to each of the structures shown; they therefore probably represent distinct structural and functional subfamilies. N, amino terminus; C, carboxyl terminus.

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 C_2H_2 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
	KOX1	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	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 Brca1
	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
	Zfp93	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

Table 1 *(continued)*

Subfamily	Protein	Species	Chromosomal localization	Number of zinc fingers	Expression pattern	Proposed function
	ZNF225	Human	19	17	Ubiquitous	Repressor of RNA pol II
	ZNF226	Human	19	17	Ubiquitous	Repressor of RNA pol II
SCAN subfamily	FPM315	Human	16p13.3	9	Ubiquitous	Represses the Col11a2 promoter
	SKAT2	Mouse	11	14	Brain, kidney and hematopoietic cells	Regulation of cytokine in T cells
	Skz1	Mouse	13	7	Ubiquitous	Repressor of RNA pol II
	ZFP95	Human	7	13	Ubiquitous	Repressor of RNA pol II
	ZNF197	Human	3p21.	20	Ubiquitous	Repressor of RNA pol II
	ZNF202	Human	11q23.3	8	Ubiquitous	Energy metabolism
	ZNF274	Human	19q	7	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 C_2H_2 zinc finger motifs found in the KRAB-containing proteins and other zinc-finger proteins are defined by the presence of the consensus sequence ϕ-X-Cys-$X_{(2-4)}$-Cys-X_3-ϕ-X_5-ϕX_2-His-$X_{(3,4)}$-His, where X represents any amino acid and ϕ 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]. In theory, proteins with 30 zinc-finger domains would bind a DNA sequence of more than 60 nucleotides. A sequence of this length would be rarely found by chance in the relatively small genomes of lower eukaryotes, consistent with the fact that KRAB-containing proteins are found only in tetrapods. One should be cautious, however, in assuming that KRAB-containing proteins always bind such long sequences, as post-translational modifications and heterodimerization with other proteins could potentially modify their binding capabilities so as to enable them to recognize shorter sequences. As studies describing DNA binding by these proteins is scant, the final answers to these provocative hypotheses will rely on further studies.

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 meditated 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, CTfin51, 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

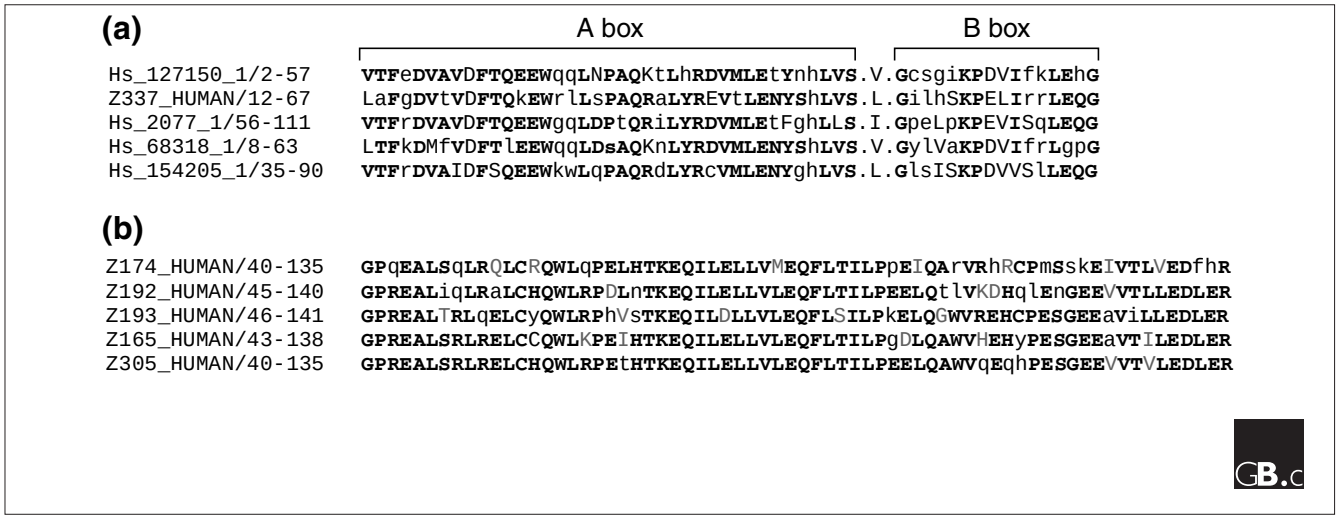

Figure 2
Alignments of the conserved KRAB and SCAN domains. **(a)** The KRAB domain, including both the A box and the B box. The A box is longer and more conserved than the B box. **(b)** The SCAN domain. This domain is found in non-zinc-finger proteins and zinc-finger proteins; the sequences shown here are for SCAN domains of KRAB-containing zinc-finger proteins. Note that these domains have a degree of conservation similar to that of the KRAB A box. Identical residues are in black, similar residues in gray and different residues in lower case. All sequences start at the first amino-acid residue.

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. Because the number and size of the nucleolus correlates with the activity level of RNA polymerase I, its repression may contribute to the disintegration of the nucleolus. Interestingly, however, the KRAB domain of Kox1, which has the same domain structure as Kid1 and therefore belongs to the same subfamily, cannot 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 C_2H_2 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.

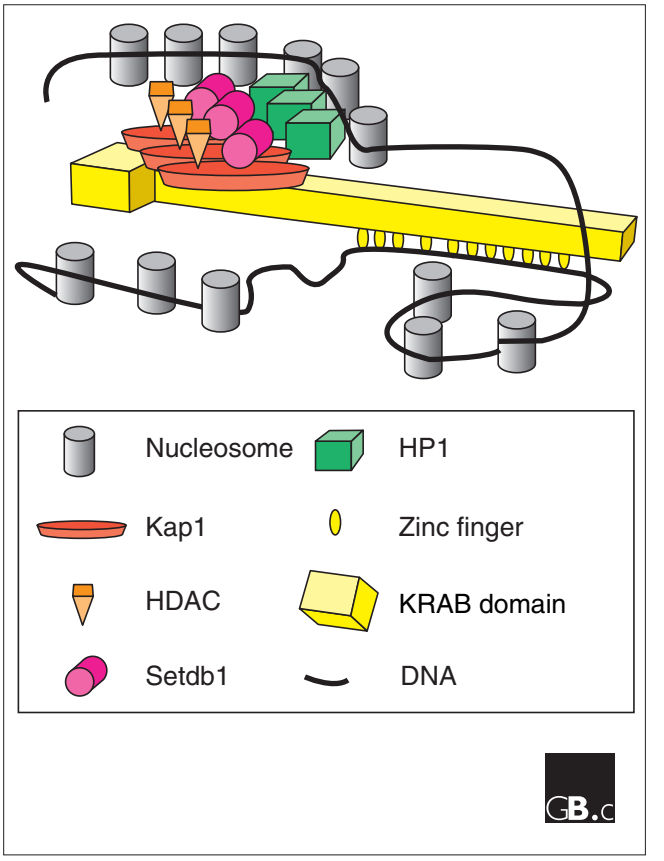

Figure 3
A current model for the complex formed by KRAB-containing proteins and other proteins. A KRAB-containing protein binds specifically to a gene promoter through its multiple zinc fingers. A trimeric Kap1 complex binds to the KRAB domain of the KRAB-containing protein and serves as a scaffold for recruitment of HP1, HDACs, and Setdb1, to form heterochromatin. Note that the figure does not include the SCAN domain because, apart from its ability to dimerize, the role of this domain remains poorly understood.

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.

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