

REVIEW

ZAS: C₂H₂ Zinc Finger Proteins Involved in Growth and Development

LAI-CHU WU¹

Departments of Molecular and Cellular Biochemistry, Internal Medicine, and Molecular Virology, Immunology and Medical Genetics, The Ohio State University, and College of Medicine and Public Health, Columbus, OH 43210

A ZAS gene encodes a large protein with two separate C₂H₂ zinc finger pairs that independently bind to specific DNA sequences, including the κB motif. Three paralogous mammalian genes, ZAS1, ZAS2, and ZAS3, and a related *Drosophila* gene, *Schnurri*, have been cloned and characterized. The ZAS genes encode transcriptional proteins that activate or repress the transcription of a variety of genes involved in growth, development, and metastasis. In addition, ZAS3 associates with a TNF receptor-associated factor to inhibit NF-κB- and JNK/SAPK-mediated signaling of TNF-α. Genetic experiments show that ZAS3 deficiency leads to proliferation of cells and tumor formation in mice. The data suggest that ZAS3 is important in controlling cell growth, apoptosis, and inflammation. The potent vasoactive hormone *endothelin* and *transcription factor AP2* gene families also each consist of three members. The ZAS, *endothelin*, and *transcription factor AP2* genes form several linkage groups. Knowledge of the chromosomal locations of these genes provides valuable clues to the evolution of the vertebrate genome.

ZAS	Separate C ₂ H ₂ zinc finger pairs	TNF signaling	Endothelin	TFAP2	Evolution
Tumorigenesis	Homologous gene clusters	Thymocyte differentiation		Growth control	

THE κB motif, 5'-GGGGACTTCC-3', first identified as a core enhancer element in the immunoglobulin κ light chain gene (62), has subsequently been found to regulate the transcription of many genes involved in immunity, inflammation, and disease [reviewed in (7,15,22,53)]. The nuclear factor κB (NF-κB) gene family is well known in encoding ubiquitous proteins that bind to the κB motif with the Rel homology domain (57). However, other cellular factors that bind to the κB motif, but are unrelated to NF-κB, have been identified in developing brains (14), in neurons (29,33,45,54,55), and in regenerating livers (70). The ZAS proteins are large zinc finger proteins. They may correspond to one or more of these κB binding factors. To date, ZAS genes in hu-

mans, mice, rats, and a related *Drosophila* gene, *Schnurri*, have been cloned and characterized. Here, we will review the structure and function of the ZAS proteins and their genetics and discuss how knowledge of the chromosomal localizations of these genes provides valuable clues about the evolution of the vertebrate genomes.

NOMENCLATURE

We propose naming this gene family ZAS and its three paralogous genes ZAS1, ZAS2, and ZAS3 in the chronological order of their discovery (Table 1). Previously, members of this family have been named as

Accepted April 4, 2002.

¹Address correspondence to Dr. Lai-Chu Wu, Davis Medical Center, Room S2077, Department of Internal Medicine, The Ohio State University, 480 West Ninth Avenue, Columbus, OH 43210. Tel: (614) 293-3042; Fax: (614) 293-5631; E-mail: wu.39@osu.edu

TABLE 1
THE ZAS GENE FAMILY

	Synonymous Names	cDNA Accession Numbers (Insert Size)	References
Vertebrates			
<i>ZAS1</i>			
Human	MHC enhancer binding protein 1 (MBP-1)	M32019 (6240 bp)	8,63
	Human immunodeficiency virus type I enhancer-binding protein 1 (HIVEP1)	J05011 (1035 bp)	37
	Zinc finger 40 (ZnF-40)		21
	Positive regulatory domain II binding factor (PRDII-BF1)	X51435 (9020 bp)	20
	α A-crystallin binding protein (α ACRYBP1)	X68946 (2520 bp)	47
Mouse		L36825-9 (8067 bp)	12
		X54250 (2497 bp)	44
Rat	α -antitrypsin binding protein 2 (AT-BP2)		
<i>ZAS2</i>			
Human	Human immunodeficiency virus type I enhancer-binding protein 2 (HIV-EP2)	M60119 (9318 bp)	48
	MHC enhancer binding protein (MBP-2)	M33920 (420 bp)	58
		X65644 (9175 bp)	78
Mouse	<i>c-myc</i> intron binding protein (MIBP1)	Y15907 (8095 bp)	18
Rat	α 1-antitrypsin binding protein 1 (AT-BP1)	X54249 (2871 bp)	44
	Angiotensinogen gene-inducible enhancer binding protein (AGIE-BP1)	M65251 (3774 bp)	56
	<i>c-myc</i> intron binding protein (MIBP1)	D37951 (9731 bp)	40
<i>ZAS3</i>			
Human	Kappa-enhancer binding protein 1 (KBP-1)	M33919 (407 bp)	58
	Human immunodeficiency virus type I enhancer-binding protein 3 (HIVEP3)	AF278765 (8546 bp)	24
Mouse		AF278766 (310 bp)	
	Recognition component (Rc)	L07911(2400 bp)	81
Rat	κ B binding and recognition component (KRC)	L46815 (8755 bp)	80
	Unnamed	BF386136 (334 bp)	
		BI288832 (505 bp)	
Invertebrates			
<i>Drosophila</i>	Schnurri, Shn	L43211 (8642 bp)	23
		U31368 (8935 bp)	64

binding proteins after individual target genes or sequences. Consequently, several names are given to the same protein. For example, *ZAS1* has been called major histocompatibility complex enhancer binding protein MBP1 (8), human immunodeficiency virus type I enhancer binding protein HIVEP1 (37), positive regulatory domain II binding factor PRDIIIF1 (20), and zinc finger 40 (21). In addition, its mouse and rat counterparts have been named α A-crystallin binding protein α ACRYBP1 (12,44) and α 1-antitrypsin promoter binding protein ATBP2 (56), respectively.

At present, the human and mouse *ZAS* genes are named *HIVEP-1*, *-2*, and *-3* in the unigene database (11). Such naming is systemic but does not fully represent these genes because the HIV enhancer is only one of their known targets and the *ZAS* genes have also been found in mice and rats. We shall name these genes and their gene products *ZAS* after a pro-

tein domain unique to this family. A *ZAS* domain is a modular protein structure consisting of a pair of consecutive C_2H_2 zinc fingers, an acidic region and a serine/threonine-rich sequence (80). Each *ZAS* protein is very large, containing 2348–2717 amino acid residues. In addition, each protein contains an amino *ZAS* domain (*ZAS-N*) and a carboxyl-*ZAS* domain (*ZAS-C*) that are widely separated in the primary sequence.

MOLECULAR CLONING: AN OVERVIEW

The first member, *ZAS1*, initially named *MBP1*, was isolated from a human B cell cDNA expression library by screening for recombinant proteins that bound to a [32 P]DNA fragment derived from the MHC class I gene enhancer sequence 5'-TGGGGAT TCCCA-3' (63). Historically, MBP1 was the first

DNA binding protein to be cloned by expression library screening procedures using ^{32}P -labeled DNA fragments containing protein binding sites as a ligand. Using similar strategy, more ZAS1 cDNAs were isolated using sequences derived from the positive regulatory domain II of the human *IFN- β* gene promoter (20), or the human immunodeficiency virus type 1 long terminal repeat (37). As in the MHC enhancer, both gene regulatory sequences harbored one or more κB -like sequences. The second member, ZAS2, was cloned by standard cDNA library screening procedures using either MBP1 or HIVEP1 cDNAs as cross-hybridization probes and has been named both *MBP2* (58) and *HIVEP2* (48).

The third family member, ZAS3, initially named *recognition component Rc*, was cloned from a mouse thymocyte cDNA expression library using the consensus signal sequences of somatic V(D)J recombination (Rss) as a ligand (81). The variable regions of the antigen receptors genes, Ig and TCR, consist of many gene segments that are brought together by somatic V(D)J recombination during B and T cell development, respectively. Rss flank each gene segment and in conjunction with the products of the *recombination activating genes*, *RAG1* and *RAG2*, mediate the somatic recombination of the antigen receptor genes [reviewed in (34,59)]. An Rss consists of a conserved heptamer sequence 5'-CACAGTG-3', a nonconserved spacer sequence of 12 or 23 bp, and a conserved nonamer sequence 5'-GGTTTTTGG-3' (3). Subsequent DNA-protein interaction studies showed that fusion proteins of Rc have dual DNA specificity for the conserved sequences of Rss and the κB motif and form highly ordered DNA-protein complexes (39). Therefore, its name was changed to κB binding and *recognition component KRc* because of its known dual DNA binding specificity (80).

Over the past 15 years, more than 20 cDNAs corresponding to nine ZAS genes have been cloned from humans, mice, and rats (Table 1). Complementary DNAs, ranging from 8.1 to 9.7 kb and harboring the largest open reading frames, have been characterized for seven ZAS genes. To date, partial cDNAs are only available for rat ZAS1 and ZAS3. Two proteins in invertebrates, Schnurri (Shn) and sem-4, have zinc fingers similar to those of the ZAS proteins. Shn is a *Drosophila* protein that plays an important role during embryogenesis (72,73). Its cDNA clones were isolated by genetic screening of fly embryos that have a dorsal-open phenotype, in which the *Shn* gene was inactivated by a P element insertion (5,23,64). A subsequent in vitro DNA site selection assay shows that Shn also binds specifically to κB -like DNA (17). Shn is a gene-specific transcription factor as well as an adaptor molecule in the signal pathway of decapen-

taplegic (dpp, a TGF- β homologue) [reviewed in (2)]. The homologies of corresponding zinc finger pairs of Shn with its mammalian counterparts are high, ranging from 66% to 76%. In addition, Shn contains 2578 amino acids and the first and second zinc finger pairs are widely separated by 1279 residues. The sequence homologies as well as the organization of two zinc finger pairs identify Shn as a member of the ZAS protein family in invertebrates. Sem-4 is a protein in *Caenorhabditis elegans* that is also important during embryogenesis (9). It contains seven C_2H_2 zinc fingers whose sequences are 39% to 56% identical to those of hZAS3. However, comparing to the ZAS proteins or to Shn, sem-4 is too small (774 residues) to be included in this group. In all, the data suggest that in the ZAS gene family there are three paralogous genes in each mammalian species examined but only one homologous gene in *Drosophila*. Therefore, the mammalian genes should be products of at least two rounds of gene duplication during the evolution from invertebrates to vertebrates.

DNA BINDING SPECIFICITY

The majority of the ZAS cDNAs were isolated by expression cloning procedures using different gene promoters or enhancers. The specific DNA binding site of Shn has been determined by an in vitro DNA site selection assay (17). The known DNA sites of the ZAS proteins and Shn are listed in Table 2. These *cis*-acting DNA elements mostly generate a consensus sequence 5'-GGGG(N_{4,5})CC-3', which is similar to the κB motif. However, in addition to the κB sequence, each mouse ZAS protein has been shown to bind specifically to distinct sequences. ZAS1 binds to a sequence 5'-GAGAAAAGCC-3' at the core enhancer of the *type II collagen gene (Col2 α 1)* (69). ZAS2 interacts with a TC-rich sequence present in the *somatostatin receptor type II (SSTR2)* promoter (18). Finally, ZAS3 binds to the RSS that mediate somatic V(D)J recombination (81). Although dual DNA binding specificity has only been shown for mouse ZAS proteins, the nearly invariant amino acid composition of corresponding zinc fingers among orthologous proteins suggests that the ZAS proteins likely have more than one DNA target.

PROTEIN DOMAINS AND MOTIFS

The structural domains and motifs of the ZAS proteins are shown in Figure 1A. Each ZAS protein contains four or five C_2H_2 zinc fingers, four of which are components of the ZAS-N or ZAS-C domains. In addition, there is a solitary middle C_2HC zinc finger

TABLE 2
KNOWN BINDING SITES OF THE ZAS PROTEINS

Gene Promoters/Enhancers	Sequence
κ B consensus sequence	GGGG(N _{4,5})CC
Major histocompatibility class I	TGGGGATTCCCA
β -Microglobulin	AAGGGACTTTCCC
Ig κ light chain	AGGGGACTTTCCC
HIV LTR (left)	AAGGGACTTTCCA
HIV LTR (right)	TGGGGACTTTCCG
Interferon- β	GTGGGAAATTCCT
Interleukin-2 receptor	AGGGGAATCTCCC
α A-cystallin	TTGGGAAATCCCA
Angiotensinogen	TTGGGATTCCCA
<i>c-myc</i> intron I	TGGGGTAGGCCG
S100A4/mts1	GGGGTTTTCCAC
Ultrabithorax	GGGGGGAGCCA CGGGTGCACCC
Distinct sites	
Rss heptamer	CACAGTG
Rss nonamer	GGTTTTTTG
Somatostatin Receptor type II	TTCCTCTTTCC
Type II collagen	TTGAGAAAAGCC

in ZAS1, ZAS3, and Shn, but not in ZAS2. Furthermore, Shn has an additional cluster of three zinc fingers near the carboxyl region, although their DNA binding ability has not been shown. The most striking features of the ZAS proteins are their very large size and the way in which the four C₂H₂ zinc fingers are organized into two widely separated pairs. Although C₂H₂ zinc fingers are common nucleic acid binding motifs, found in the largest group of transcriptional proteins in metazoans, their sequence and organization in the ZAS proteins are unique:

- First, the sequence identity among the ZAS zinc fingers is very high, extending beyond the backbone residues and including the linker between consecutive zinc fingers (Fig. 1B). The highly conserved zinc fingers underscore the fact that the ZAS proteins bind to similar DNA sequences.
- Second, a sequence comparison has identified 39 C₂H₂ zinc finger families from the available protein sequences of fruit flies, nematodes, and humans (32). The ZAS proteins have been found to form a distinct C₂H₂ zinc finger family.
- Third, C₂H₂ zinc fingers can be subdivided into three classes based on their organization (28). The subclasses are multiple-adjacent, triple, and separated-paired zinc fingers. The last group contains only a small number of proteins: ZAS, basonuclein, and tramtrack (28).
- Fourth, the overall organization of zinc finger pairs, an acidic domain and a Ser/Thr-rich region to form a composite ZAS domain, is unique to the ZAS proteins. In essence, the zinc fingers of the ZAS proteins are distinct from others using several different criteria.

Ten conserved regions, including the zinc fingers, are found in the ZAS proteins (24). Two of them are

found only in the ZAS proteins but not in Shn or in the PROSITE database. The first region contains ~100 amino acid residues and is located between the ZAS domains. It starts with a stretch rich in proline and glutamic acid, follows by a putative nuclear localization signal “PKKKRLR,” and ends with a serine stripe (24). A serine stripe was first identified in basonuclein in which eight serine residues are located on one side of a putative α -helix wheel (75). Basonuclein has three separated-paired zinc fingers. As described above, such configuration has resulted in basonuclein being placed in a subclass of C₂H₂ zinc fingers with the ZAS proteins (28). Basonuclein is probably involved in growth. It is expressed in proliferating cells (71) and activates the ribosomal RNA genes during mouse oogenesis (74). The second conserved region, the linker region, contains 27 residues (24,44). It is located near the carboxyl region of the ZAS proteins. The conservation of the middle and linker regions in the ZAS proteins suggests that they are acquired to perform important functions in vertebrates.

Throughout the ZAS molecules are many potential sites for protein modifications, including *N*-glycosylation sites and phosphorylation sites for cAMP, protein kinase C, casein kinase, and tyrosine kinase. Significantly, three or four tyrosine kinase phosphorylation sites are clustered within or near the ZAS-C domain (Fig. 1A). The phosphorylation status of these sites has been shown to affect DNA binding affinity of a ZAS protein, although the mechanism is

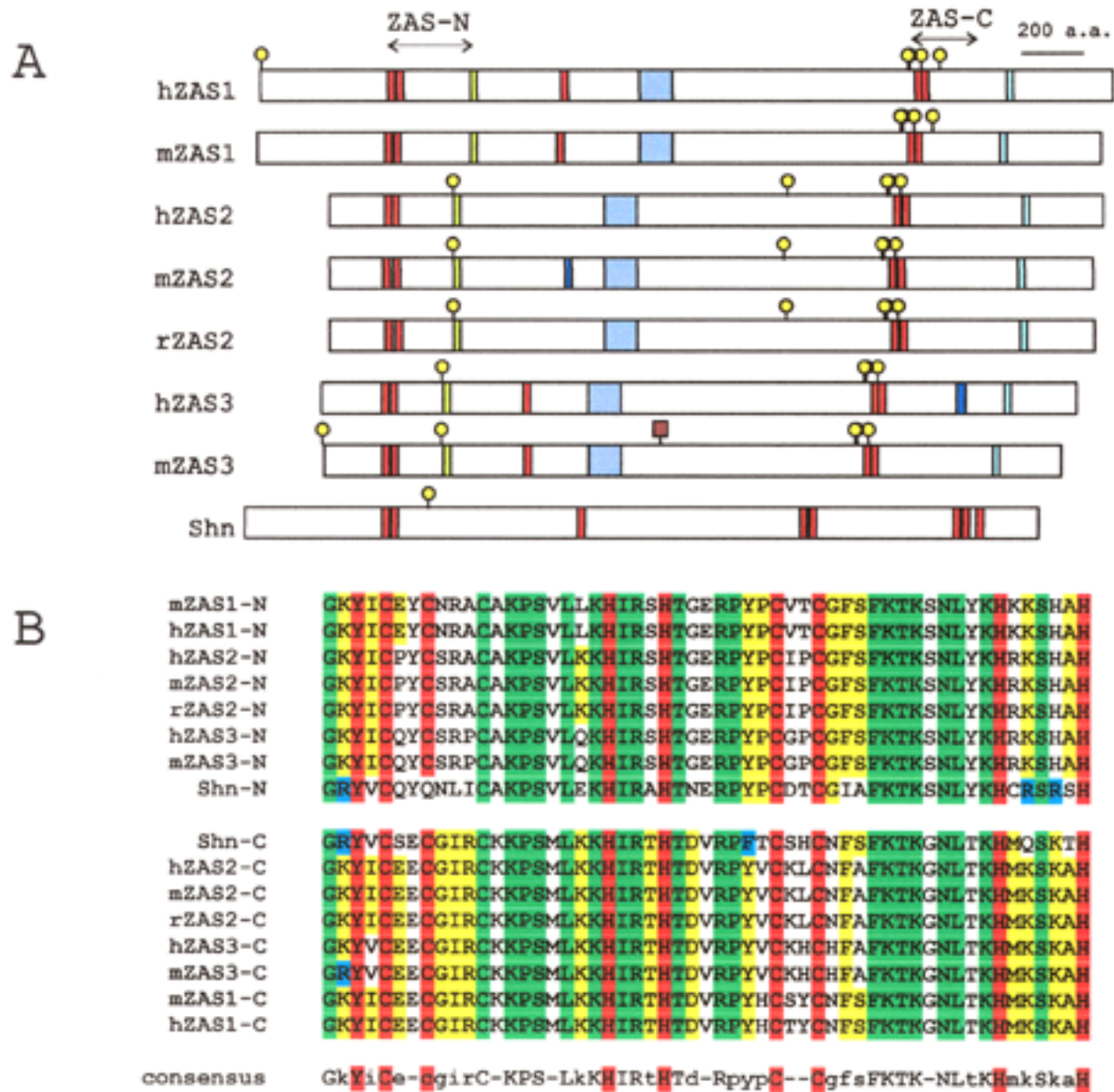


Figure 1. The protein structure of the ZAS gene family. (A) Protein domains and motifs. Red boxes, C_2H_2 zinc fingers; green boxes, serine-rich regions; purple boxes, conserved middle region; light blue boxes, conserved linker region; dark blue boxes, leucine zippers; square lollipop, GTPase motif; and yellow lollipops, tyrosine phosphorylation sites. (B) Conservation of the zinc finger pairs. Completely conserved residues are shown in green, identical residues are shown in yellow, similar residues are shown in cyan, and different residues are shown in white. In the consensus sequence, identical residues are shown in upper cases, similar residues are shown in lower cases, and gaps are nonconserved residues.

unknown. The DNA binding affinity of a fusion protein containing the ZAS-C domain of mZAS3 was increased ninefold after phosphorylation by the epidermal growth factor receptor kinase, a tyrosine kinase (6). In addition, a 115-kDa mZAS3 protein isoform has been shown to be a tyrosine-phosphorylated protein because antibodies against phospho-tyrosine abolished its DNA binding in electrophoretic mobility shift assays (79). Of interest, the DNA binding affinity of the 115-kDa protein also decreased upon

upregulation of *RAG1/2* or activation of V(D)J recombination, suggesting that the function of mZAS3 may be related to the developmental stages of lymphocytes (79). Furthermore, there are 7 to 12 copies of the Ser/Thr/ProXArg/Lys (SPXK) motif in each ZAS protein (80). SPXK motifs are frequently found in gene regulatory proteins (67). It can bind to AT-rich DNA sequences (13) and may be sites of protein phosphorylation (31). The protein domains and motifs present in the ZAS proteins are compatible with

their function in DNA binding and transcription regulation.

In addition to the domains or motifs shared by all ZAS proteins, some motifs are found in only one or two ZAS proteins. A leucine zipper, with one leucine in every six residues, is present in mZAS2 at residues 751–772. This motif may be involved in protein–protein interactions. A leucine zipper is also found in hZAS3 at residues 2024–2052. There are three conserved motifs for a GTPase present in mZAS3 including an ATP/GTP binding site motif A (P-loop) GGPSGGKS (at residues 1076–1083), DSEGQ (at residues 1798–1802), and TKND (at residues 1951–1954) (80). The presence of these GTPase motifs suggests that mZAS3 might use GTP as an energy source or as an allosteric effector. Specific motifs may confer additional functions to individual ZAS proteins.

TISSUE DISTRIBUTION

In adult mouse tissues, *ZAS1* and *ZAS2* are ubiquitously expressed (12,47), whereas *ZAS3* is only expressed in lymphoid and neural tissues (38,80,81). In addition, the expression of all three ZAS genes can be induced by mitogens, such as PHA/PMA and lipopolysaccharide, suggesting that they may be nuclear targets for cell signaling (8,25,48,51). Furthermore, the ZAS genes are probably involved in ontogenesis because their transcripts are detected in developing mouse embryos by in situ hybridization experiments (18,25,69). However, the embryonic stage at which significant amounts of transcripts are detected for each gene differs: *ZAS1* is expressed first at E8.5 (69), *ZAS2* second at E10.5 (18), and *ZAS3* last at E13.5 (25). In addition, *ZAS1* and *ZAS2* are expressed in a variety of developing tissues, whereas *ZAS3* is mainly expressed in the trigeminal ganglion, dorsal root ganglia, cerebral cortex, and thymus (25). In late-stage embryos, starting from E16.5, the expression of *ZAS3* is higher in the thymic cortex than in the medulla. The developing thymus cortex contains largely pro-T and pre-T lymphocytes undergoing the first V(D)J recombination to generate their TCR chains. As the cortical thymocytes mature, they migrate towards the medulla. Therefore, *ZAS3* is expressed in a region of the thymus where V(D)J recombination is taking place. The different spatial and temporal expression pattern of *ZAS3*, compared with those of *ZAS1* or *ZAS2*, suggests that *ZAS3* may perform specific functions during lymphoid and neural development. These observations also suggest that the regulation of the ZAS genes has diverged during evolution.

PROTEIN ISOFORMS AND ALTERNATIVE RNA PROCESSING

The largest open reading frame of each ZAS protein contains two ZAS domains. Immunoblot analysis showed that in addition to a large protein of >200 kDa, two or more smaller proteins reacted with antibodies against *ZAS1* (30) or *ZAS3* (4,79). Those smaller proteins can be protein isoforms generated by alternative splicing and/or protein processing. Multiple *ZAS1* and *ZAS3* transcripts, some expressed in tissue-specific manners, have been detected by Northern blot analysis (12,44,47,80,81). Alternative splicing events of *ZAS1* and *ZAS3* have been shown by characterization of cDNAs, ribonuclease protection experiment, and RT-PCR (38,46).

Alternative splicing of mZAS3 involves exon 4, exon 6, and an unusual alternative splicing event involving a sequence within exon 4 (38). As for other ZAS genes, exon 4 of mZAS3 is gigantic of 5487 bases in size (see below). Exon 4 of mZAS3 encodes the amino 1654 amino acid residues, including the ZAS-N domain. Because this exon contains the putative translation initiation site, mZAS3 transcripts lacking exon 4 probably initiate translation at an alternate site in an upstream exon and encode truncated proteins lacking the ZAS-N domain. Furthermore, some mZAS3 transcripts lack a sequence of 459 nucleotides within exon 4. Significantly, that sequence encodes the ZAS-N domain. In addition, it starts with “GC” and ends with “AG,” dinucleotides matching a variant splice donor site and the consensus splice acceptor site, respectively. Therefore, that 459 nucleotide sequence can be regarded as an unusual intron, which, when retained in mRNA, encodes a DNA- and protein-interacting structure. Because alternative splicing of the corresponding regions has not been shown in *ZAS1*, *ZAS2*, or *Shn*, the described sequence within exon 4 of mZAS3 represents a novel conversion of exon sequences into an intron during evolution. Exon 6 contains 176 nucleotides and encodes zinc finger 4 and part of zinc finger 5. The alternative splicing of exon 6 occurs in a cell-specific manner: it is present in most thymic transcripts but is mostly spliced out from brain transcripts (38).

Alternative splicing of *ZAS1* also involves sequences encoding the ZAS-N or ZAS-C regions (46). The alternative splicing events, defined for *ZAS1* and *ZAS3*, would lead to the production of truncated proteins containing only the ZAS-N domain, only the ZAS-C domain, or no ZAS domain at all. These truncated proteins would be expected to have DNA binding properties and transcriptional functions different from those of the full-length proteins. In this context, it has been shown that the full-length *ZAS1* activates the HIV-LTR enhancer (61), whereas the two smaller

isoforms, lacking the ZAS-N or ZAS-C domains, repress transcription (46).

Alternate transcription termination has been found to occur only in *hZAS3*. The human *ZAS3* gene has an additional 310-bp exon, exon 5b, between exon 5 and exon 6 (24). Exon 5b was probably inserted into *hZAS3* after divergence and speciation because no homologous sequences were identified in *mZAS3* or other family members. Exon 5b encodes 69 amino acid residues, a 3' UTR of 103 nucleotides, and a hexanucleotide AAUAAA located 39 nucleotides from the 3' end. For most eukaryotic mRNAs, the sequence AAUAAA is present in similar 3' location and serves as a signal for polyadenylation. Therefore, transcription termination of *hZAS3* can occur at the last exon (exon 9) or exon 5b, producing a longer 9.5-kb transcript or a shorter 7-kb transcript, respectively. The shorter transcript lacks the last four exons of *hZAS3* and encodes a truncated protein without the ZAS-C domain. Because alternative splicing of *hZAS3* also involves exon 6 (24), proteins with similar carboxyl-terminal truncations can be generated by splicing out exon 6 or by transcription termination at exon 5b.

FUNCTION IN TRANSCRIPTION AND SIGNAL TRANSDUCTION

The ZAS proteins carry out both transcriptional and nontranscriptional functions by binding directly to DNA targets or by associating with other transcription factors or signaling molecules. Human *ZAS1* and *mZAS3* have been shown to activate transcription of HIV-LTR and the metastasis-associated gene *S100A4/mts1*, respectively, in reporter gene assays (27,61). In cells stably transfected with a *ZAS3* expression vector, the expression of both endogenous *S100A4/mts1* and reporter genes under the control of the *S100A4/mts1* composite enhancer were upregulated (27). Further analysis showed that *mZAS3* antibodies can recognize a DNA-protein complex formed between nuclear extracts, prepared from *S100A4/mts1*-expressing cells, and a *S100A4/mts1* enhancer element, Sb, suggesting that *ZAS3* contributes to the regulation of the transcription of *S100A4/mts1* via Sb, a κ B-like sequence (16,27).

In addition to binding directly to target sites, ZAS can regulate transcription through the association with other transcriptional proteins. *Shn*, in response to *dpp*, associates with Mad/med and represses the transcription of *Brinker* (41,72). *Brinker* encodes a nuclear protein, which serves as a feedback regulator to *dpp* signaling [review in (2)]. Mouse *ZAS2* associates with a basic helix-loop-helix transcription factor, SEF-2, and together they activate the *SSTR2* pro-

motor (18). Finally, *mZAS1* binds to the *Col2 α 1* promoter, inhibits further binding of the transcription activator Sox9 to a nearby target site, and thereby represses *Col2 α 1* gene expression (69). The large size and the presence of multiple domains and motifs in the ZAS proteins probably allow them to perform multifaceted functions.

Shn mediates the signaling of *dpp*, a TGF- β homologue. Recently, the TNF receptor-associated factor 1 (TRAF1) has been identified as a *ZAS3*-associated protein in a yeast two-hybrid library screen, suggesting that the mammalian ZAS proteins may be involved in signal transduction as well (51). Further studies show that *ZAS3* negatively regulates the expression and signaling of TNF- α (51). *ZAS3* represses TNF- α signaling by inhibiting both NF- κ B-dependent transactivation and c-Jun amino-terminal kinase (JNK) phosphorylation. The effect of *ZAS3* was mediated partially through its interaction with TRAF2, an adaptor protein that transduces the interaction of plasma membrane receptors with specific ligands to NF- κ B and JNK signaling (82). Consistent with the ability of a NF- κ B-dependent signal to protect against TNF- α -induced apoptosis (53), overexpression of *ZAS3*, which represses NF- κ B-signaling, results in increases of TNF- α -induced apoptosis (51). Therefore, *ZAS3* plays a critical role in controlling growth, inflammatory and apoptotic responses via NF- κ B activation.

CONTROL OF NORMAL GROWTH

Although *ZAS3* is expressed specifically in normal lymphoid and neural tissues, its expression, mostly alternatively spliced transcripts, has been detected in cell lines of diverse origins. The mechanism and implication of aberrant expression of *ZAS3* in cell lines are unknown. In cell lines, disruption of *ZAS3* expression by long-term transfection with a vector directing the expression of *ZAS3* antisense transcripts resulted in accelerated cell proliferation (4). In the human epithelial cell line HeLa, downregulation of *ZAS3* further conferred anchorage independence of growth and promoted nuclear division without cell division, which led to the formation of multinucleated giant cells that eventually underwent mitotic cell death (4). The accelerated growth rate and abnormal growth phenotypes observed in *ZAS3*-deficient cells suggest that *ZAS3* probably inhibits abnormal cell growth, by monitoring and slowing down the cell cycle and/or by facilitating apoptosis. A role in controlling growth has also been shown in *Shn*. *Shn* expressed in cyst cells restricts proliferation of adjacent spermatogonial cells (42). Furthermore, allelic loss on human chromosome at 1p34, the chromosomal locus of *ZAS3*, has been frequently reported in a variety

of tumors, including breast cancer (19,26,76,77), liver cancer (35,52,66), and B cell lymphoma (10,36). Furthermore, changes in gene expression of *ZAS1* and *ZAS2* have been associated with poor prognosis in chronic lymphocytic leukemia patients having the 11q23 deletion (1). Therefore, appropriate expression of the ZAS proteins, at least in the case of *ZAS3*, may be required to maintain normal growth.

Evidence generated in animal models substantiates that ZAS plays an important role in growth control and could represent an important factor in the etiology of human diseases. Homozygous *ZAS3* embryonic stem (*ZAS3*^{-/-} ES) cells were generated by homologous recombination (8). These cells were used to perform the RAG2 complementation experiment to determine whether *ZAS3* is important in lymphoid development. The results showed that although the lymphoid compartments were reconstituted in younger chimera, the CD4⁺CD8⁺ double positive thymocytes were depleted in older mice. A progressive defect of thymocyte development, a block in the differentiation of immature CD4⁺CD8⁺ double positive cells into mature single positive cells, was also observed in *ZAS2* knockout mice (68). Evidently, the ZAS genes may play vital roles in the development of thymocytes. One unexpected finding in the above experiments was the development of tumor. The RAG2^{-/-}*ZAS*^{-/-} chimeric mouse with the highest *ZAS3*^{-/-} ES cell contribution developed an axillary tumor spontaneously (10). That tumor grew aggressively from barely detectable at 3 weeks of age to more than 20% of the total body mass by 5 weeks. DNA analysis showed that the tumor mass was entirely derived from *ZAS*^{-/-} ES cells. The tumor was classified as a malignant teratoma due to the presence of malignant neoplasms that contain poorly differentiated carcinoma cells and ectopic existence of different tissues, including hyaline cartilage, skeletal muscle, fat, and primitive neural elements. The formation of a tumor in a *ZAS3*-deficient mouse suggests that *ZAS3* might prevent neoplasia. Taken together, the phenotypes observed for *ZAS3* deficiency in a different context, abnormal growth in cell lines and tumor formation in mice, suggest that *ZAS3* is involved in controlling normal growth.

PHYLOGENY

Other than those highly conserved regions, the overall sequences of paralogous ZAS proteins have relatively low homologies (Fig. 2). Among orthologous proteins, the divergence of *ZAS1* is the most and *ZAS2* is the least. Because only the *ZAS2* protein in rats is available, when this sequence was incorpo-

rated with the others in the phylogenetic analysis, the program erroneously placed *ZAS2* into a group evolutionarily distinct from the others (compare Fig. 2B and C). In addition, the sequence homology between *hZAS3* and *mZAS3* is more significant in the protein-coding region than in the untranslated regions (Fig. 3), in line with higher mutation rates of nonprotein-coding DNA. Among the ZAS proteins, significant sequence homology, with more than 50% identity, is further limited to the region between ZAS-N and ZAS-C domains and the linker region. Furthermore, the sequence homology between *ZAS3* and *ZAS1* is disrupted to below 50% after the middle conserved region and before zinc finger 4. Therefore, more in-depth sequence analysis suggests that *ZAS3* is more related to *ZAS2* than to *ZAS1*. Finally, only the first two zinc finger pairs of Shn show significant homology to the ZAS protein in that sequence alignment. The conservation of the zinc fingers from invertebrates to vertebrates suggests that the ZAS proteins share conserved DNA binding functions.

GENE STRUCTURES

The structures of the human *ZAS1*, *ZAS2*, and *ZAS3* genes, determined by comparing their cDNA with genomic DNA sequences, are largely similar (Fig. 4). Each gene spans a relatively large genomic region of over 200 kb and contains at least 10 exons. Notably, exon 4 of each gene is unusually large, ranging from 5574 nucleotides to 5981 nucleotides. Considering that the average vertebrate exon size is 137 bp and that very few are greater than 600 bp in length, the size of exon 4 of ZAS is striking. Each exon 4 of *ZAS2* and *ZAS3* contains 5' untranslated sequences. However, exon 4 of *ZAS1* is completely protein coding. The putative translation codon of *ZAS1* is at exon 2. Therefore, the composition of exon 4 suggests that *ZAS3* and *ZAS2* are more similar to each other than to *ZAS1*. As discussed above, alternative splicing of exon 4 have been shown for *ZAS1* and *ZAS3* transcripts (38,46). Such transcripts encode truncated proteins without the ZAS-N domain. Upstream of exon 4 are several small exons that spread over a relatively large genomic region. There are no significant homologies of those upstream exons among paralogous ZAS genes or when compared with those of Shn. Exons upstream of exon 4 of individual ZAS genes, therefore, may be acquired after gene duplications or have diverged significantly during evolution. Although the 5' ends of the ZAS genes have not been reported, the 5' untranslated regions (5'-UTR) for *rZAS2* and *hZAS3* are at least 766 and 888 nucleotides, respectively. The average length of

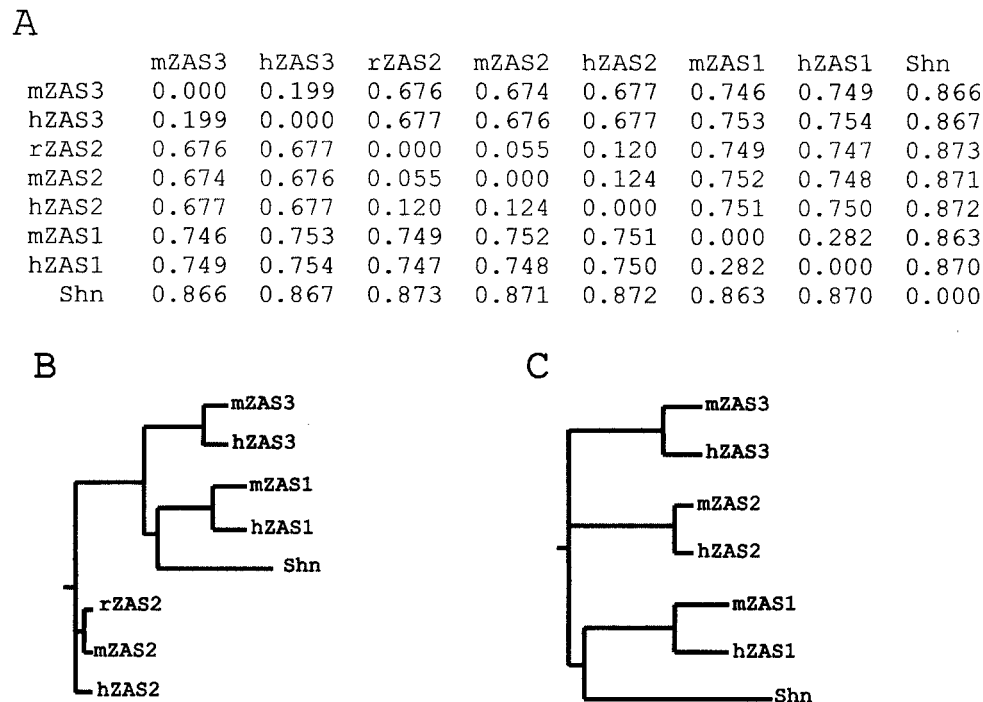


Figure 2. Phylogenetic analyses of the ZAS protein family. (A) Distance matrix scores between ZAS proteins. h, human; m, mouse; and r, rat. (B) Phylogenetic tree of seven full-length ZAS proteins and Shn. (C) Phylogenetic tree of human and mouse ZAS proteins and Shn.

5'-UTR of human transcripts is 210 nucleotides and rodent is 186 nucleotides (43). Therefore, the 5'-UTR of the ZAS genes are relatively long. Although a solitary zinc finger is found in ZAS1 and ZAS3 but not in ZAS2, sequence homology and composition of exon 4 suggest that ZAS2 and ZAS3 are more similar to each other than to ZAS1. The exon-intron organization of Shn is different from those of the mammalian ZAS genes (Fig. 4).

The corresponding exons 5, 6, and 7 of paralogous and orthologous ZAS genes are highly conserved with respect to their size, sequence, and exon-intron boundaries. Conservation of the exon-intron boundaries and alternative splicing events involving one or more of these exons suggest that the function of the ZAS genes is subjected to the control of RNA processing. As described above, *hZAS3* has an additional exon, exon 5b, inserted between exon 5 and exon 6. Such an exon is not found in *mZAS3* or in any other family members. Furthermore, Southern blot analysis suggests that sequences homologous to exon 5b of *hZAS3* are present only in primates. Therefore, the evolution of the ZAS genes is dynamic in that each gene has acquired different upstream exons, and with insertion of an internal exon (exon 5b of *hZAS3*) and conversion of part of an exon into an intron (in the case of *mZAS3*) for individual genes.

THE LINKAGE OF ZAS WITH *ENDOTHELIN* AND *TRANSCRIPTION FACTOR AP2*

Human *ZAS1*, -2, and -3 have been mapped to chromosomal loci on 6p24, 6q24, and 1p34, respectively (24,50,65). Another gene family of potent vasoactive hormones, *endothelins*, also consists of three members, *EDN1*, *EDN2*, and *EDN3*. The *EDN* genes are located on different chromosomes, at 6p23-p24, 1p34, and 20q13, respectively. Notably, *ZAS1-EDN1* and *ZAS3-EDN2* are mapped to the same chromosomal loci, respectively. The close linkage of *ZAS1* and *EDN1* has been confirmed by a large-scale gene map constructed using expression sequence tagged (EST) sequences to array PAC clones on a YAC clone backbone on the human 6p23-p25 region (50). That gene map contains a contig of genes in the order: *cen-CD83-ZAS1-EDN1-TFAP2 α -BMP6-DSP-tel*. A comparison of the genomic DNA sequence (accession number Z98050; Sanger Center, Cambridge, England) with the cDNA sequences of *ZAS1* (accession number nm_002114) and *EDN1* (accession number nm_001955) establishes that *ZAS1* is located 125 kb upstream of *EDN1* in the same transcriptional orientation (Fig. 4). Similar sequence analysis has established that the *ZAS3* and *EDN2* genes are only ~25 kb apart. Probably, mouse *ZAS1-EDN1* and *ZAS3-*

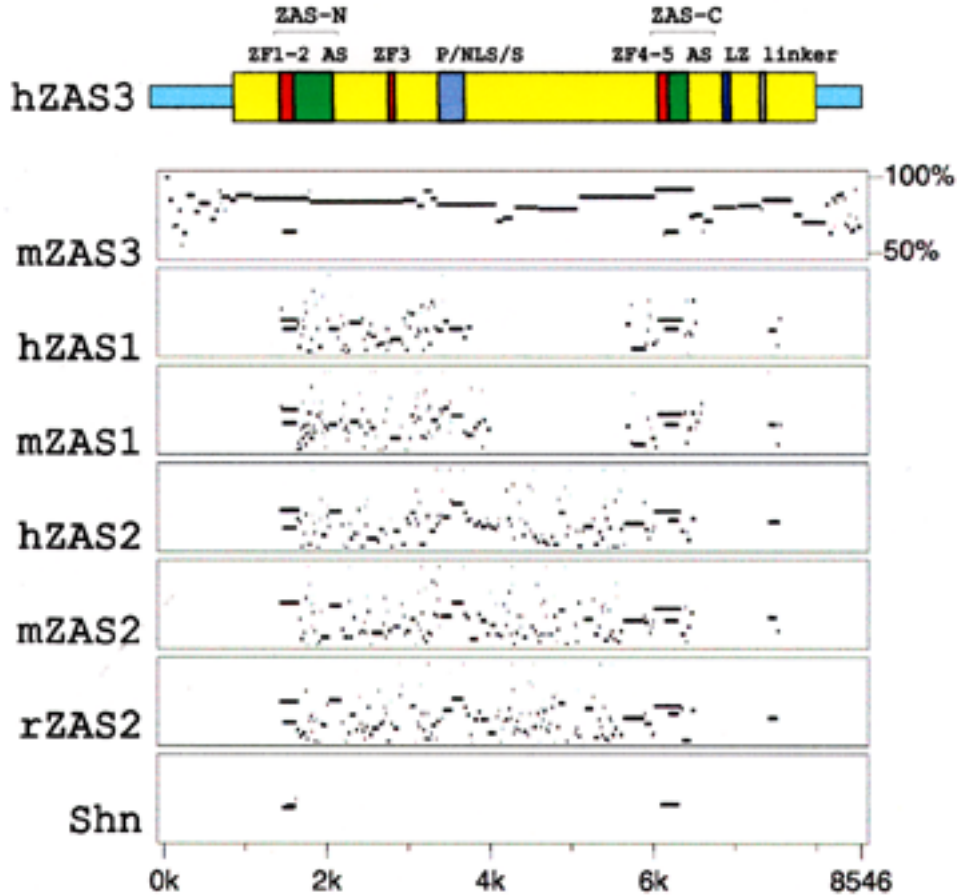


Figure 3. Conservation of the protein-coding regions of the ZAS genes. The sequence of the longest cDNA of each ZAS gene was retrieve from the GenBank databases and compared with the hZAS3 cDNA using the computer program PipMaker (60). The corresponding hZAS3 protein is shown at the top as a reference and a graphical output of the sequence alignments is shown below. The x-axis shows the nucleotide numbering of the hZAS3 cDNA sequence. Protein-coding region is shown in thicker box and untranslated regions are shown in thinner boxes. ZF, zinc finger; P/NLS/S, prolines/nuclear localization signal/serines; AS, acidic residues and serine-rich region; and LZ, leucine zipper.

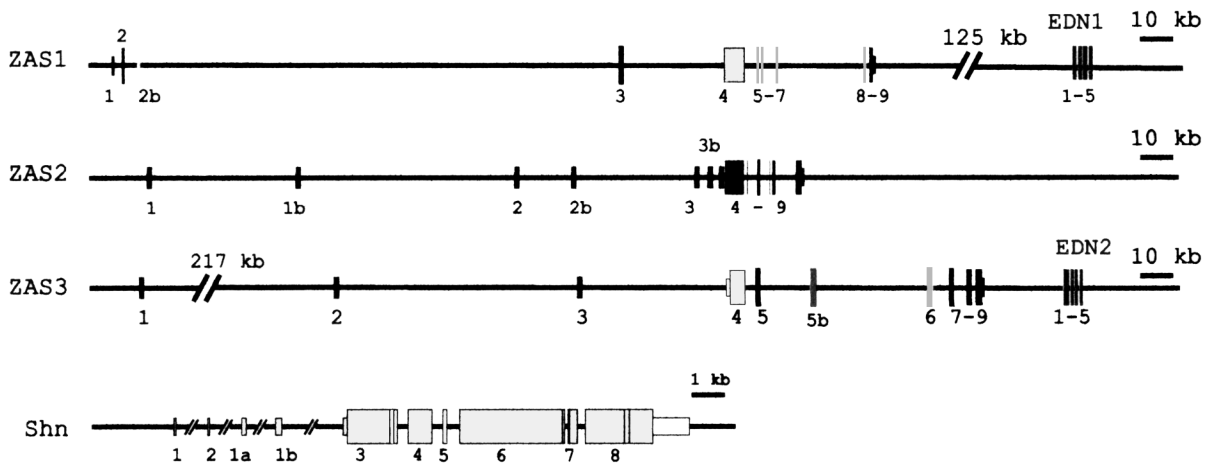


Figure 4. The exon-intron organization of the mammalian ZAS and *Shn* genes. Thicker boxes or lines represent protein-coding sequences and thinner boxes or lines represent untranslated sequences. Exons that have been shown to be involved in alternative splicing are shown in red. The additional transcription termination exon, exon 5b, of hZAS3 is shown in green. The regions in *Shn* encoding for zinc fingers are shown in yellow. *EDN*, endothelin gene.

TABLE 3
CHROMOSOMAL LOCI OF *ZAS*, *EDN*, AND *TFAP2* GENES
IN HUMANS AND MICE

Genes	Human	Mouse
<i>ZAS1</i>	6p24	13 24.0 cM
<i>EDN1</i>	6p24	13 26.0 cM
<i>TFAP2α</i>	6p24	13 25.0 cM
<i>ZAS2</i>	6q23-q24	not available
<i>EDN3</i>	20q13	2 104.0 cM
<i>TFAP2γ</i>	20q13	2 H3-H4
<i>ZAS3</i>	1p34	4 52.00 cM
<i>EDN2</i>	1p34	4 1.00 cM
<i>TFAP2β</i>	6p12	1 A2-A4

EDN2 are neighboring genes as well because each gene pair has been mapped at close loci in chromosome 13 and chromosome 4, respectively (Table 3). The close linkage of the *ZAS* and *EDN* genes suggests that the two gene families likely have evolved together.

Another gene, transcription factor (*TF*)*AP2 α* , originally mapped within the described 6p23-p25 contig, also belongs to a three-member gene family. The *TFAP2* gene family includes three human genes, *TFAP2 α* , *TFAP2 β* , and *TFAP2 γ* . The chromosomal locations of members of human *ZAS*, *EDN*, and *TFAP2* families are shown in Figure 5 and Table 3. The nine genes are distributed into different linkage

groups on three human chromosomes: *ZAS3-EDN2* are on chromosome 1; *ZAS2*, *TFAP2 β* , and *ZAS1-EDN1-TRAP2 α* are on chromosome 6; and *TFAP2 γ -EDN3* are on chromosome 20. Using those gene loci as bearings, we propose an evolution scheme of these three gene families from a single progenitor gene cluster consisting of *-ZAS-EDN-TFAP2-* (Fig. 6). First, tandem gene duplications of the progenitor gene cluster formed three gene clusters (referred to as homologous clusters hereafter). Second, DNA breaks occurred between and within the homologous clusters. Third, chromosomal fragments containing *TFAP2 γ -EDN3* or *ZAS3-EDN2* were excised, translocated to different chromosomes, and eventually became chromosome 20 and chromosome 1, respectively. The original broken chromosome further underwent an inversion event including *ZAS2* and the centromere. This event is classified as a pericentric chromosome inversion. Finally, the broken chromosomal pieces religated and evolved to form chromosome 6.

The proposed evolutionary scheme suggests that homologous clusters of *-ZAS-EDN-TFAP2-* were expanded by tandem duplications of an ancestral gene cluster. Although it is possible that genes could be shuffled and brought together fortuitously during evolution, the fact that the *ZAS*, *EDN*, and *TFAP2* genes form more than one linkage group makes this possibility unlikely. Comparative genome analysis

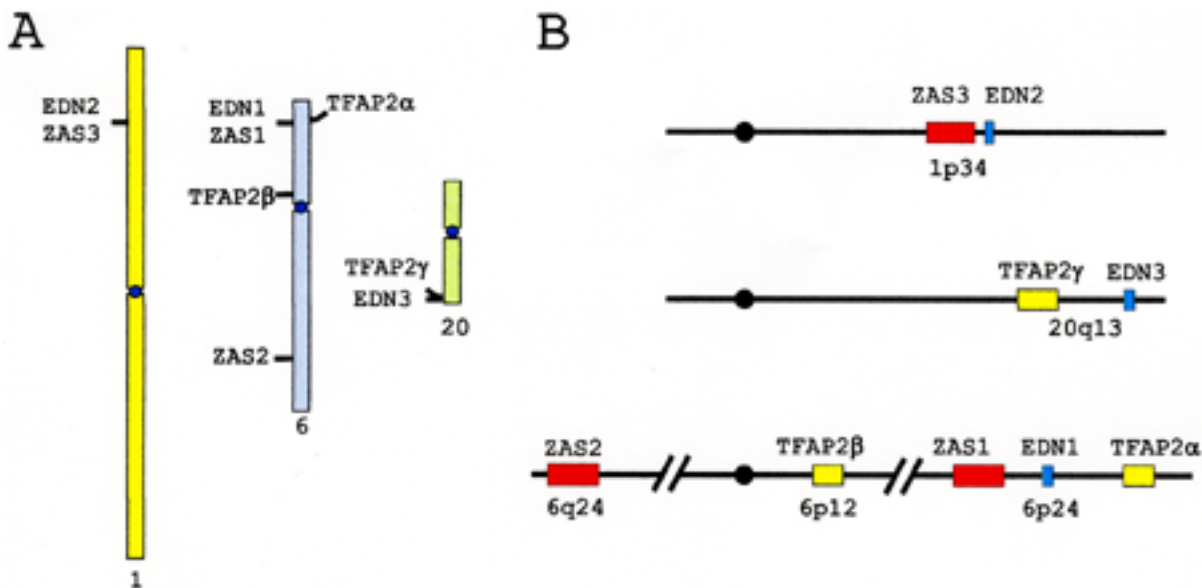


Figure 5. The chromosomal locations of the human *ZAS*, *EDN*, and *TFAP2* genes. (A) Gene loci distributed on three chromosomes. (B) Relative locations of the genes showing different linkage groups. To enhance the visual, *ZAS* genes are shown in red, *TFAP2* genes are shown in yellow, and *EDN* genes are shown in blue.

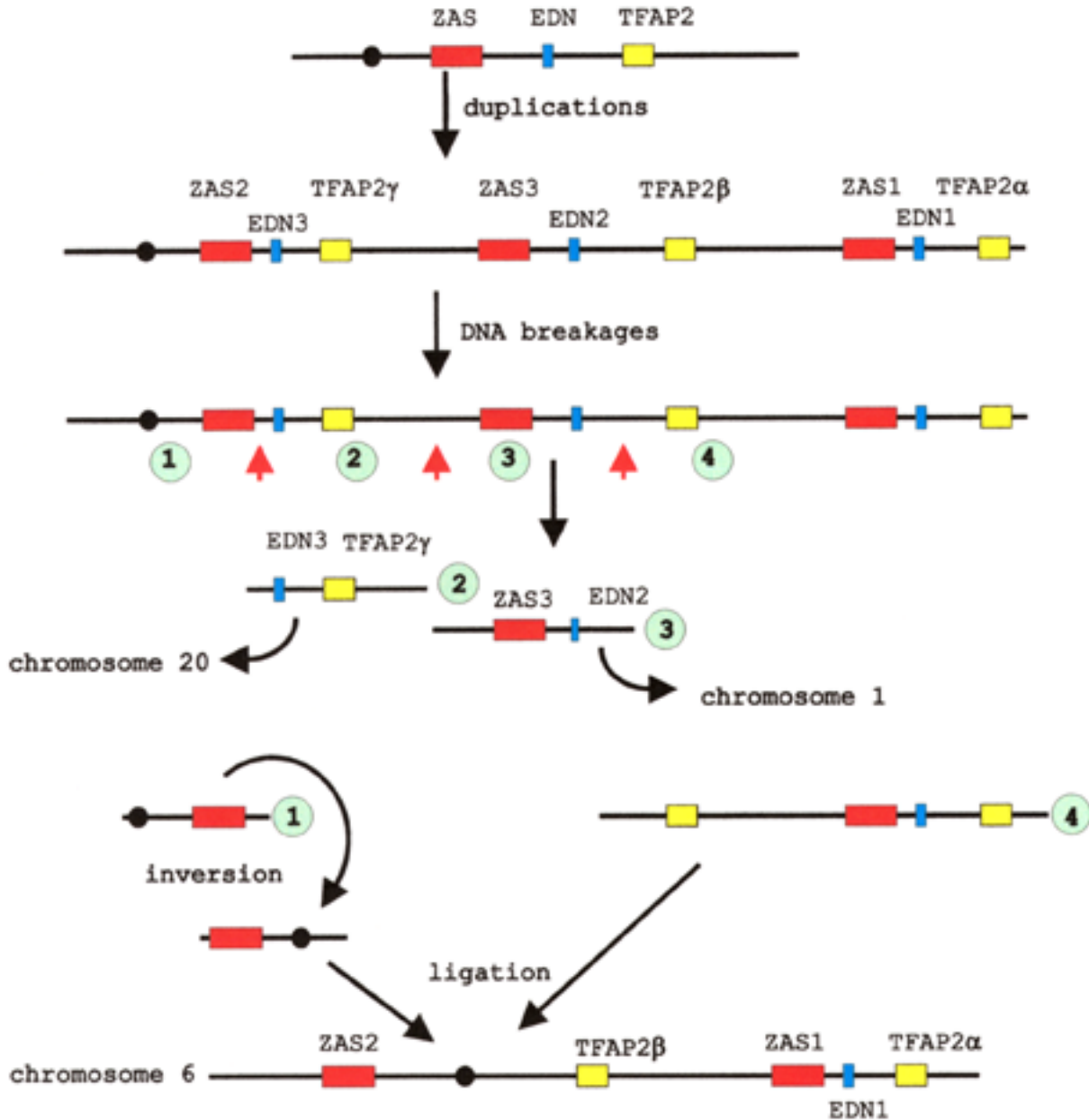


Figure 6. A hypothetical evolutionary scheme of the *ZAS*, *EDN*, and *TFAP2* gene families. Red arrows represent DNA breakages, black circles represent centromeres, and a curved arrow represents a pericentric chromosome inversion.

has suggested two rounds of total genome doubling in early vertebrate evolution (49). In the case of whole chromosome duplications, one of the tetraploidy chromosomes needed to be totally or with the region containing a homologous cluster lost. In addition, *TFAP2 β* and *ZAS2* would be excised from their corresponding homologous clusters from chromosomes 1 and 20, respectively. However, the possibility of having both genes to be incorporated into chromosome 6 is remote. Therefore, at least for the part of the genome containing the ancestral *ZAS*, *EDN*,

and *TFAP2* gene cluster, the vertebrate genomes were evolved from the invertebrate genomes by tandem duplications of subchromosomal regions, which was followed by a relatively short period of extensive DNA breakage and translocation.

CONCLUSIONS AND FUTURE PROSPECTS

The *ZAS* proteins are transcriptional proteins with structurally distinct separate C_2H_2 zinc finger pairs

that bind the κ B motif. Recent results show that they are important transcription factors regulating specific gene expression and are adaptor molecules in TNF and dpp/TGF- β signal transduction pathways. Because of their large size and the presence of many structural domains for DNA-protein and protein-protein interactions, we envision that they can serve as docking sites for proteins to perform vital cellular processes. As would be expected for genes that are involved in controlling growth and development, the expression of *ZAS* is subjected to complex regulations: induction by mitogens, formation of protein isoforms (probably with antagonistic functions to full-length proteins), and modification by various posttranslational mechanisms or signals. Experimental evidence showing that *ZAS3* is involved in controlling growth includes accelerated cell growth and tumorigenesis as outcomes of *ZAS3* deficiency. The frequent deletion of the chromosomal region where *ZAS3* resides in a variety of tumors also favors the notion that *ZAS3* may function as a tumor suppressor gene. Further dissection of the functional

roles of the *ZAS* proteins, identification of their target genes, and definition of their role in regulating transcription and cell signaling will advance our understanding in transcription regulation and tumorigenesis.

The reconstruction of the phylogenetic history of genomes is important in understanding the evolution of genetic complexity. One application of the human genome project is to define the chromosomal locations of genes. Knowledge about the locations and linkages of the *ZAS*, *EDN*, and *TFAP2* genes is instrumental to a retrocognition of the evolution of these gene families. Our scheme suggests that the expansion of the vertebrate genome could be partially due to tandem duplication of gene clusters. At present, there is no obvious relationship in the expression or function among those linked genes. Whether there is an advantage of maintaining some of the linkage among members of the *ZAS*, *EDN*, and *TFAP2* gene families or the remaining links are merely fading footprints of evolution is an interesting question that remains to be answered.

REFERENCES

1. Aalto, Y.; El-Rifa, W.; Vilpo, L.; Ollila, J.; Nagy, B.; Vihinen, M.; Vilpo, J.; Knuutila, S. Distinct gene expression profiling in chronic lymphocytic leukemia with 11q23 deletion. *Leukemia* 15:1721–1728; 2001.
2. Affolter, M.; Marty, T.; Vigano, M. A.; Jazwinska, A. Nuclear interpretation of Dpp signaling in *Drosophila*. *EMBO J.* 20:3298–3305; 2001.
3. Akira, S.; Okazaki, K.; Sakano, H. Two pairs of recombination signals are sufficient to cause immunoglobulin V-(D)-J joining. *Science* 238:1134–1138; 1987.
4. Allen, C. E.; Wu, L. C. Downregulation of the large zinc finger protein KRC promotes cell growth, disrupts a cell cycle check point and induces mitotic cell death. *Exp. Cell Res.* 206:346–356; 2000.
5. Arora, K.; Dai, H.; Kazuko, S. G.; Jamal, J.; O'Connor, M. B.; Letsou, A.; Warrior, R. The *Drosophila* schnurri gene acts in the Dpp/TGF beta signaling pathway and encodes a transcription factor homologous to the human MBP family. *Cell* 81:781–790; 2002.
6. Bachmeyer, C.; Mak, C. H.; Yu, C. Y.; Wu, L. C. Regulation by phosphorylation of the zinc finger protein KRC that binds the kappa B motif and V(D)J recombination signal sequences. *Nucleic Acids Res.* 27:643–648; 1999.
7. Baeuerle, P. A.; Henkel, T. Function and activation of NF-kappa B in the immune system. *Annu. Rev. Immunol.* 12:141–179; 1994.
8. Baldwin, A. S.; LeClair, K. P.; Singh, H.; Sharp, P. A. A large protein containing zinc finger domains binds to related sequence elements in the enhancers of the class I major histocompatibility complex and kappa immunoglobulin genes. *Mol. Cell. Biol.* 10:1406–1414; 1990.
9. Basson, M.; Horvitz, H. R. The *Caenorhabditis elegans* gene sem-4 controls neuronal and mesodermal cell development and encodes a zinc finger protein. *Genes Dev.* 10:1953–1965; 1996.
10. Benn, D. E.; Dwight, T.; Richardson, A. L.; Delbridge, L.; Bambach, C. P.; Stowasser, M.; Gordon, R. D.; Marsh, D. J.; Robinson, B. G. Sporadic and familial pheochromocytomas are associated with loss of at least two discrete intervals on chromosome 1p. *Cancer Res.* 60:7048–7051; 2000.
11. Boguski, M. S.; Schuler, G. D. ESTablishing a human transcript map. *Nat. Genet.* 10:369–371; 1995.
12. Brady, J. P.; Kantorow, M.; Sax, C. M.; Donovan, D. M.; Piatigorsky, J. Murine transcription factor alpha A-crystallin binding protein I. *J. Biol. Chem.* 270:1221–1229; 1995.
13. Brzeski, J.; Grycuk, T.; Jerzmanowski, A.; Lipkowski, A. W.; Rudnicki, W.; Lesyng, B. Binding of SPKK- and APKK-peptide motifs to AT-rich DNA. Experimental and theoretical studies. *Acta Biochim. Pol.* 45:221–231; 1998.
14. Cauley, K.; Verma, I. M. Kappa B enhancer-binding complexes that do not contain NF-kappa B are developmentally regulated in mammalian brain. *Proc. Natl. Acad. Sci. USA* 91:390–394; 1994.
15. Chen, F.; Castranova, V.; Shi, X.; Demers, L. M. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. *Clin. Chem.* 45:7–17; 1999.
16. Cohn, A. M.; Hjelmsoe, I.; Wu, L. C.; Guldberg, P.; Lukanidin, E. M.; Tulchinsky, E. M. Characterization

- of Sp1, AP-1, CBF, KRC binding sites and minisatellite DNA as functional elements of the metastasis-associated mts1/S100A4 gene intronic enhancer. *Nucleic Acids Res.* 29:3335–3346; 2001.
17. Dai, H.; Hogan, C.; Gopalakrishnan, B.; Torres-Vazquez, J.; Nguyen, M.; Park, S.; Raftery, L. A.; Warrior, R.; Arora, K. The zinc finger protein schnurri acts as a smad partner in mediating the transcriptional response to decapentaplegic. *Dev. Biol.* 227:373–387; 2000.
 18. Dörflinger, U.; Pscherer, A.; Moser, M.; Rummele, P.; Schüle, R.; Buettner, R. Activation of somatostatin receptor II expression by transcription factors MIBP1 and SEF-2 in the murine brain. *Mol. Cell. Biol.* 19:3736–3747; 1999.
 19. Emi, M.; Yoshimoto, M.; Sato, T.; Matsumoto, S.; Utada, Y.; Ito, I.; Minobe, K.; Iwase, T.; Katagiri, T.; Bando, K.; Akiyama, F.; Harada, Y.; Fukino, K.; Sakamoto, G.; Matsushima, M.; Iida, A.; Tada, T.; Saito, H.; Miki, Y.; Kasumi, F.; Nakamura, Y. Allelic loss at 1p34; 13q12; 17p13.3; and 17q21.1 correlates with poor postoperative prognosis in breast cancer. *Genes Chromosomes Cancer* 26:134–141; 1999.
 20. Fan, C. M.; Maniatis, T. A DNA-binding protein containing two widely separated zinc finger motifs that recognize the same DNA sequence. *Genes Dev.* 4:29–41; 1990.
 21. Fountain, J. W.; Dracopoli, N. C.; Housman, D. E.; Baldwin, A. S. MspI RFLP detected by a ZNF-40 cDNA sequence. *Nucleic Acids Res.* 19:2514; 1991.
 22. Ghosh, S.; May, M. J.; Kopp, E. B. NF-kappa B and Rel proteins: Evolutionarily conserved mediators of immune responses. *Annu. Rev. Immunol.* 16:225–260; 2002.
 23. Grieder, N. C.; Nellen, D.; Burke, R.; Basler, K.; Affolter, M. Schnurri is required for *Drosophila* Dpp signaling and encodes a zinc finger protein similar to the mammalian transcription factor PRDII-BF1. *Cell* 81:791–800; 1995.
 24. Hicar, M. D.; Liu, Y.; Allen, C. E.; Wu, L. C. Structure of the human zinc finger protein HIVEP3: Molecular cloning, expression, exon–intron structure, and comparison with paralogous genes HIVEP1 and HIVEP2. *Genomics* 71:89–100; 2001.
 25. Hicar, M. D.; Robinson, M. L.; Wu, L. C. Embryonic expression and regulation of the large zinc finger protein KRC. *Genesis* 33:8–20; 2002.
 26. Hirano, A.; Utada, Y.; Haga, S.; Kajiwara, T.; Sakamoto, G.; Kasumi, F.; Nakamura, Y.; Emi, M. Allelic losses as prognostic markers for breast cancers. *Int. J. Clin. Oncol.* 6–12; 2001.
 27. Hjelmsoe, I.; Allen, C. E.; Cohn, M.; Tulchinsky, E.; Wu, L. C. The kappa B and V(D)J recombination signal sequence binding protein KRC regulates transcription of the mouse metastasis associated gene mts1. *J. Biol. Chem.* 275:913–920; 2000.
 28. Iuchi, S. Three classes of C₂H₂ zinc finger proteins. *Cell. Mol. Life Sci.* 58:625–635; 2001.
 29. Kaltschmidt, C.; Kaltschmidt, B.; Neumann, H.; Wekerle, H.; Baeuerle, P. A. Constitutive NF-kappa B activity in neurons. *Mol. Cell. Biol.* 14:3981–3992; 1994.
 30. Kantorow, M.; Becker, K.; Sax, C. M.; Ozato, K.; Piatigorsky, J. Binding of tissue-specific forms of alpha A-CRYBP1 to their regulatory sequence in the mouse alpha A-crystallin-encoding gene: Double-label immunoblotting of UV-crosslinked complexes. *Gene* 131:159–165; 1993.
 31. Kennelly, P. J.; Krebs, E. G. Consensus sequences as substrate specificity determinants for protein kinases and protein phosphatases. *J. Biol. Chem.* 266:15555–15558; 1991.
 32. Knight, R. D.; Shimeld, S. M. Identification of conserved C₂H₂ zinc-finger gene families in the Bilateria. *Genome Biol.* 2:1–8; 2001.
 33. Korner, M.; Rattner, A.; Mauxion, F.; Sen, R.; Citri, J. A brain-specific transcription activator. *Neuron* 3:563–572; 1993.
 34. Lewis, S. M. The mechanism of V(D)J joining: Lessons from molecular, immunological, and comparative analyses. *Adv. Immunol.* 56:27–150; 1994.
 35. Li, S. P.; Wang, H. Y.; Li, J. Q.; Zhang, C. Q.; Feng, Q. S.; Huang, P.; Yu, X. J.; Huang, L. X.; Liang, Q. W.; Zeng, Y. X. Genome-wide analyses on loss of heterozygosity in hepatocellular carcinoma in Southern China. *J. Hepatol.* 34:840–849; 2001.
 36. Lucas, M. M.; Shi, X.; Rothstein, T. L. Abnormal kappa B-binding protein in the cytoplasm of a plasmacytoma cell line that lacks nuclear expression of NF-kappa B. *Mol. Immunol.* 30:479–489; 1993.
 37. Maekawa, T.; Sakura, H.; Sudo, T.; Ishii, S. Putative metal finger structure of the human immunodeficiency virus type 1 enhancer binding protein HIV-EP1. *J. Biol. Chem.* 264:14591–14593; 1989.
 38. Mak, C.-H.; Li, Z.; Allen, E. C.; Liu, Y.; Wu, L. C. KRC transcripts: Identification of an unusual alternative splicing event. *Immunogenetics* 48:32–39; 1998.
 39. Mak, C. H.; Strandmann, J.; Wu, L. C. The V(D)J recombination signal sequence and kappa B binding protein Rb binds DNA as dimers and forms multimeric structures with its DNA ligands. *Nucleic Acids Res.* 22:383–390; 1994.
 40. Makino, R.; Akiyama, K.; Yasuda, J.; Mashiyama, S.; Honda, S.; Sekiya, T.; Hayashi, K. Cloning and characterization of a c-myc intron binding protein MIBP1. *Nucleic Acids Res.* 22:5679–5685; 1994.
 41. Marty, T.; Müller, B.; Basler, K.; Affolter, M. Schnurri mediates Dpp-dependent repression of brinker transcription. *Nat. Cell Biol.* 2:745–749; 2000.
 42. Matunis, E.; Tran, J.; Gönczy, P.; Caldwell, K.; DiNardo, S. punt and schnurri regulate a somatically derived signal that restricts proliferation of committed progenitors in the germline. *Development* 124:4383–4391; 1997.
 43. Mignone, F.; Gissi, C.; Liuni, S.; Pesole, G. Untranslated regions of mRNAs. *Genome Biol.* 3:1–10; 2002.
 44. Mitchelmore, C.; Traboni, C.; Cortese R. Isolation of two cDNAs encoding zinc finger proteins which bind to the alpha 1-antitrypsin promoter and to the major

- histocompatibility complex class I enhancer. *Nucleic Acids Res.* 19:141–147; 1991.
45. Moerman, A. M.; Mao, X.; Lucas, M. M.; Barger, S. W. Characterization of a neuronal kappa B-binding factor distinct from NF-kappa B. *Mol. Brain Res.* 67: 303–315; 1999.
 46. Muchardt, C.; Seeler, J. S.; Nirula, A.; Shurland, D. L.; Gaynor, R. B. Regulation of human immunodeficiency virus enhancer function by PRDII- BF1 and c-rel gene products. *J. Virol.* 66:244–250; 1992.
 47. Nakamura, T.; Donovan, D. M.; Hamada, K.; Sax, C. M.; Norman, B.; Flangan, J. R.; Ozato, K.; Westphal, H.; Piatigorsky, J. Regulation of the mouse alpha A-crystallin gene: Isolation of a cDNA encoding a protein that binds to a cis sequence motif shared with the major histocompatibility complex class I gene and other genes. *Mol. Cell. Biol.* 10:3700–3708; 1990.
 48. Nomura, N.; Zhao, M. J.; Nagase, T.; Maekawa, T.; Ishizaki, R.; Tabata, S.; Ishii, S. HIV-EP2, a new member of the gene family encoding the human immunodeficiency virus type 1 enhancer-binding protein. Comparison with HIV-EP1/PRDII-BF1/MBP-1. *J. Biol. Chem.* 266:8590–8594; 1991.
 49. Ohno, S. Gene duplication and the uniqueness of vertebrate genomes circa 1970–1999. *Semin. Cell Dev. Biol.* 10:517–522; 1999.
 50. Olavesen, M. G.; Bentley, E.; Mason, R. V. F.; Stephens, R. J.; Ragoussis, J. Fine mapping of 39 ESTs on human chromosome 6p23-p25. *Genomics* 46:303–306; 1997.
 51. Oukka, M.; Kim, S. T.; Lugo, G.; Sun, J.; Wu, L. C.; Glimcher, L. H. A mammalian homolog of *Drosophila* schnurri, KRC, regulates TNF receptor-driven responses and interacts with TRAF2. *Nat. Immunol.* 9: 121–131; 2002.
 52. Parada, L. A.; Hallen, M.; Tranberg, K. G.; Hagerstrand, I.; Bondeson, L.; Mitelman, F.; Johansson, B. Frequent rearrangements of chromosomes 1, 7, and 8 in primary liver cancer. *Genes Chromosomes Cancer* 23:26–35; 1998.
 53. Perkins, N. The Rel/NF-kappa B family: Friend and foe. *Trends Biochem. Sci.* 25:434–440; 2002.
 54. Rattner, A.; Korner, M.; Walker, M. D.; Citri, Y. NF-kappa B activates the HIV promoter in neurons. *EMBO J.* 12:4261–4267; 1993.
 55. Recio, J. A.; Aranda, A. Activation of the HIV-1 long terminal repeat by nerve growth factor. *J. Biol. Chem.* 272:26807–26810; 1997.
 56. Ron, D.; Brasier, A. R.; Habener, J. F. Angiotensinogen gene-inducible enhancer-binding protein 1, a member of a new family of large nuclear proteins that recognize nuclear factor kappa-B-binding sites through a zinc finger motif. *Mol. Cell. Biol.* 11:2887–2895; 1991.
 57. Rushlow, C.; Warrior, R. The rel family of proteins. *Bioessays* 14:89–95; 1992.
 58. Rustgi, A. K.; Van't Veer, L. J.; Bernards, R. Two genes encode factors with NF-kappa B- and H2TF1-like DNA-binding properties. *Proc. Natl. Acad. Sci. USA* 87:8707–8710; 1990.
 59. Schatz, D. G.; Oettinger, M. A.; Schlissel, M. S. V(D)J recombination: Molecular biology and regulation. *Annu. Rev. Immunol.* 10:359–383; 1992.
 60. Schwartz, S.; Zhang, Z.; Frazer, K. A.; Smit, A.; Riemer, C.; Bouck, J.; Gibbs, R.; Hardison, R.; Miller, W. PipMaker—a web server for aligning two genomic DNA sequences. *Genome Res.* 10:577–586; 2000.
 61. Seeler, J. S.; Muchardt, C.; Suessle, A.; Gaynor, R. B. Transcription factor PRDII-BF1 activates human immunodeficiency virus type 1 gene expression. *J. Virol.* 68:1002–1009; 2002.
 62. Sen, R.; Baltimore, D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 46: 705–716; 1986.
 63. Singh, H.; LeBowitz, J. H.; Baldwin, A. S.; Sharp, P. A. Molecular cloning of an enhancer binding protein: Isolation by screening of an expression library with a recognition site DNA. *Mol. Cell. Biol.* 52:415–423; 1988.
 64. Staehling-Hampton, K.; Laughon, A. S.; Hoffmann, F. M. A *Drosophila* protein related to the human zinc finger transcription factor PRDII/MBP1/HIV-EP1 is required for dpp signaling. *Development* 121:3393–3403; 1995.
 65. Sudo, T.; Ozawa, K.; Soeda, E. I.; Nomura, N.; Ishii, S. Mapping of the human gene for the human immunodeficiency virus type 1 enhancer binding protein HIV-EP2 to chromosome 6q23-q24. *Genomics* 12:167–170; 1992.
 66. Sun, M.; Eshleman, J. R.; Ferrell, L. D.; Jacobs, G.; Sudilovsky, E. C.; Tuthill, R.; Hussein, M. R.; Sudilovsky, O. An early lesion in hepatic carcinogenesis: Loss of heterozygosity in human cirrhotic livers and dysplastic nodules at the 1p36-p34 region. *Hepatology* 33:1415–1424; 2001.
 67. Suzuki, M. SPXX, a frequent sequence motif in gene regulatory proteins. *J. Mol. Biol.* 207:61–84; 1989.
 68. Takagi, T.; Harada, J.; Ishii, S. Murine Schnurri-2 is required for positive selection of thymocytes. *Nat. Immunol.* 2:1048–1063; 2001.
 69. Tanaka, K.; Matsumoto, Y.; Nakatani, F.; Iwamoto, Y.; Yamada, Y. A zinc finger transcription factor, alpha A-crystallin binding protein 1, is a negative regulator of the chondrocyte-specific enhancer of the alpha 1(II) collagen gene. *Mol. Cell. Biol.* 20:4428–4435; 2000.
 70. Tewari, M.; Dobrzanski, P.; Mohn, K. L.; Cressman, D. E.; Hsu, J. C.; Bravo, R.; Taub, R. Rapid induction in regenerating liver of RL/IF-1 (an I kappa B that inhibits NF-kappa B, RelB-p50, and c-Rel-p50) and PHF, a novel kappa B site-binding complex. *Mol. Cell. Biol.* 12:2898–2908; 1992.
 71. Tian, Q.; Kopf, G. S.; Brown, R. S.; Tseng, H. Function of basoonuclin in increasing transcription of the ribosomal RNA genes during mouse oogenesis. *Development* 128:407–416; 2001.
 72. Torres-Vazquez, J.; Park, S.; Warrior, R.; Arora, K. The transcription factor Schnurri plays a dual role in mediating Dpp signaling during embryogenesis. *Development* 128:1657–1670; 2001.

73. Torres-Vazquez, J.; Warrior, R.; Arora, K. Schnurri is required for dpp-dependent patterning of the *Drosophila* wing. *Dev. Biol.* 227:388–402; 2000.
74. Tseng, H. Basonuclin, a zinc finger protein associated with epithelial expansion and proliferation. *Front. Biosci.* 3:985–988; 1998.
75. Tseng, H.; Green, H. Basonuclin: A keratinocyte protein with multiple paired zinc fingers. *Proc. Natl. Acad. Sci. USA* 89:10311–10315; 1992.
76. Tsukamoto, K.; Ito, N.; Yoshimoto, M.; Kasumi, F.; Akiyama, F.; Sakamoto, G.; Nakamura, Y.; Emi, M. Allelic loss on chromosome 1p is associated with progression and lymph node metastasis of primary breast carcinoma. *Cancer* 82:317–322; 1998.
77. Utada, Y.; Emi, M.; Yoshimoto, M.; Kasumi, F.; Akiyama, F.; Sakamoto, G.; Haga, S.; Kajiwara, T.; Nakamura, Y. Allelic loss at 1p34-36 predicts poor prognosis in node-negative breast cancer. *Clin. Cancer Res.* 6:3193–3198; 2000.
78. van 't Veer, L. J.; Lutz, P. M.; Isselbacher, K. J.; Bernards, R. Structure and expression of major histocompatibility complex-binding protein 2, a 275-kDa zinc finger protein that binds to an enhancer of major histocompatibility complex class I genes. *Proc. Natl. Acad. Sci. USA* 89:8971–8975; 1992.
79. Wu, L. C.; Hicar, M. D.; Hong, J.-W.; Allen, C. E. The DNA-binding ability of HIVEP3/KRC decreases upon activation of V(D)J recombination. *Immunogenetics* 53:564–571; 2001.
80. Wu, L. C.; Liu, Y.; Strandtmann, J.; Mak, C.-H.; Lee, B.; Li, Z.; Yu, C. Y. The mouse DNA binding protein Rc for the kappa B Motif of transcription and for the V(D)J recombination signal sequences contains composite DNA-protein interaction domains and belongs to a new family of large transcriptional proteins. *Genomics* 35:415–424; 1996.
81. Wu, L. C.; Mak, C.-H.; Dear, N.; Boehm, T.; Feroni, L.; Rabbitts, T. H. Molecular cloning of a zinc finger protein which binds to the heptamer of the signal sequence for V(D)J recombination. *Nucleic Acids Res.* 21:5067–5073; 1993.
82. Yeh, W.-C.; Shahinian, A.; Speiser, D.; Kraunus, J.; Billia, F.; Wakeham, A.; de la Pompa, J. L.; Ferrick, D.; Hum, B.; Iscove, N.; Ohashi, P.; Rothe, M.; Goeddel, D. V.; Mak, T. W. Early lethality, functional NF-kappa-B activation, and increased sensitivity to TNF-induced cell death in TRAF2-deficient mice. *Immunity* 7:715–725; 1997.