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Review

# Three classes of $C_2H_2$ zinc finger proteins

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**Abstract.** $C_2H_2$ zinc finger proteins probably comprise the largest family of regulatory proteins in mammals. Most zinc fingers bind to a cognate DNA. In addition to DNA, many of the proteins also bind to RNA or protein, and some bind to RNA only. The binding properties depend on the amino acid sequence of the finger domains and of the linker between fingers, as well as on the higher-order structures and the number of fingers. $C_2H_2$ zinc finger proteins contain from 1 to more than 30 figures. Based on the number and the pattern of the fingers, most of the proteins can be classified into one of three groups: triple-$C_2H_2$, multiple-adjacent-$C_2H_2$, and separated-paired-$C_2H_2$ finger proteins. In contrast to proteins with triple-$C_2H_2$ fingers, proteins with multiple-adjacent-$C_2H_2$ fingers can bind multiple, different ligands. Proteins with a number of separated-paired fingers bind to the target by means of only a single pair.

**Key words.** Zif268; Sp1; WT1; KLF; Ikaros; TFIIIA; dsRBP-Zfa; Basonuclin.

## Introduction

Zinc finger proteins are a class of regulatory proteins that participate in a variety of cellular activities, such as development, differentiation, and tumor suppression. The zinc finger is a small peptide domain with a special secondary structure stabilized by a zinc ion bound to the Cys and His residues of the finger. Differential use of the two residues gives rise to several types of zinc finger, such as $C_2H_2$, $C_2HC$, $C_2C_2$, $C_2HC$ $C_2C_2$, and $C_2C_2$ $C_2C_2$ [1–3]. Of these, $C_2H_2$ is known as the classical zinc finger and is often described as $CX_{2-4}CX_{12}HX_{2-6}H$, to show the intervals between the zinc-binding residues. The finger contains two to three $\beta$ strands in its N-terminal sequence and one $\alpha$ helix in the C-terminal half of the $X_{12}H$. The primary role of $C_2H_2$ fingers is to bind to DNA segments with a specific affinity conferred by several amino acid residues present in the $\alpha$ helix of the finger and with support provided by conserved linkers present between fingers. $C_2H_2$ proteins together with other factors participate in controlling transcription of target genes.

There are many $C_2H_2$ zinc finger proteins in mammals and lower eukaryotes, and even in prokaryotes. Such proteins are estimated to comprise as much as 1% of total mammalian proteins. One hundred thirty-three species of $C_2H_2$-type zinc finger cDNAs have been identified in human brain alone [4–6]. Some $C_2H_2$ finger proteins can bind to RNA or protein in addition to DNA, and others bind to RNA alone: in general, the greater the number of fingers, the more fingers with specific affinity for different ligands. For example, proteins with 3 fingers, may bind DNA, RNA or protein through the fingers, whereas proteins with 6, 9, or 29 fingers can have two to three kinds of binding activities mediated by different fingers. In this review, $C_2H_2$ finger proteins will be divided into three groups: (i) triple-fingered, (ii) multi-adjacent-fingered, and (iii) separated-paired-fingered (table 1). Binding of the different fingers will be compared with that of Zif268, the paradigm of the triple-fingered group.

GAGA factor is the only protein that contains a single $C_2H_2$ finger. The finger binds the target DNA in conjunction with thc adjacent, two basic DNA-binding domains,

Table 1. Some of $C_2H_2$ zinc finger proteins

| Zinc finger | Number of $C_2H_2$ | Number of conseved linkers | Ligand | Reference |
|---|---|---|---|---|
| Triple-$C_2H_2$ ($tC_2H_2$) finger proteins | | | | |
| BKLF | 3 (3) | 2 | DNA | 7 |
| SP1 | 3 (3) | 2 | DNA, DNA-RNA | |
| | | | Protein (GATA-1, GKLF) | 8–10 |
| Zif268 | 3 (3) | 2 | DNA, RNA | 11, 12 |
| Multiple-adjacent-$C_2H_2$ ($maC_2H_2$) finger proteins | | | | |
| JAZ | 4 (0) | 0 | dsRNA, DNA-RNA | 13 |
| WT1 | 4 (3) | 3 | DNA, ssRNA | 12, 14, 15 |
| GL1 | 5 (4) | 3 | DNA | 16 |
| PRDI-BF1 | 5 (2) | 4 | DNA | 17 |
| Gfi-1 | 6 (3) | 2 | DNA | 18, 19 |
| Ikaros | 6 (4) | 2 | DNA, protein (Ikaros, Sin) | 20 |
| MTF-1 | 6 (4) | 3 | DNA, ion (zinc) | 21, 22 |
| dsRBP-Zfa | 7 (0) | 0 | dsRNA, DNA-RNA | 23 |
| p43 | 9 (0) | 0 | 5S RNA | 24 |
| TFIIIA | 9 (3) | 2 | DNA, 5S RNA | 25–29 |
| Roaz | 29 (7) | 1 | DNA, protein (Roaz, Olf-1/EBF) | 30 |
| Separated-paired-$C_2H_2$ ($spC_2H_2$) finger proteins | | | | |
| TTK | 2 x1 (2 x1) | 0 | DNA | 31 |
| PRDII-BF1 | 2 x2 (2 x2) | 1 x2 | DNA | 32 |
| Basonuclin | 2 x3 (2 x1) | 0 | DNA | 33, 34 |

The total number of zinc fingers in a protein and the number of those possessing high affinity DNA-binding activity are shown outside and inside the parentheses, respectively. The number of $spC_2H_2$ fingers are shown by a formula, 2 x(number of pair). RPDII-BF1 contains another tpye of zinc finger, but is not included in this table. Conserved linker suggests TGEKP and the homologue, which are usually present inregions that contain DNA-binding activity. ssRNA, dsRNA, and DNA-RNA represent single-stranded RNA, double-stranded RNA, and DNA/RNA hybrid nucleic acid, respectively. Binding to dsRNA is non-sequence specific, but to other RNAs is sequence specific. Binding to protein is mediated through the $C_2H_2$ zinc finger.

but the finger alone does not [35, 36]. The finger will not be discussed in this review.

## Triple-$C_2H_2$ ($tC_2H_2$) zinc fingers

### Zif268

Zif268, also known as Krox-24, NGFI-A, and Egr1, is perhaps the most extensively studied of all the zinc finger proteins. It contains three $C_2H_2$ fingers, each of which forms two $\beta$ strands and one $\alpha$ helix (figs. 1, 2A), and also contains linkers between the fingers. Amino acid residues in the $\beta$ strands and $\alpha$ helix as well as those of linkers are well conserved within this protein. The three zinc fingers are located in tandem repeats with consistent span and interval. This feature suggested what was later proven: that each finger interacts with one or more nucleotides. The crystal structure of the zinc finger-target DNA complex demonstrates in detail how the fingers of Zif268 align with the DNA duplex and how each finger interacts with nucleotides of the DNA [11, 37]. All the Zif268 fingers bind in the major groove of the target DNA duplex anti-parallel to the primary strand 5′-$G^1C^2G^3T^4G^5G^6G^7C^8G^9G^{10}$-3′, with which most of the residue contacts are made (fig. 2B), making hydrogen bonds to nucleotide bases and the phosphate backbones, and wrapping around the DNA for almost one turn. The DNA takes a form between the B and A form so that the major groove is still wide but can also be deep [37, 39]. The three fingers bind to adjacent subsites: finger 3 to 5′- $G^1C^2G^3$-3′, finger 2 to 5′-$T^4G^5G^6$-3′, and finger 1 to 5′- $G^7C^8G^9$-3′. All the fingers bind very similarly to two of the three bases of its subsite and to one base at an adjacent subsite. The binding affinity decreases in the order 2 > 3 > 1 [40]. An important determinant of the specific binding is the side chain of amino acid residues at position –1, 2, 3, and 6 of the $\alpha$ helix. A residue at each of those positions often contacts a base of a subsite [11, 37]. $R^{-1}$ and $R^6$, when present, bind to G at the first and the third positions of each subsite, respectively. Residue D at position 2 of all three fingers contacts the complementary strand at the C or A upstream of the GC pair bound by $R^{-1}$. Residues at position 1, 4, and 5 do not participate in specific base recognition, but smaller and uncharged residues at these posi-

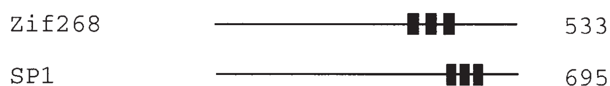


Figure 1. Intramolecular distribution of $C_2H_2$ fingers in $tC_2H_2$ finger proteins. Horizontal lines and black boxes indicate the proteins and the $C_2H_2$ fingers, respectively. The total residue number of the protein is shown next to the N terminus.

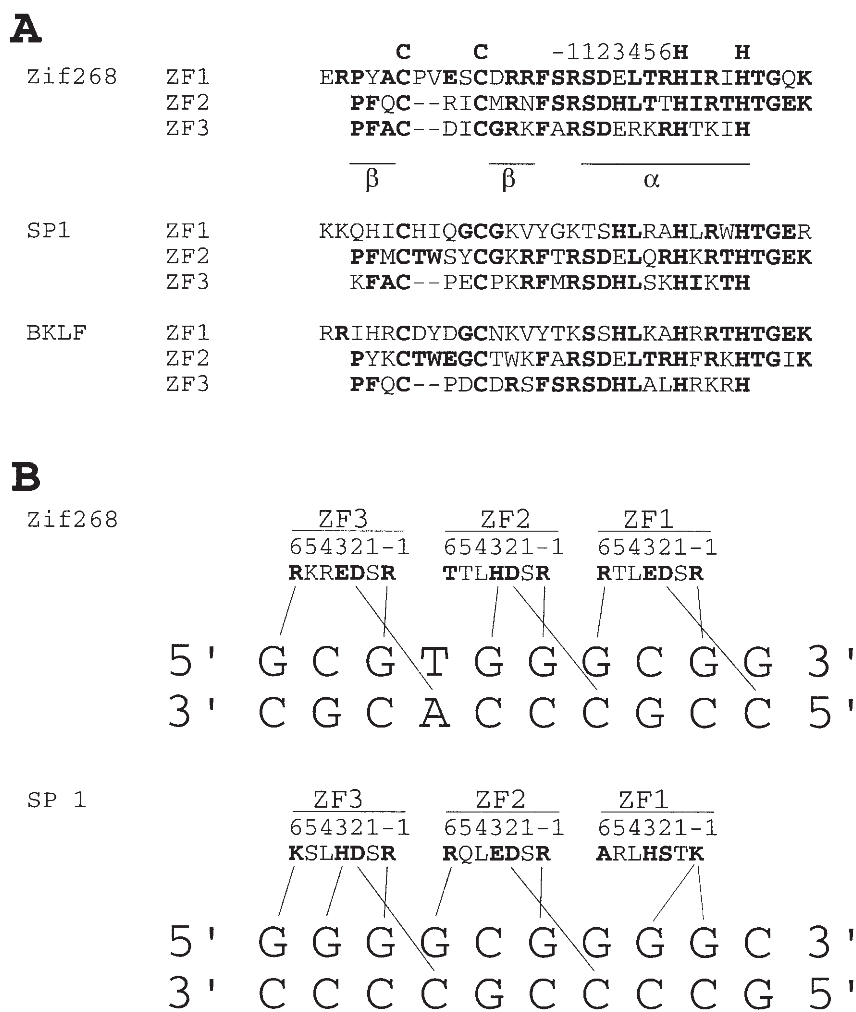


Figure 2. (*A*) Alignment of Zif268, SP1, and BKLF. ZF1, ZF2, and ZF3 indicate zinc finger 1, 2, and 3, respectively, numbering from the N terminus. C, H, and numbers above ZF1 of Zif268 represent the canonical residues, Cys and His, and residue number in the $\alpha$ helix, respectively. $\alpha$ and $\beta$ indicate the $\alpha$ helix and $\beta$ strand, respectively. Space in sequences shows a gap. Amino acid residues that most frequently appear in the nine-finger alignment are shown in bold. When two different residues are tied for the highest frequency in the alignment, only one of them is in bold. (*B*) Models of the residue-base contacts in the Zif268-DNA and the SP1-DNA complexes. Solid lines between zinc fingers and nucleotide sequences show residue-base contacts. The model of Zif268 is derived from results of the DNA-complex crystal structure [11, 37], and that of SP1 from mutational analysis results [38]. Note that zinc fingers mainly bind to the primary strands, which are presented in the anti-parallel direction to zinc fingers (fingers 3 → 1 versus DNA 5′ → 3′) [11].

tions improve the specificity of the fingers. Perhaps such amino acid residues avoid non-specific contacts with the phosphate backbone [41].

The conserved linkers between adjacent fingers are also important. Upon formation of the finger-DNA complex, the conserved K residue of the TG(Q/E)KP sequences located between fingers 1 and 2, and between fingers 2 and 3 (fig. 2A) interacts with the phosphate backbone. Moreover, linkers contact the C terminus of the adjacent $\alpha$ helix through a hydrogen bond, stabilizing the zinc finger and the DNA complex (C-capping) [37, 42]. The role of the linker in the specificity of binding has also been revealed by the effect of substituting conserved residues of the linker [43, 44]. Zif268 derivatives differentially recognize the methylated and non-methylated DNA [45].

Based on the Zif268 finger-DNA interactions and the mutational analysis of Zif268 and other zinc fingers, a residue-base recognition code has been proposed [46–51]. If a code for all 64 triplets were established, it would be valuable for residue-base recognition chemistry as well as for the design of a zinc finger protein targeting a specific gene, and controlling its expression at will. This approach might permit the restoration of gene expression where promoter mutations prevent binding of a cognate transcription factor. The proposed code predicts specific zinc finger-DNA base bindings, but there are exceptions. Residue-base pairing during phage display selection includes some that are not expected and does not always include those that are expected. These inconsistencies result from (i) so-called context-dependent effects caused

by adjacent fingers and (ii) the effect of 'inside environments' such as residue size and species within a finger [41, 48, 49, 52–54]. Thus, only 'a conditional code' that is valid in certain environments exists for residue-base contacts. In the absence of an absolute code, phage display is satisfactory for identifying residue-base interaction, and it should be possible to construct a $C_2H_2$ finger domain that interacts with any target DNA sequence with high affinity [41, 52, 54–58].

## A family of KLF (Krüppel-like factors)

Mammalian proteins of this family, similar to the original Krüppel [7], have three $C_2H_2$ fingers at the C terminus, but differ from the $C_2H_2$ protein just described in possessing a different type of finger 1. This family includes SP1, which has three zinc fingers that bind to a GC-rich duplex with a 5′-$G^1G^2G^3G^4C^5G^6G^7G^8G^9$-3′ primary strand. SP1, like other members of this family, possesses fingers 2 and 3, whose amino acid sequences resemble those of Zif268, but finger 1 of SP1 differs from the others (fig. 2A). This suggests that finger 1 of SP1 may behave differently from the others in binding to DNA and, indeed, experimental results show that finger 1 contributes only weakly to high-affinity binding [38]. Finger 1 of SP1 interacts with the DNA through only one residue ($K^{-1}$) while fingers 2 and 3 interact through four and three residues, respectively (fig. 2B): H3 of SP1 finger 1 might be expected to bind to the adjacent G as it does in finger 3 and in finger 2 of Zif268, but this binding is apparently prevented by the large side chain of $K^{-1}$, which can bind to more than one base [38, 48, 52]. Fifteen other zinc finger proteins of the mammalian KLF family, including BKLF (basic KLF) (fig. 2A), have fingers similar to SP1 fingers [7]. These finger proteins should bind weakly to the target DNA at finger 1, like SP1, by the one residue-base contact.

In addition to DNA, SP1 binds to the primary DNA strand of DNA-RNA heteroduplexes, although the biological role of the duplexes is not known [8]. This finger protein also binds to GATA-1, a $C_2C_2$-type transcription factor, by interaction of its three fingers with the GATA-1 C-terminal $C_2C_2$. In this way, SP1 synergistically activates the reporter genes that have binding sites for either SP1 or GATA-1 or both [10]. Furthermore, SP1 binds to GKLF (gut-enriched KLF) through its fingers. This binding renders SP1 inactive for *CYP1A1* gene transcription [9]. Similar binding also occurs between SP1 and GATA-2 and GATA-3, and between EKLF (erythroid-KLF) and GATA-1. These findings suggest that $C_2H_2$ fingers are important not only for protein-DNA but also for protein-protein interactions. $tC_2H_2$ zinc fingers would be able to bind to only one ligand at a time, DNA, RNA, or protein, since the binding sites overlap. But, the dimerized or multimerized proteins may be able to bind the different ligands simultaneously through multiple $tC_2H_2$ fingers.

## Multiple-adjacent-$C_2H_2$ ($maC_2H_2$) zinc fingers

### $maC_2H_2$ finger proteins often have more than one binding activity

Proteins of this group contain four or more finger located close to one another at similar intervals (fig. 3). TFIIIA, containing nine fingers, is such an example of $maC_2H_2$. This protein completes its binding reaction in 1 h [60], while a recombinant protein with tripled-SP1 fingers, with a total of nine fingers, needs 72 h to complete the reaction sequence, even though the fingers bind with a better affinity than the parent finger protein to an expanded target [61]. Under the same conditions the parent finger protein, with tripled-SP1 fingers, completes the binding reaction in 0.5 h. The rapid TFIIIA reaction probably occurs because only a small fraction of the nine fingers participate in the high-affinity finger-DNA interaction. A crystal structure of fingers 1–6 with the DNA shows that fingers 1–3 wrap smoothly around the major groove of DNA like Zif268, but fingers 4–6 run along one side of the DNA duplex and form an open, extended structure (fig. 4) [59]. Although fingers 7–9 are expected to wrap around the major groove of DNA, their contribution to TFIIIA-DNA complex formation is much weaker than that of fingers 1–3 [29, 62]. Fingers 7–9 might be able to wrap around the major groove independently of and simultaneously with fingers 1–3 because of the intervention of fingers 4–6. In other $maC_2H_2$ finger proteins, such as WT1 and Roaz, as in the case of TFIIIA, participation of fingers in DNA binding is limited to 24–75% of the total number of fingers (table 1), leaving the remaining fingers free to engage in other roles, such as binding to RNA and protein, as will be discussed

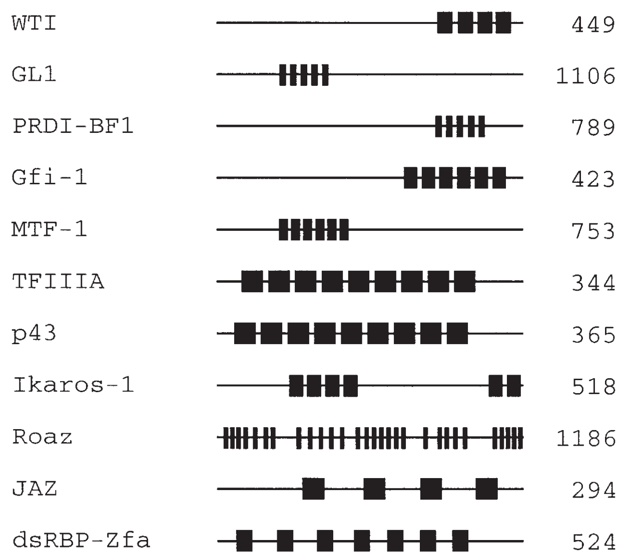

Figure 3. Intramolecular distribution of $C_2H_2$ fingers in $maC_2H_2$ finger proteins.

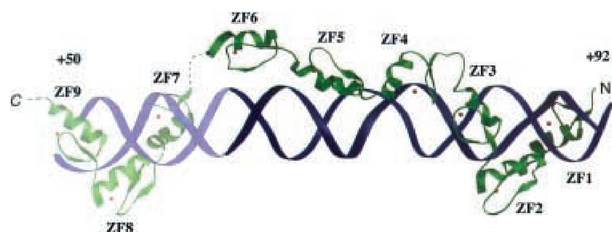


Figure 4. A model of the TFIIIA-DNA complex. The model is reproduced from the report of Nolte et al. [59] . Purple, green, and red colors indicate DNA, TFIIIA zinc fingers, and zinc ion, respectively. The model of the DNA complex of fingers 1–6 is derived from the crystal structure, that of fingers 7–9 is from biochemical analyses.

below. As research proceeds on $maC_2H_2$ zinc finger proteins, it should become clearer whether the binding mode of TFIIIA is common among other $maC_2H_2$ proteins. Many $maC_2H_2$ zinc finger proteins are now known and some contain over 30 zinc fingers [63, 64].

Although many $maC_2H_2$ finger proteins bind to RNA as well as to DNA, two $maC_2H_2$ finger proteins, dsRBP-Zfa with seven fingers and JAZ with four fingers, bind primarily to dsRNA [13, 23]. This feature of $maC_2H_2$ fingers will also be briefly discussed below.

## WT1: a DNA- and RNA-binding $maC_2H_2$ zinc finger

WT1 has a total of four fingers (fig. 3). Fingers 2–4 are 64% identical to the Zif268 fingers, but finger 1 differs in sequence from the others (fig. 5A). Fingers 2–4 bind to the cognate DNA but finger 1 is less important. This conclusion comes from the finding that mutations in fingers 2–4 decrease the specific DNA binding more severely than those in finger 1; in addition, a fragment with fingers 2–4 has a higher affinity for a DNA probe than the entire groups of fingers [14, 15].

WT1 includes a group of isoforms whose molecular weights are 52–65 kDa. These isoforms result from alternative splicing of the immature mRNA at two sites and translation initiation at two sites [66]. Alternative splicing at exon 5 and the two different translation start sites do not affect DNA binding, but an alternative splicing after exon 9 may insert KTS into the linker between fingers 3 and 4. The insertion converts the conserved

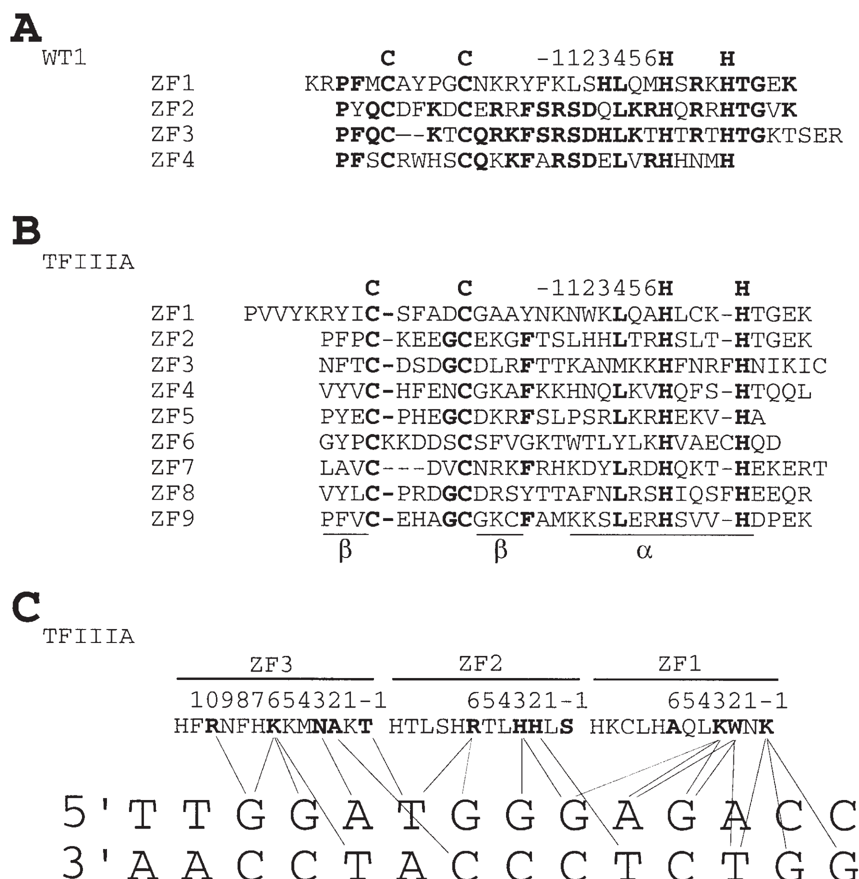


Figure 5. Examples of multiple fingers. (*A*) and (*B*) show an alignment of the WT1 and the TFIIIA fingers, respectively. Bold indicates amino acid residues conserved 50% or more among fingers of each protein. (*C*) A model of the residue-base contact of the TFIIIA-DNA complex. The model is drawn according to the publication by Wuttke et al. [65]. $K^{-1}$ and $K^3$ of finger 1, $H^3$ of finger 2, and $K^6$ of finger 3 are flexible in the solution structure so that they can contact more than one base.

amino acid sequence to TGKTSEKP and concomitantly decreases the binding activity. An NMR study showed that the insertion breaks the C capping of finger 3 in the finger peptide-DNA complex [67]. These facts demonstrate that the conserved linker residue is important in the binding. Besides its DNA binding, WT1 binds to specific single-stranded RNAs. WT1 is able to bind to different RNA sequences, using different fingers to bind each RNA. WT1 finger 1 is more important than finger 4 in binding to RNA transcribed from the mouse insulin-like growth factor 2 gene (*Igf-2*), while it does not contribute to the binding to RNA species identified by the SELEX method (systematic evolusion of ligands by exoponential enrichment). The SELEX-identified RNA species compete with the cognate DNA for the binding, and the KTS insertion in the linker disturbs the RNA binding [12, 15].

### TFIIIA: DNA- and 5S RNA-binding ma$C_2H_2$ zinc finger protein

TFIIIA, which contains a tandem array of nine zinc finger domains, binds to the 5S RNA gene promoter, consisting of three short elements within the coding sequence: a 5′ A block, an intermediate element (IE), and a 3′ C block. The control region is over 55 bp long. The sequence of *Xenopus laevis* TFIIIA (fig. 5B) reveals three features. (i) No clear homology is present among the nine fingers except for L and F residues, which contribute to the conformation of the fingers but not to the specific interaction of the fingers with cognate bases. (ii) Of eight linkers, only two are typical: TGEKP between finger 1 and 2 and TGEKN between fingers 2 and 3. (iii) The length of the linkers is very short between fingers 5 and 6, and between fingers 6 and 7. As already described, TFIIIA is similar to Zif268 in that fingers 1–3 bind in the DNA major groove wrapping around the duplex and making the typical contact with bases by residues at positions –1, 2, 3, and 6 (fig. 5C) [59, 65, 68]. This TFIIIA-DNA complex structure is compatible with an earlier model made with results from biochemical and genetic studies. A mutational analysis of the entire TFIIIIA suggests that the residue-base contacts in living cells activate 5S RNA gene transcription [69]. When the finger peptide contacts the cognate DNA, C-capping occurs: each conserved linker contacts the C terminus of the adjacent $\alpha$ helix through the canonical G of the linkers (TGEKN and TGEKP) [42]. The DNA-induced C-capping provides substantial binding energy to the DNA complex. This stabilization by C-capping of the DNA complex is observed in many fingers including those of Zif268, WT1, GL1, YY1, and MEY. TFIIIA binding to the cognate DNA occurs quite efficiently in vitro, but it is hindered by histones in vivo, where H3/H4 tail domains are the major arbiters of TFIIIA access to DNA [70].

The way that TFIIIA fingers 1–3 bind differs from that of other fingers [65, 68]. (i) More residues of each finger participate in the base contact, including a novel base contact through $R^{10}$ of finger 3. (ii) Single fingers of TFIIIA tend to share a single base of the subsite with the neighboring fingers more often than do Zif268 fingers. (iii) Residues K and H of TFIIIA fingers undergo dynamic conformational fluctuations in the solution structure and contact more than one base. (iv) TFIIIA fingers interact with the neighboring fingers [65, 68]. This occurs between fingers 1 and 2 and between fingers 2 and 3 when a fragment of fingers 1–3 binds to DNA.

The most prominent feature of TFIIIA comes from the fingers 4–6 that traverse the minor groove without wrapping around the DNA duplex (fig. 4). The finger structure allows the protein to stretch over the promoter containing widely separated cis elements: box C, IE, and box A [59, 71, 72]. Although finger 5 contacts IE, the sequence including fingers 4–6 appears to be a large connective between two DNA-binding domains, fingers 1–3 and fingers 7–9. This connective has a role in binding to 5S RNA to store it until it is required for ribosome assembly. TFIIIA binds to the RNA with a dissociation constant of 1.2 nM; this affinity appears to be entirely due to fingers 4–7, since a fragment containing only those fingers has similar affinity. A fragment of fingers 4–6 shows good binding to the central core of the RNA, and the binding is strongly reduced by mutations in finger 4 or 6. Fingers 4–7 appear to provide almost all the free energy for TFIIIA-5S RNA binding [28, 73, 74]. In contrast to fingers 4–6, finger 7 binds to the helix II-loop B region [74, 75]. As in DNA binding, TFIIIA binds 5S RNA through the $\alpha$ helix of the fingers, but uses the residues only at positions –1 and 2 of the helix [73].

*Xenopus* has another 5S RNA-binding zinc finger protein present in the 7SRNP complex, called p43 [24]. p43 is nearly identical to TFIIIA with respect to protein size, zinc finger length, and linker length. Yet it neither binds to the 5S RNA gene promoter nor has homology to the TFIIIA transactivation domain present in the C terminus. Thus, p43 has nothing to do with DNA binding, but binds to 5S RNA mainly by the N-terminal fingers in order to store the RNA [27]. The conserved linkers of TFIIIA are not present in p43. Swapping of the linkers between the N-terminal finger peptides of TFIIIA and p43 demonstrates that the conserved linker of TFIIIA is necessary for DNA binding but is not required for RNA binding by either protein [76].

### Ikaros and Roaz: DNA- and protein-binding ma$C_2H_2$ zinc fingers

Some ma$C_2H_2$ finger proteins bind to DNA and protein. Ikaros is such an example. Ikaros contains six zinc fingers: the four N-terminal fingers bind to the DNA and the two C-terminal fingers bind to each other to form a homodimer [77], i.e., the two functionally independent fin-

ger groups are located at opposite ends of the protein (fig. 3). Binding of the N-terminal fingers to the sequence GGGAA is stronger in the presence of fingers 5–6, suggesting that the homodimerization strengthens the DNA binding. The canonical C and H residues of fingers 5–6 are essential for the homodimerization but the residues at positions –1, 2, and 3 are dispensable, indicating that the mode of the protein-protein interaction differs from that of the conventional protein-DNA interaction. There are two Ikaros homologues, Alios and Helios; all three proteins dimerize with themselves, or with each other, and they activate reporter genes with the consensus promoter sequence GGGAA [20, 78]. They are co-localized in macromolecular nuclear structures and involved in normal hematopoietic development. Both Ikaros and Alios interact with Sin family proteins to recruit histone deacetylases and repress the target genes, but the two proteins use neither finger 5 nor 6 for this heterodimerization and instead use an N-terminal finger as well as a C-terminal non-finger region [79].

Another example is Roaz, which has 29 fingers. Roaz binds to the dyad sequence of GCACCC separated by 2 bp. Fingers 1–7 are responsible for the binding, but it is more efficient when Roaz participates as a homodimer. The dimerization is abolished when fingers 25–29 are deleted together with the C-terminal flanking sequence. Roaz also binds to another protein, Olf-1/EBF, rendering it unable to transcribe both olfactory marker protein and type III adenylyl cyclase genes. Finger 29 is essential for the heterodimerization to Olf-1/EBF but the N-terminal and middle finger regions are also involved, suggesting that several interfaces may participate in the heterodimerization [30].

## dsRBP-Zfa and JAZ: RNA-binding $maC_2H_2$ zinc fingers

dsRBP-Zfa is a finger protein that was found in a *Xenopus* ovary cDNA library as the first $C_2H_2$ zinc finger with dsRNA-binding activity [2, 23]. This protein has seven zinc fingers but it differs from other conventional $maC_2H_2$ zinc finger proteins in that linkers are much longer (34–44 versus 6–8 amino acid residues) and they do not have the consensus linker sequence (fig. 3). The three N-terminal fingers do not share strong similarity in their primary structure, but the four C-terminal fingers, including the linkers, are nearly identical to each other. dsRBP-Zfa binds to dsRNA with an apparent Kd of 0.5 nM but has virtually no binding activity to ssRNA and dsDNA. The binding to dsRNA shows no sequence specificity, but the finger greatly prefers to bind to an A-form helix. dsRBP-Zfa binds to RNA-DNA hybrids as well with an apparent $K_d$ of 1.7 nM. Unlike SP1, which prefers to bind to hybrids containing DNA as the primary strand, dsRBP-Zfa does not show such a preference. Both fingers 1–3 and fingers 5–7 bind to a 36-bp dsRNA with a high affinity, similar to that of the full-length protein, but fingers 1–3 show three shifts in the mobility shift RNA-binding assay, whereas fingers 5–7 show two shifts. This result indicates that three fragments of fingers 1–3 and two fragments of fingers 5–7 bind to the RNA strand. This suggests that, like the TFIIIA-DNA complex, fingers 1–3 wrap around the dsRNA and fingers 5–7 bind to it while extended [80].

JAZ is widely expressed in mouse and human tissues. It has four zinc fingers, each of which is homologous to the finger at the corresponding location of dsRBP-Zfa [13]. JAZ also has longer linkers consisting of 28–38 amino acid residues, a sequence that is not homologous to dsRBP-Zfa linkers. The fingers preferentially bind to dsRNA and RNA-DNA hybrids over DNA. Any two fingers in a row sustain the binding activity but two fingers separated by a sequence of more than one span do not show the high affinity binding, suggesting that linker length is important for optimal binding. The zinc fingers are also needed for nuclear localization and forced expression of JAZ induces apoptosis in murine fibroblast cells.

## Separated-paired-$C_2H_2$ ($spC_2H_2$) zinc fingers

Tramtrack (TTK) has two C2H2 zinc fingers (fig. 6, 7 A). The crystal structure of the DNA complex reveals that the two fingers contact 5′-$A^1G^2G^3A^4T^5$-3′ overlapping a recognition site at $G^3$ and the overall mode of the contact is similar to that of Zif268 [31]. TTK finger 2 binds to the DNA making residue-base contact at positions –1, 2, an 3 as does Zif268 (fig. 7B). Finger I also interacts with DNA but forms a different contact at position 2. Another difference of TTK is that it does not contain, TGERP, the common linker sequence among $tC_2H_2$ and $maC_2H_2$ fingers, but has KRNVKVYP instead (fig. 7A). This linker appears to be flexible, disordered, and does not seem to influence the protein-DNA binding. TTK has an extra $\beta$ strand N-terminal to the conventional $\beta$ strands, but it may have nothing to do with the DNA binding and instead participate in maintaining the protein structure.

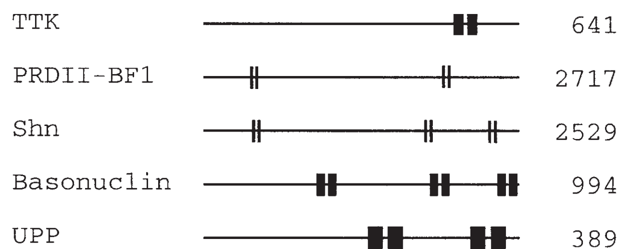


Figure 6. Intramolecular distribution of $C_2H_2$ fingers in $spC_2H_2$ finger proteins.

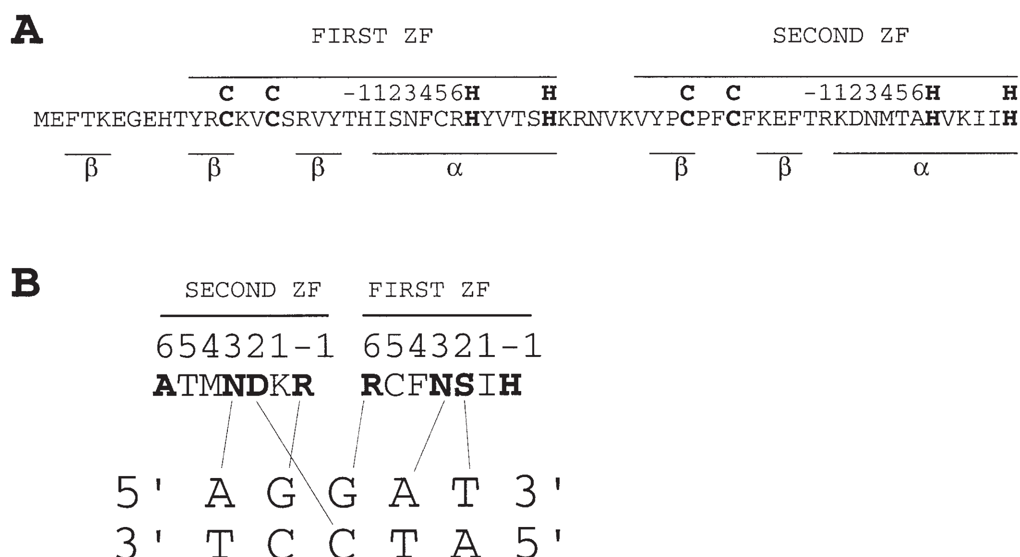


Figure 7. TTK fingers and its DNA complex.

Some proteins contain a few sp$C_2H_2$ fingers whose pairs are widely separated (fig. 6). For example, RPDII-BF1 has two pairs that are separated by 1630 residues [32]. The pairs of PRDII-BF1 are homologous to each other (fig. 8 A) and each pair binds to the same DNA sequence motif with similar affinities and methylation interference patterns. PRDII-BF1 has been suggested to be involved in the assembly of a multicomponent DNA-protein complex by binding simultaneously to widely separated recognition sequences, or it can alternatively bind to the same DNA sequence on different chromosome to bring two different promoters to a common site within the nucleus. Shn is another example of sp$C_2H_2$ fingers, but it has three pairs (fig. 6, 8 A). Similarly, Sal has three paired fingers [81]. Shn and Sal are similar to PRDII-BF1 in finger sequence, and all three finger proteins contain TGEK-like linkers. These observations suggest that each finger pair of Shn and Sal behaves independently as do those of PRDII-BF1. Although each has an extra zinc finger ($C_2HC$ in RPDII-BF1 and Shn, $C_2H_2$ in Sal) whether the extra finger is involved in any binding activity is not known.

Some sp$C_2H_2$ fingers belong to a different subgroup. Examples are Disco of *Drosophila,* unnamed protein product (UPP) of human, and basonuclin of human and mouse (fig. 6, 8 B). These proteins have an atypical primary structure. They contain 6–8 residues between the two His residues of the second finger, and these residues

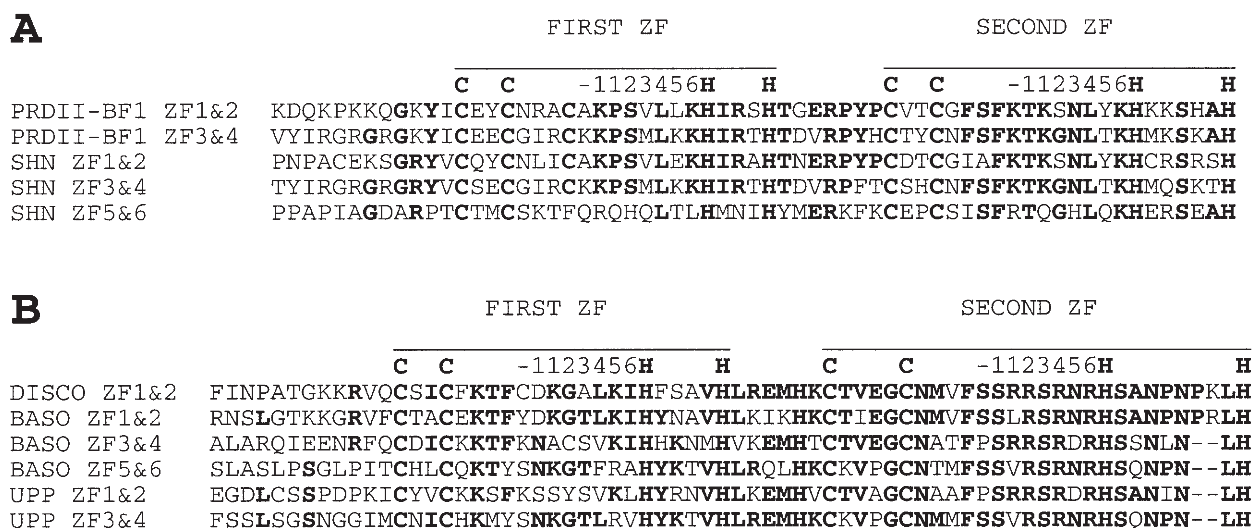


Figure 8. A family of pair fingers. Bold indicates amino acid residues conserved 50% or more among zinc fingers of a subfamily. (*A*) Alignment of a PRDII-BF1 subfamily. (*B*) Alignment of a basonuclin subfamily. Accession number of UPP is BAA90908.

are highly conserved. A large number of the residues may build an extra contact with a base and the phosphate backbone of the target DNA, strengthening the two-zinc finger binding.

Basonuclin has been demonstrated to bind to the ribosomal RNA gene (rDNA) promoter and the basonuclin gene itself [33, 34]. It binds to two dyad sequences, GGA-$CAX_2TGTCC$ and $CGCGTCGCCX_{18}GGCGACGCG$, located side by side within 80 bp of the rDNA pomoter. The first zinc finger pair (fingers 1 and 2) alone can also bind to this region, while the second pair (fingers 3 and 4) cannot [33]. The second pair is possibly engaged in homodimerization of basonuclin and the dimer binds to the dyad sequences through the first pair. Basonuclin has so far been identified only in keratinocytes, hair follicles, and reproductive germ cells [82X–85]. These cells may need sudden increases in rRNA synthesis for conversion from a resting to a growing state. For these purposes, a cell-type-specific transcription factor (basonuclin) may be required.

## Conclusion

The probable primary role of $C_2H_2$ zinc finger proteins is to bind to a specific region of DNA and to participate in regulating gene expression. Many investigators have attempted to understand how zinc fingers specifically recognize the cognate DNA, and a pattern is now emerging. Fragments with two to three consecutive $C_2H_2$ zinc fingers are able to bind to the cognate DNA, and those fingers are often responsible for the binding of $tC_2H_2$, $maC_2H_2$, and $spC_2H_2$ zinc finger proteins. The fingers wrap around the major groove of the DNA and contact cognate bases through residues of the $\alpha$ helix at positions –1, 2, 3, 6, and 10, and the finger-DNA complexes are stabilized by C-capping. Different amino acid residues at the critical positions of the $\alpha$ helix confer different binding specificities on the numerous zinc finger proteins. The stoichiometric residue-base contact can be disturbed by 'context-dependent effects', 'inside environments,' and unusual linkers.

In addition to DNA, many $C_2H_2$ zinc finger proteins bind to RNA. The mode of this binding is not clear, but the binding ability of different species and structures of RNA, such as dsRNA, ssRNA, and DNA-RNA heteroduplexes, indicates that the mode of $C_2H_2$ zinc finger-RNA binding is not as simple as that of dsDNA binding. $C_2H_2$ zinc finger proteins commonly use their fingers to bind to proteins and form homo- or heterodimers. The canonical His and Cys residues arc essential but residues at positions –1, 2, and 3 are apparently dispensable in this binding, suggesting that the mode of finger-finger binding differs from that of finger-DNA binding.

Some $tC_2H_2$ zinc finger proteins use the same fingers to bind DNA or protein, but $maC_2H_2$ zinc finger proteins often have some fingers that bind to DNA and others that bind to protein and RNA. Repetition of fingers and combination of various fingers expands the ability of the proteins to bind many different ligands, possibly simultaneously.

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*Note added in proof.*
International Human Genome Sequencing Consortium estimates, that seven hundred-six human genes encode $C_2H_2$ zinc finger proteins. (2001) Nature 409: 860–921.


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