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REVIEW PAPER

Keep Your Fingers Off My DNA: Protein–Protein Interactions Mediated by C2H2 Zinc Finger Domains

Kathryn J. Brayer · David J. Segal

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Abstract Cys2-His2 (C2H2) zinc finger domains (ZFs) were originally identified as DNA-binding domains, and uncharacterized domains are typically assumed to function in DNA binding. However, a growing body of evidence suggests an important and widespread role for these domains in protein binding. There are even examples of zinc fingers that support both DNA and protein interactions, which can be found in well-known DNA-binding proteins such as Sp1, Zif268, and Ying Yang 1 (YY1). C2H2 protein–protein interactions (PPIs) are proving to be more abundant than previously appreciated, more plastic than their DNA-binding counterparts, and more variable and complex in their interactions surfaces. Here we review the current knowledge of over 100 C2H2 zinc finger-mediated PPIs, focusing on what is known about the binding surface, contributions of individual fingers to the interaction, and function. An accurate understanding of zinc finger biology will likely require greater insights into the potential protein interaction capabilities of C2H2 ZFs.

Keywords Transcription factors · Protein–DNA interactions · Protein chemistry · Structural biology · Functional annotations

K. J. Brayer
Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA

D. J. Segal (✉)
Genome Center and Department of Pharmacology, University of California, Davis, Genome Center/Pharmacology, 4513 GBSF, 451 Health Sciences Dr, Davis, CA 95616, USA
e-mail: djsegal@ucdavis.edu

Introduction

Zinc finger domains (ZFs) are protein structures stabilized by the coordinated binding of a zinc ion. Although there are 20 different types of ZF domains, each categorized by the structure of their zinc stabilizing amino acids [1, 2], the most common type is the Cys2-His2 (C2H2) type (Fig. 1). The C2H2, or "classical" zinc finger, comprise a large group of proteins containing the consensus sequence (F/Y)-X-C-$X_{2\text{-}5}$-C-X_3-(F/Y)-X_5-Ψ-X_2-H-$X_{3\text{-}4}$-H, where X is any amino acid and Ψ is any hydrophobic residue [3]. This motif, which self-folds to form a $\beta\beta\alpha$ structure, obtains its name from the coordinated binding of a zinc ion by the two conserved cysteine and histidine residues [4–7]. Natural variants that contain cysteine as the final zinc-chelating residue (C2HC) also fold into the same structure [8].

Zinc finger proteins (ZFP) may contain between 1 and 40 ZF domains, which are frequently arranged in groups or clusters of tandem repeats. C2H2 ZFs were initially identified in the DNA-binding domain of transcription factor TFIIIA in *Xenopus laevis*, which has nine fingers [9]. Since their discovery, the C2H2 ZFP have grown to be recognized as an important class of genomic regulators, in part because of their broad distribution, but also because of their significant expansion within the genomes of eukaryotes. Found throughout all kingdoms, the C2H2 domain is not only ubiquitous, but is also one of the most common protein domains found within many eukaryotic proteomes (Table 1). In humans, recent estimates propose that approximately 3% of genes code for C2H2 proteins, making them the second most prevalent protein motif [2, 10, 11]. Understanding the functionalities that ZFs impart therefore provides insights into one of the largest superfamilies of proteins in the human genome.

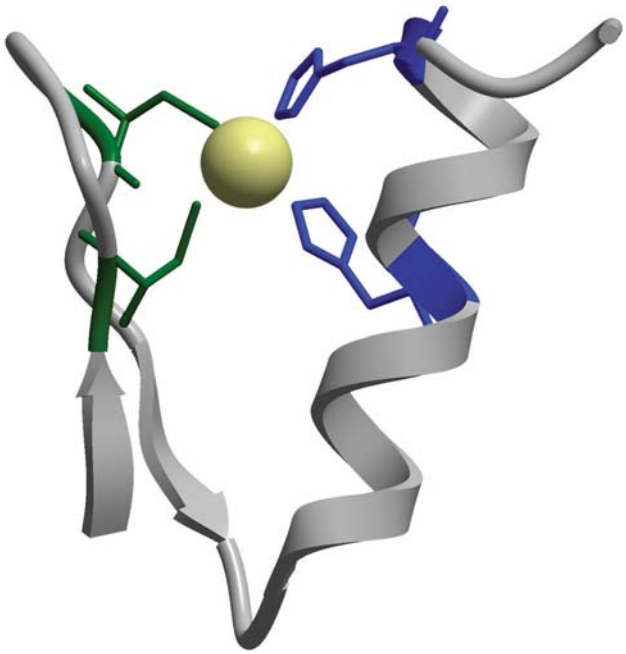


Fig. 1 The canonical C2H2 zinc finger structure. A ribbon diagram of the third C2H2 domain from TFIIIA in *Xenopus laevis* (PDB accession number 1TF3) is showing the canonical stabilization of the $\beta\beta\alpha$ fold by the coordination of a zinc ion (yellow) by two cysteine (green) and two histadine (blue) residues

The many C2H2 ZFPs that have not yet been functionally characterized are generally assumed to have DNA-binding capabilities. However, C2H2 domains have proven to be as diverse functionally as they are abundant, having been shown capable of interacting with RNA and protein [12–15]. In multi-finger proteins, typically only 3–4 ZFs are involved in DNA binding. The remaining fingers are frequently involved in other types of interactions [16–24]. In other cases, such as the 3-finger proteins Zif268/EGR1 and SP1, the ZFs seem to play a dual role of providing both DNA and protein-binding functions [25–27]. Therefore, while it may be reasonable to predict that an uncharacterized C2H2 ZFP might bind DNA, this assumption is likely insufficient to describe the full interaction potential, and thus the full function, of the protein.

Recently, several structural and functional studies of ZF-mediated protein–protein interactions (PPI) have been published. Previous descriptions of this field focused on a few well-characterized examples (e.g., FOG1, the IKAROS family, ATF2, rOAZ), prompting questions of whether PPIs represented a limited or widespread functionality of C2H2 ZFs. Here we review the current information of more than 100 PPIs mediated by C2H2 domains. Where information is available, the binding surface and contribution of specific fingers to the interaction are described. This information suggests the role of ZFs in mediating PPIs has probably been underappreciated and likely under-annotated. The full significance of PPIs to the biology of C2H2 ZFPs remains to be explored.

Surface Diversity

Over the past 20 years, numerous studies have examined the DNA-protein interactions mediated by C2H2 domains. Early biochemical [28] and structural [7] studies of ZF protein such as Sp1, Krox-20 and Zif268 revealed that amino acids in positions −1, 2, 3, and 6 of the α-helix contact specific nucleotides within the major groove of DNA (Fig. 2). Since then, numerous studies have confirmed a critical role for these amino acid positions in DNA binding [3]. A small group of "non-canonical" zinc fingers

Table 1 Distribution and abundance of the C2H2 zinc finger domain across taxa

Taxon	Number of proteins with C2H2 domains[a,b]	Ranking within proteome[b]	Coverage (%)[c]
Archaea	68		
Bacteria	153		
Eukaryotes	13,617		
Arabidopsis thaliana	164	50	0.5
Caenorhabditis elegans	216	13	1
Danio rerio	216	9	1.8
Drosophila melanogaster	349	2	2.1
Gallus gallus	74	18	1.4
Homo sapiens	1,055	2	2.8
Mus musculus	837	4	2.5
Rattus norvegicus	183	8	1.5
Saccharomyes cerevisiae	47	19	0.8
Viruses	74		

[a] Reference [10]

[b] Reference [11]

[c] Percentage of proteome containing proteins with C2H2 domains [11]

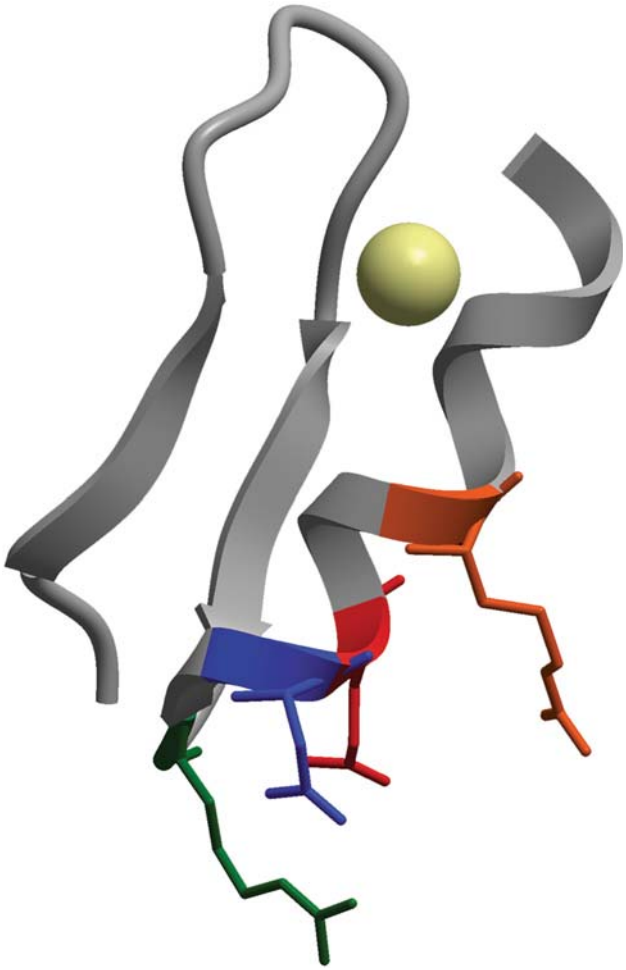


Fig. 2 A DNA-binding zinc finger. A ribbon diagram shows a DNA-binding C2H2 finger from Zif268 (PDB accession 1ZAA). Amino acids involved in contacting DNA are highlighted at positions −1 (green), 2 (blue), 3 (red), and 6 (orange)

appear to use more diverse sets of DNA interactions [3]. However, the ZF-DNA contacts are virtually always mediated by amino acids located in the N-terminal portion of the α-helix.

Unlike ZF-DNA interactions, the interacting residues responsible for specific ZF PPIs have been significantly less studied. In most cases, little is known about the binding surface due to a lack of structural information. Only a handful of the C2H2 domains known to be involved in PPIs have solved structures, and only a few scanning mutagenesis studies have been conducted to examine the location of critical residues. However, there is compelling evidence to expect a diverse assortment of protein-binding surfaces. This evidence comes quite unexpectedly from a class of scorpion toxins that bind potassium channels. Many of these toxins have a nearly identical structure to C2H2 ZFs, and some bind their target channel using amino acids similar to those used by C2H2 ZFs to bind DNA (reviewed in [29]). However, others contact their cognate channel using amino acids projecting from the β-strands, while others use residues from the loop region between the α-helix and β-strands.

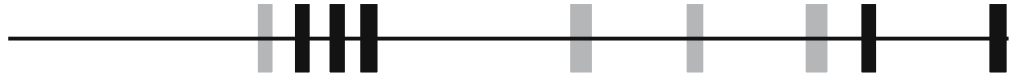
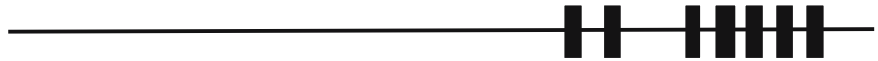
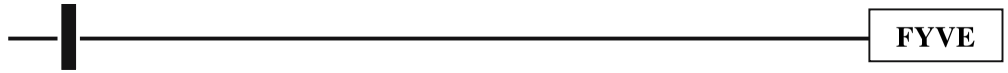
In the following sections we present structural information regarding the binding surface of PPIs mediated by C2H2 and C2HC ZFs. Indeed, there are examples of ZFs using the "DNA face" of the α-helix, an alternative face of the α-helix, the β-strands, the loop region, and even more complex combinations of these elements. Given the variety of interaction surfaces presented by the relative few interfaces that have been characterized, it seems reasonable to expect that ZFs have the potential to interact with many proteins, and that ZF PPIs may be more common that previously thought.

α-Helix Binding Using DNA-Binding Residues

The best-characterized ZF PPIs are those between *Friend of Gata1 (FOG1)* and its binding partners, the globin transcription factor GATA1 and Transforming Acidic Coiled-coil 3 (TACC3). FOG1 contains a total of nine ZFs (Table 2); however, only four are of the classic C2H2 type. The remaining fingers substitute a conserved cysteine for the final histidine [36]. FOG1 is known to interact with GATA1 using fingers 1, 5, 6, and 9, which are variant C2HC fingers [17]. Recently, Liew et al. [37] determined the structure of the interaction between the C2HC finger 1 of the *Drosophila* FOG and a segment of the murine GATA-1. The variant C2HC was found to be structurally identical to C2H2 ZFs, except for subtle differences in the C-terminal end of the α-helix (Fig. 3a; [8]). (The reader should not confuse these C2HC variants with the C-X_2-C-X_4-H-X_4-C class of ZF. This latter class, which includes the well-studied NCp7 nucleocapsid ZFs of HIV, is also referred to as C2HC but has a completely different protein structure [38]). As shown in Fig. 3b, the residues in the α-helix of the FOG C2HC domain contact GATA, primarily through polar and hydrophobic interactions. Interestingly, despite similarities in both sequence and structure, C2HC and C2H2 domains are not interchangeable. Matthews et al. [39] demonstrated that mutation of the C2HC domains of FOG fingers 1 and 9 to C2H2 domains inhibited their ability to interact with GATA without disrupting their ability to fold.

The function of the N-terminal cluster of FOG1 was unknown until recently. This cluster of fingers is comprised of one variant C2HC finger, followed by three classic C2H2 fingers. DNA-binding studies failed to demonstrate DNA binding by the cluster, suggesting that these fingers do not participate in DNA interactions [40]. After conducting yeast-two hybrid and immunoprecipitation experiments to find and map the PPI between the third

Table 2 Binding surfaces for protein–protein interactions involving C2H2 domains

Protein[a]	Binding surface	Partner	Architecture[b]	References
FOG1	α helix of F1 of F3	Gata1 TACC3		[23]
Eos	α helix of Fs 5–6	Eos Ikaros Pegasus Trps1		[30]
Gli	α helix, Fs 1–2	Intra-protein		[31]
Zap1	α helix, Fs 1–2	Intra-protein		[32]
EEA1	β1 of F1	Rab5		[33]
Zac1	β1 of F2	Zac1		[34]
MBP-1	Linker	Intra-protein		[35]

[a] All proteins from human except Zac1 (*Mus musculus*) and Zap1 (*Saccharomyces cerevisiae*)

[b] Linear representation of the domain structure of proteins. Black boxes, C2H2 zinc finger domain; gray boxes, variant C2HC zinc finger domains; FYVE, FYVE zinc finger domain

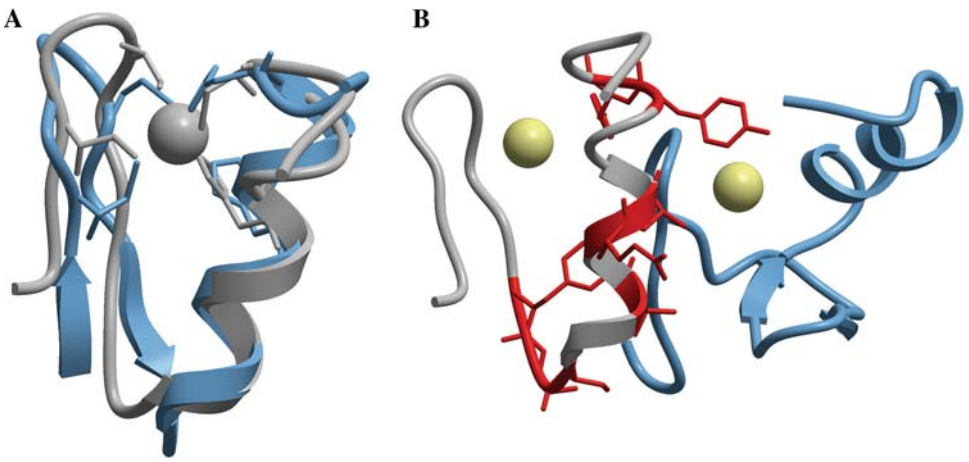


Fig. 3 C2HC zinc finger domains. (**a**) Superimposed ribbon diagrams of the third C2H2 domain from TFIIIA in *Xenopus laevis* (PDB 1TF3, shown in blue) and the first C2HC domain of *Drosophila* FOG (PDB 1y0j, shown in gray) demonstrate that C2HC has the same structure as the classic C2H2 domains. (**b**) Ribbon diagram of the protein–protein interaction between the first C2HC domain of *Drosophila* FOG and the N-terminal treble-cleft zinc finger of murine GATA-1 [37]. FOG amino acids critical to the interaction are displayed in red

(classic) zinc finger of FOG1 and TACC3, Simpson et al. [23] combined NMR and alanine mutagenesis to pinpoint critical amino acid involved in the interaction. Interestingly, they demonstrated that amino acids in the α-helix of FOG1 formed the binding surface for the interaction and that residues in positions normally involved in contacting DNA, positions −1, 2, 3, and 6, were also utilized for protein interactions (Fig. 4). Additionally, they showed that the interacting surface was longer than that required for DNA binding. It included residues along the entire length of the α-helix, as well as residues located before and after the helix that were oriented into the binding face [23].

Protein interactions between members of *the Ikaros family* of proteins have also been examined and partially mapped to specific amino acid positions. Ikaros is the founding member of a family of proteins composed of five proteins—Ikaros, Aiolos, Helios, Eos, and Pegasus—all of which maintain a conserved, characteristic domain architecture, containing two clusters of C2H2 domains, an N-terminal cluster comprised of 3–4 domains and a C-terminal cluster of two fingers (Tables 2 and 3). Forming either self-associations or associations with other members of the family, all members of this family use their C-terminal fingers mediate PPIs; and, use their N-terminal fingers to bind DNA [19, 20, 22, 58, 59] (Table 3). Trichophinophalageal syndrome 1 (Trps1) contains two C-terminal ZF domains that are homologous to the C-terminal fingers in the Ikaros family. Interestingly, Trps1, which is not a member of the Ikaros family, forms a PPI with the family member Eos, although not the others [60, 61].

Westman et al. [30] solved the structure of the C-terminal cluster of two domains in Eos and used alanine mutagenesis to determine amino acids critical for interactions with Ikaros, Pegasus, Trps1, and itself. As with zinc

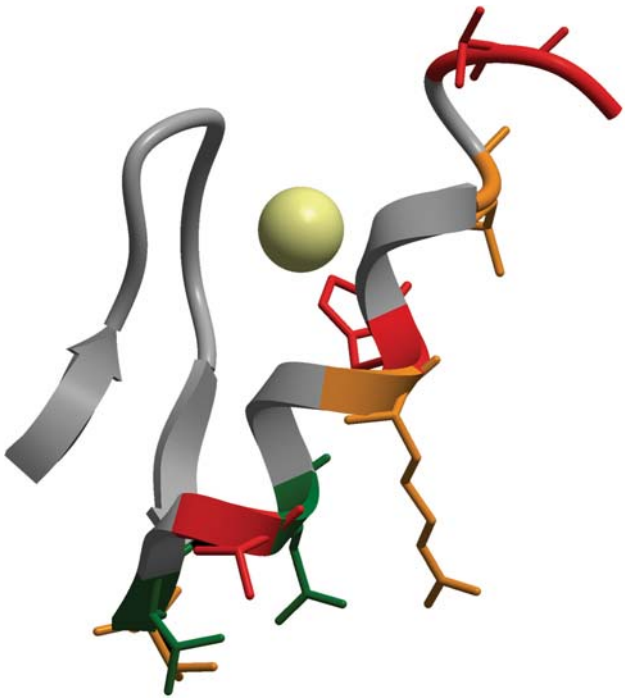
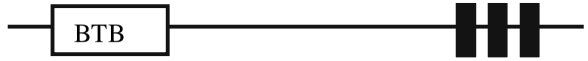


Fig. 4 A protein-binding finger. A ribbon diagram shows the third finger of FOG1 (PDB accession 1SRK). Amino acids critical to the interaction with TACC3 as determined by NMR titration (red), mutation analysis (green), or both (orange) are shown

finger 3 from FOG1, Westman et al. demonstrated that the binding surface for the PPI ran along the α-helix region of finger 5. Scanning mutagenesis again highlighted the importance of amino acids in positions 2, 3, 5, and 6 for maintaining the interaction, particularly in finger 5. Additionally, as shown in Fig. 5a and b, they determined that amino acids located in the turn between the β-sheets in both fingers also participated in the interaction, and that

Table 3 C2H2 Domains involved in protein–protein interactions and their binding partners

Protein[a]	C2H2 domains	Partner(s)	Architecture[b]	References
rOAZ	29	O/E1		[41]
OAZ	14–10	SMAD-1, -4		[42]
PLZF	1–3	RARα, GR, ERα		[43]
	1–6	GATA2		[44, 45]
	1–9	BCL6		[46]
	6–7	HB-EGF-C		
FAZF	1–3	Gata2		[44]
Sp1	1–3	BCoR, NCoR, SMRT, p300, SWI/SNF, TAF1		[47] [48–50]
	1–2	E2F1		[51]
YY1	1	ATF/CREB		[52]
	1	Sp1		[26, 27]
	1–2	YAF2		[53]
	1–4	SMAD4		[54]
Rag1	A	Self		[55]
	B	Rag2		[56]
Zac1	6–7	p300		[57]
BCL6	1–3	PLZF		[45]

[a] All proteins from human except rOAZ (*Rattus norvegicus*)

[b] Linear representation of the domain structure of protein. Black boxes, C2H2 zinc finger domain; BTB, Broad-Complex, Tramtrack, and Bric-a-Brac domain; R, Ring finger domain

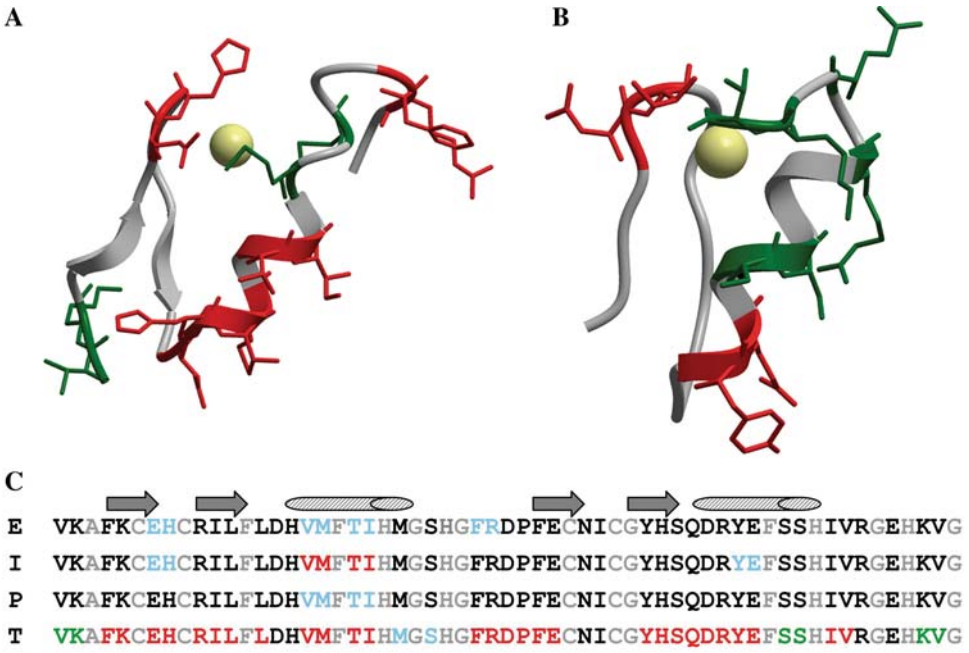


Fig. 5 Critical amino acids in Eos-mediated protein interactions. Homology models of (**a**) finger 5 and (**b**) finger 6 of Eos (based on PDB accession 1SRK and 1PAA, respectively) showing amino acids critical to PPIs with Eos, Ikaros, Pegasus, or Trps1. Amino acid positions in which alanine substitution disrupted homodimerization and/or interaction with Ikaros and Pegasus are shown in red. Since most alanine substitutions disrupted the interaction between Eos and Trps1, the positions for which alanine mutation still allowed interactions are shown in green. (**c**) Scanning alanine mutagenesis revealed amino acids critical to the Eos with either Eos (E), Ikaros (I), Pegasus (P), and Trps1 (T)—as indicated on the left. Domain structural elements are indicated above with arrows representing β-sheets and tubes representing α-helices. Alanine mutations with a strongly (red) or moderately/weakly (blue) negative effect on yeast growth in a yeast-two hybrid are shown. Alanine mutations that still allowed interactions are shown in green. Positions not affected by alanine substitution are black, positions that were not tested are light gray

these residues were also likely oriented toward the binding surface. The alanine substitutions had dissimilar effects on the various PPIs, indicating an underlying selectivity in these interactions (Fig. 5c).

α-Helix Binding, Non-DNA Binding Residues

One of the earliest examples of a PPI between C2H2 domains was observed between finger 1 and finger 2 in the crystal structure of *glioma-assocated protein 1 (Gli1)* [31]. The five-finger Gli1 protein forms several complex interactions, with most fingers able to interact with both protein and DNA (Tables 2 and 4) [31]. Fingers 2–5 bind DNA [31]. Fingers 3–5 mediate a PPI with multiple members of the Zic family of proteins; however, neither the contribution of individual fingers nor the binding surface has been elucidated (Table 4, [80]). Remarkably, while finger 2 is binding DNA, it also forms numerous inter-finger contacts with finger 1 (Fig. 6a, [31]).

More recently, Wang et al. [32] reported a similar finding in the *Saccharomyces cerevisiae* protein *Zap1* after solving the structure using NMR. Zap1 contains seven C2H2 domains arrayed in two clusters (Table 2). The C-terminal cluster of Zap1 contains five domains and is known to bind DNA [32]. The N-terminal cluster contains two domains that interact with each other. A comparison of the Gli1 and Zap1 structures revealed striking similarities in their interaction surfaces [32]. In each case the interaction occurs in the α-helical region; however, amino acid positions typical to DNA-protein interactions are not used. Instead the interacting face is located about a quarter-turn counterclockwise to the DNA-binding surface (when looking down the axis of the alpha helix) (Fig. 6a and b). Despite similarities in the binding faces, there is little overlap in the positional location of amino acids required for the interactions, with the exception of α-helix position 8, located in the C-terminal end of the helix [31, 32]. In both Gli1 and Zap1, amino acids at position 8 in both finger 1 and finger 2 form inter-finger contacts [32].

β-Sheet Binding

Not all PPIs depend on α-helical amino acids; nor do all C2H2 PPIs preclude DNA binding. One example of this is

Table 4 Proteins containing C2H2 domains implicated in protein–protein interactions

Protein	Alias/Symbol	Fingers	Partner	References
Aiolos	ZnFN1A3, IKZF3	5–6	Ikaros, Aiolos, Pegasus, Helios	[19, 20, 22]
AT-binding transcription factor 1	ZFHX3, ATBF1	22–23	PIAS3	[62]
B-cell CLL/lymphoma 11A	Bcl11a (MGI), EVI9, CTIP1	1–2	ARP1	[63]
		4	Self	[64]
B-cell CLL/lymphoma 11B	ZfphRit1 alpha, CTIP2	2–4	Tat, HP1	[65]
		5–7	Tat, HP1	[65]
B-cell CLL/lymphoma 6	Znf 51, BCL6	1–3	PLZF	[45]
		3–6	HDAC5/HDAC7	[66, 67]
		4–5	ETO	[68]
		All	LRF	[69]
		All	Miz1	[70]
		All	c-Jun, JunD, JunB	[71]
Bone marow zinc finger 2	ZNF224, BMZF2	6–10	WT1 -KTS	[72]
CCCTC-binding factor	CTCF	All	YB-1	[73]
		All	YY1	[74]
DAZ interacting protein 1, testis 1	DZIPt1	1	DAZ	[75]
DAZ interacting protein 1, testis 2	DZIPt2	1	DAZ	[75]
Early growth response 1	Zif263, ZNF225, Egr1	All	RELA	[25]
Early hematopoietic zinc finger	ZNF521, Evi3	All	SMAD1, SMAD4	[76]
Ecotropic viral integration site 1	Evi1, PRDM3	1, 6	Gata1	[77]
Eos	ZNFN1A4, IKZF4	5–6	Eos, Ikaros, Pegasus, Trps1	[18, 22, 30]
Fanconi anemia zinc finger protein	ZBTB32, FAZF, ZNF538	All	GATA2	[44]
FLT3-interacting zinc finger 1	ZNF798, Fiz1	1–4 or 7–11	Flt3	[78]
GLI-Kruppel family member GLI3	Gli3	3–5	Smad1/2	[79]
Glioma-associated oncogene 1	Gli1	3–5	Zic-1, -2, -3	[80]
Growth factor independent 1	ZNF163, GFI1	3–5	PU.1	[81]
		All	MTG8 -human	[82]
Helios	ZNFN1A2, IKZF2	5–6	Helios, Ikaros, Aiolos	[19]
Hunchback	hb	5–6	hunchback	[83]
Ikaros	ZNFN1A1, IKZF1	5–6	Ikaros, Aiolos, Helios, Eos, Pegasus	[24, 83]
KRAB box containing zinc finger protein	Krim1 (RGD)	2	MYC	[84]
Kruppel-like factor 1 (erythroid)	KLF1, EKLF	All	FLI1	[85]
Kruppel-like factor 13	KLF13, FKLF2	All	CBP/p300	[86]
Lola locus isoform 3D	LOLA3D	1–2	JIL1 Kinase	[87]
Myc-associated zinc finger protein-related factor	ZNF278, PATZ1, MAZR	All	MITF	[88]
NRC-interacting factor 1	Zfp335	5–6	NRC	[89]
Pegasus	ZNFN1A5, IKZF5	4–5	Pegasus, Eos, Ikaros, Aiolos	[22]
Polymerase (DNA directed), eta	POLH	1	Ubiquitin	[90]
Promyelocytic leukemia zinc finger protein	ZNF145, ZBTB16	1–3	RARa, GR, ERa	[43]
		1–6	GATA2	[44]
		6–7	HB-EGF-C	[46]
		All	BCL6	[45]
RE1-silencing transcription factor	REST, NRSF	9	Co-REST	[91]
Recombination activating gene 1	Rag1	B	RAG2	[56]

Table 4 continued

Protein	Alias/Symbol	Fingers	Partner	References
Recombination activating gene 1	Rag1	A	Rag1	[55]
ROAZ	ZFP423	29	Olf-1	[41]
Schnurri	shn	1–2, 4–8	MAD	[92]
Senseless	Sens	2–3	Scute	[93]
Sequoia	seq	All	dsh	[94]
Serendipity	SRY	6	Self	[95]
Smad- and Olf-interacting zinc finger protein	ZNF423, OAZ	14–19	SMAD-1, -4	[42]
Sp1 transcription factor	Sp1	1	E2F1	[51]
		All	BCoR, NCoR, SMRT	[47]
		All	E2F	[96]
		All	Huntingtin	[97]
		All	MYC	[98]
		All	TAF1	[50]
		All	YY1	[26, 27]
Sp2 transcription factor	Sp2	1	E2F1	[51]
Sp3 transcription factor	Sp3	1	E2F1	[51]
Sp4 transcription factor	Sp4	1	E2F1	[51]
Transcription factor 8	Tcf8, ZEB1	5	Oct1	[99]
Trichorhinophalangeal syndrome 1	Trps1, zfp GC79	8–9	Eos	[30]
Uncoordinated protein 98	unc-98	All	UNC-97 (pinch)	[100]
Wilms tumor 1 (-KTS)	WT1 (-KTS)	1–2	WTAP	[101]
		1–2	CBP/p300	[102]
		All	BMZF2, Ciao-1, Par4	[72, 102]
		All	NHRPU	[103]
Wilms tumor 1 (+KTS)	WT1 (+KTS)	1–2	CBP/p300	[102]
		2–4	U2AF65	[104]
Ying Yang 1	YY1	1	ATF/CREB	[52]
		1–2	Sp1	[26, 27]
		1–2	YAF2	[53]
		2–4	TAFII55	[105]
		3–4	Adenovirus E1A	[106, 107]
		All	CTCF	[74]
		All	MYC	[108]
		All	Smad4	[54]
		All	TBP, CBP	[105]
		All	TFIIB	[105]
Zic family member 1	Zic1	3–5	Gli-1, -2, -3	[80]
Zic family member 2	Zic2	3	Ku70, Ku80, PARP, RHA	[109]
Zinc finger 148	ZNF148, ZBP89	All	P53	[110]
Zinc finger and BRCA1-interacting protein with a KRAB domain 1	ZNF350, ZBRK1	7–8	BRCA1	[111]
Zinc finger and BTB domain-containing protein 7A	ZBTB7A	All	BCL6	[69]
Zinc finger protein 161	Zfp106, zf5	1–5	Self	[112]
Zinc finger protein 219	zfp219	7–9	mSufu	[113]
Zinc finger protein 251	znf251	1–5	Smad1	[114]
Zinc finger protein 295	znf295, zbtb21	1–9	Zfp161	[115]
Zinc finger protein 41	znf41	9–16	Smad2	[114]
Zinc finger protein 484	ZNF484	2–5	Smad8	[114]
Zinc finger protein 512	ZNF512B	3–5	Many	[114]

Table 4 continued

Protein	Alias/Symbol	Fingers	Partner	References
Zinc finger protein 512	ZNF512	5–6	Many	[114]
Zinc finger protein 76	znf76	1–5	Smad1	[114]
Zinc finger protein 8	znf8	1–6	Many	[114]
Zinc finger protein 8	Znf8	All	Smad8a	[114]
Zinc finger protein 83	znf83	8–15	Smad3, Smad8	[114]
Zinc finger, X-linked, duplicated A	ZXDA	All	ZXDC	[116]
Zkscan17 (MGI)	zfp496	All	jumonji/jarid2	[117]

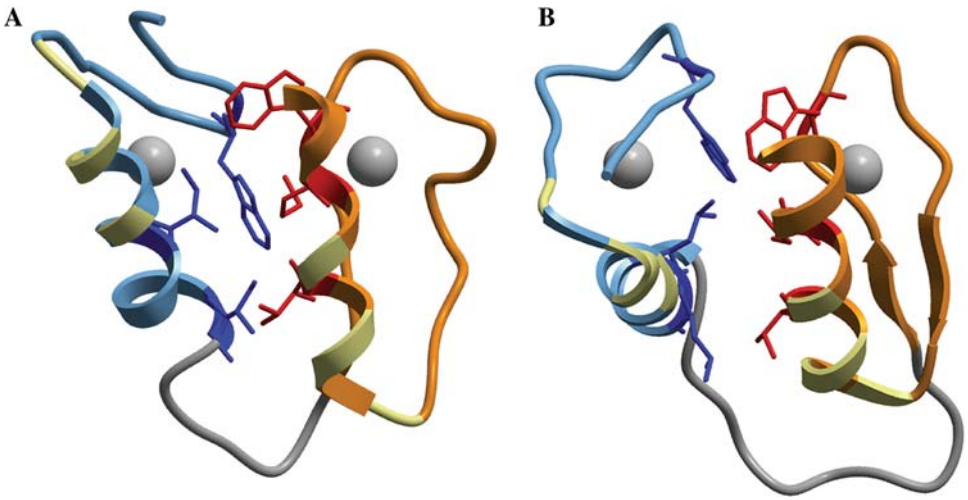


Fig. 6 α-helix inter-finger protein contacts. Ribbon diagrams depict fingers 1 (blue) and 2 (orange) of (**a**) Gli1 (2GLI) and (**b**) Zap1 (1ZW8). Interacting amino acids are shown as sticks. Positions typically involved in DNA binding by C2H2 domains are shown in yellow

seen in *early endosome antigen 1 (EEA1)*. EEA1 contains only one C2H2 domain located in the N-terminal region of the protein. It also contains a FYVE domain in the C-terminal region (Table 2). While both the C2H2 and FYVE domains are important for PPI with Rab5, the C2H2 domain is sufficient for interaction with Rab5 [33]. By combining scanning alanine mutagenesis with surface plasmon resonance (SPR) to measure PPI affinity, Merithew et al. [33] was able to localize the PPI to the first β-sheet (Fig. 7). In particular, mutation of either the phenylalanine or the isolucine residues located at the beginning of the β sheet, immediately before the first cysteine, caused a 100-fold decrease in affinity. Other substitutions, located on the same binding face but different regions of the C2H2 fold, had less of an effect on affinity, decreasing it by 10-fold [33].

Zac1, a protein involved in regulating apoptosis and cell-cycle arrest, contains seven C2H2 domains, some of which mediate PPIs (Table 2, [34, 118, 119]). By combining gel-shift experiments with scanning mutagenesis, Hoffmann et al. [34] demonstrated that fingers 2–4 and 6–7 bound DNA using residues in positions –1, 2, 3, and 6 of the α-helix [34]. Using co-immunoprecipitation of in vitro translated proteins, they further demonstrated that Zac1 formed homodimers. Homodimerization was dependent on fingers 1 and 2 [34], indicating a role in both protein and DNA binding for finger 2. As summarized in Fig. 8, a series of protein deletions enabled Hoffmann et al. [34] to

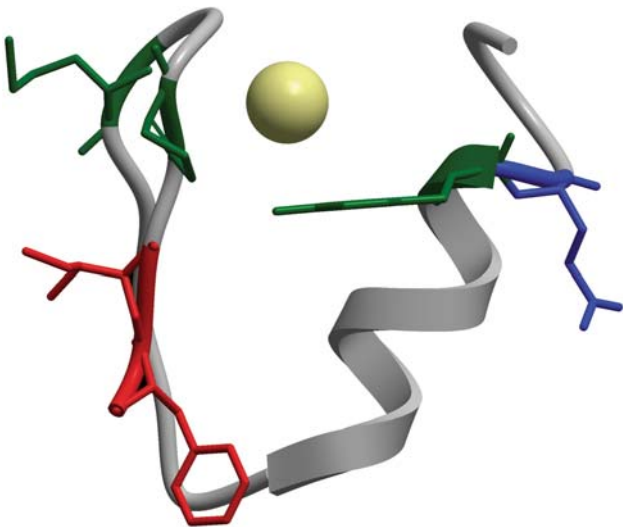


Fig. 7 Amino acids in EEA1 important for dimerization with Rab5. A homology model of EEA1 (based on 1PAA) shows the amino acids contributing to Rab5 binding. Critical residues determined by alanine mutation and surface plasmon resonance are colored according to fold decrease in binding affinity following mutation to alanine. Red ~ 100 fold decrease, green ~ 30- to 40-fold decrease, blue ~ 5-fold

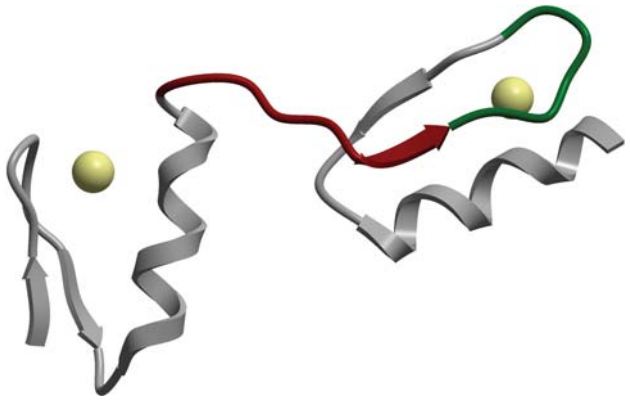


Fig. 8 Location of PPI binding surfaces in Zac1. A homology model of Zac1 (based on 1UBD) shows amino acids in fingers 1–2 critical for homodimerization. Residues colored red and green delineate finger regions that are strongly or moderately required, respectively

narrow the site of the PPI to a span of amino acids starting in the linker region between fingers 1 and 2 through the first β-strand of finger 2. The importance of specific amino acids was not elucidated.

Similar to GLI and Zap1, intra-finger PPIs can also be mediated by amino acids in the β-sheets. *Major histocompatibility complex enhancer binding protein 1 (MBP1)* contains five C2H2 domains arranged into a cluster of two fingers, a single finger, and a second cluster of two fingers. This protein also forms inter-finger bonds (Table 2, Fig. 9). However, the intra-protein interaction observed in MBP1 is very different than that seen in GLI and Zap1 (Figs. 6 and 9). Omichinski et al. [35] solved the structure of a synthetic peptide corresponding to the C-terminal pair of C2H2 domains of MBP1. As can be seen in Fig. 9, inter-finger contacts are made between a threonine located in the C-terminal end of the helix of finger 5, a valine in linker region, and a lysine located in the loop between the β-sheet and α-helix of finger 6 [35]. Although these fingers are known to interact with DNA [120–123] and the inter-finger contacts do not involve residues typically involved in DNA interaction, the effect of the interaction on DNA binding is not known.

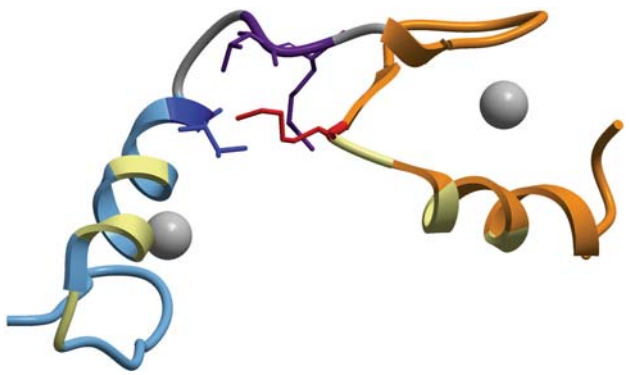


Fig. 9 Inter-finger contacts by MBP1 finger 5 (blue) and finger 6 (orange). Critical amino acids are shown as sticks. Residues that typically contact DNA are colored yellow. Zinc ions shown as gray circles

Interaction Diversity

Many Fingers, Many Partners

Human *Olf1/Early B-cell Factor 1-associated zinc finger protein (OAZ)* was one of the first proteins recognized for its ability to mediate hetero-PPIs with its C2H2 ZF domains. A large ZFP containing 30 C2H2 domains arranged into six clusters (Table 3), OAZ uses two different sets of zinc fingers to interact with two different DNA sequences [41, 42]. Using yet other sets of ZFs, OAZ can homodimerize or interact with at least three other proteins [41, 42, 124]. Working with the rat ortholog, rOAZ, Tsai and Reed [41, 124] determined that fingers 1–7 bind DNA, and fingers 25–29 mediate homodimerization as well as an interaction with Olf1/Early B-cell Factor (O/E1). Tsai and Reed [41] also examined the contribution of particular fingers to the interactions. Using "broken-finger" mutants, in which asparagine was substituted for the first histidine residue, they found zinc finger 29 to be critical for interactions with O/E1. However, this mutant had no significant effect on homodimerization. Tsai and Reed [41] were unable to localize the homodimerization surface, and concluded that the interaction surface was likely either distributed across several fingers or the fingers had redundant functions.

More recently, Hata et al. [42] reported fingers 9–13 of human OAZ bound to a different DNA target than the target of fingers 2–8 (homologous to fingers 8–12 and 1–7, respectively, of rOAZ). They also reported human OAZ interacting with SMAD1 and SMAD4 to regulate mesoderm and neural development [42]. This interaction was mediated using fingers 14–19 (homologous to fingers 13–18 in rOAZ). Importantly, they found that the OAZ interaction with O/E1 inhibited the OAZ-SMAD1/4 interaction, suggesting that these were two separate transcriptional pathways [42].

Another protein that interacts with a number of different proteins using C2H2 domains is *promyelocytic leukemia zinc finger (PLZF)*. The protein was first discovered as a fusion protein with retinoic acid receptor alpha (RARα) in acute promyelocytic leukemia patients with a t(15:17) translocation [125, 126]. PLZF contains nine C2H2 domains as a single cluster located in the C-terminus and an N-terminal Broad-Complex, Tramtrack and Bric-a-Brac (BTB) domain (Table 3). Fingers 3–7 are known to bind a GTACAGTT(C/G)CAT DNA consensus sequence [127].

PLZF also forms PPIs with several different proteins, each of which requires different combinations of fingers and produces various outcomes. Using GST-pull down assays, Martin et al. [43] demonstrated binding of PLZF fingers 1–3 to full length RARα [43, 128]: The authors also examined the ability of PLZF to interact with other nuclear receptors, including 9-cis RXRα, estrogen receptor alpha (ERα), glucocorticoid receptor (GR) and vitamin D receptor (VDR). The first three fingers of PLZF were able to interact with ERα, GR, and VDR but not RXRα, although the basis of this specificity was not determined [43, 128]. In this role, since interaction with PLZF caused a decrease in the transcriptional activities of RARα, ERα, GR, and VDR, PLZF appeared to be acting as a transcriptional repressor [43]. However, when examining the effect of the interaction on transcription, the authors noted that although PLZF seemed to have similar affinity for ERα, GR, and VDR based on the GST-pull down results, the effect on transcription activation by the nuclear receptor varied [43].

Promyelocytic leukemia zinc finger also acts as a transcriptional inhibitor via its interaction with Gata2 [44]. Tsuzuki and Enver [44] also found another C2H2 protein, *Fanconi anemia-related zinc finger protein (FAZF)*, which interacts with Gata2 using its ZFs. Although FAZF only contains three C2H2 domains, FAZF is structurally similar to PLZF because it also contains an N-terminal BTB domain and C-terminal C2H2 domains (Table 3). Despite having nearly 70% homology to the three fingers of FAZF, the last three fingers of PLZF (fingers 7−9) failed to pull-down Gata2. FAZF fingers 1–3 were able to interact with Gata2 [44].

Nanba et al. [46] reported a third PPI for PLZF with a distinct function. They found that fingers 6–7 of PLZF interact with C-terminal remnant of heparin-binding epidermal growth factor-like protein (HB-EGF-C). HB-EGF-C is formed following the proteolytic cleavage of membrane bound proHB-EGF. Upon cleavage, it translocates to the nucleus where it interacts with PLZF [46, 129]. The interaction of PLZF with HB-EGF-C results in nuclear export of PLZF [46]. Unlike the examples above, the PPI appears to disrupt PLZF-mediated repression of cyclin A. Thus this interaction results in transactivation [46].

A Few Fingers, Many Partners

In addition to proteins that use different zinc fingers to interact with different proteins, there are also examples of proteins that seem to use the same zinc finger(s) to interact with several different proteins. One of these, *specificity protein 1 (Sp1)*, is ubiquitously expressed in human tissues and contains three C2H2 domains (Table 3). Sp1 binds to the consensus sequence 5′-GGGCGG-3′ in GC-rich promoters found in many genes [131, 134]. Sp1 serves mainly as an activator of transcription for housekeeping genes and genes involved in growth regulation, but it can also act as a repressor in certain circumstances [47, 48, 130–140]. The ability of Sp1 to act as a repressor or activator depends, in part, on promoter access. Of particular interest are recent findings demonstrating interactions between the C2H2 domains of Sp1 and proteins responsible for various types of chromatin-remodeling proteins, including p300, SWI/SNF, and TAF1 [50]. Suzuki et al. [48] found that the ZFs of Sp1 interacted with the acetyltransferase region of p300. This interaction lead to acetylation of Sp1 and DNA binding by the zinc fingers. DNA binding by the zinc fingers in turn inhibited both the interaction with p300 and the subsequent acetylation of the zinc fingers [48]. Similarly, Kadam et al. [49] demonstrate a PPI between members of the ATP-dependent chromatin-remodeling complex SWI/SNF and Sp1 zinc fingers. Specifically, GST-pull down assays showed Sp1 zinc fingers were able to interact with BRG1, BAF170, and BAF155 [49]. Finally, Sp1 zinc fingers have also been shown to interact with the histone chaperon protein TAF1, resulting in the inability of Sp1 to bind DNA [50].

The function of Sp1 has been shown to vary depending on the co-regulators with which it interacts. This is true even for the interactions mediated by the zinc fingers. Interactions between the zinc fingers of Sp1 and nuclear corepressor protein (NCoR), B-cell lymphoma 6 protein (BCL6) interacting corepressor protein (BCoR), and silencing mediator for retinoid and thyroid receptor protein (SMRT) result in repression of transcription [47]. Contrary to these results, the interaction between E2F1 and the zinc fingers of Sp1 results in activation [51]. Rotheneder et al. [51] narrowed the site of the E2F1-Sp1 interaction to the start of zinc finger 1 through the β-sheets of zinc finger 2. It is unclear to what extent Sp1 might use different combinations of fingers for each PPI.

Ying Yang 1 (YY1) regulates a broad range of genes, both cellular and viral. YY1's functional versatility is likely due to its plasticity in recognizing DNA, the wide distribution of its binding sites in both distal and proximal promoter regions, its ubiquitous expression, and its interactions with a wide variety of co-factors [141–143]. Containing four C2H2 domains, YY1 can act as either a transcriptional activator or repressor, and is also known to participate in a wide variety of PPIs (Table 3). YY1 interacts with Sp1, resulting in transcriptional activation [26, 27]. Like Sp1, the binding surface of YY1 was narrowed to the start of finger 1 through the β-sheet of finger 2, although in this interaction all three fingers of Sp1 were used [26, 27].

Ying Yang 1 also interacts with ATFa2, a member of the ATF/CREB family, using the same region. Zhou et al. [52] demonstrated that YY1 can interact with ATFa2 in vitro

and in vivo, using fingers 1 and 2. The interaction resulted in repression of transcription from the c-fos promoter [52]. YY1-associated factor 2 (YAF2) also interacts with the finger 1–finger 2 region of YY1. However, this interaction results in proteolytic cleavage of YY1 rather than directly activating or repressing transcription [53].

Not all interactions involving YY1 are limited to the first two zinc fingers (Table 3). For example, Kurisaki et al. [54] demonstrated an interaction between YY1 and the DNA-binding domain of SMADs. Subsequent to testing several truncated proteins, the authors found all four zinc fingers of YY1 were required for the interaction. YY1 also displays specificity for the different SMADs, having highest affinity for SMAD4, then Smad1 and Smad3, and having the weakest affinity for Smad2 [54]. Since binding of YY1 to the SMADs inhibits their ability to bind DNA, YY1 acts as a transcriptional repressor in this context [54].

Other Domains, Other Interactions

In addition to containing variable numbers of ZF domains, ZFPs frequently contain a wide variety of other types of domains. KRAB, BTB, and SCAN are the most common types [144]. Not surprisingly, several examples of these multi-fingered, multi-domain proteins are involved in PPIs, often with several different binding partners. In many cases, the relative contribution of each domain type is unclear.

The homodimeriztion interface of *recombination activating gene 1 (RAG1)*, which is comprised of both a C2H2 domain and a Ring domain, is the best example of a PPI involving a ZF and another type of domain. RAG1 is an important member of the V(D)J recombination protein complex. It contains two widely spaced C2H2 domains, referred to as ZFA and ZFB, and a Ring domain located ~20 amino acids N-terminal to ZFA (Table 3). Early studies determined that RAG1 forms homo-oligomers, mediated through ZFA in cooperation with the adjacent Ring domain [55]. Structural analysis, however, revealed the dimer interface to be located in the linker region joining the C2H2 domain and the Ring domain [145]. The C2H2 domain is not part of the interface but acts as a critical scaffolding element [55, 145], similar to the structural role played by the C2H2 domain in the nuclease I-TevI [146]. RAG1 also interacts with RAG2; however, this interaction is mediated by ZFB [56]. The RAG1–RAG2 heterodimer is critical to the initiation of recombination since RAG2 serves to stabilize RAG1's interaction with the DNA at the cleavage site, possibly by altering the conformation or orientation of RAG1 [147, 148].

As discussed above in greater detail, *Zac1* forms homodimers using the second of its seven C2H2 domains [34]. Zac1 also interacts with p300 through its zinc fingers (Table 3). Using deletion experiments, Hoffmann et al. [57] demonstrated that different combinations of Zac1 fingers interact with different regions of p300. In particular, fingers 6–7 were required for the interaction with the KIX and CH3 domains of p300, whereas finger 2 was critical for the interaction with the HAT domain of p300. While the zinc fingers of Zac1 are sufficient for binding to p300, proper function also requires the interaction of the C1 region of Zac1 with the KIX domain of p300 [57]. It appears that simultaneous binding of the zinc fingers and C1 with p300 confers an allosteric change to p300 increasing histone and acetyl-CoA binding, and therefore increasing catalytic activity [57]. Amazingly, this complex set of interactions appears to also occur when fingers 2, 6, and 7 are simultaneously binding DNA [57].

B-cell lymphoma 6 protein is a transcriptional repressor that is required for B and T cell development and also has roles in oncogenesis [45, 149–151]. Dhordain et al. [45] characterized a PPI between BCL6 and PLZF. As detailed in the previous section, PLZF is involved in many protein interactions using different combinations of its zinc fingers. Unlike the previously described interactions, which rely exclusively on C2H2 domains, PLZF's interaction with BCL6 relies on the combination of a BTB domain and zinc fingers [45]. Similarly, both the BTB and zinc fingers domains of BCL6 are involved in the interaction [45].

Uncharted Domains

The examples above (Tables 2–3) describe PPIs mediated by C2H2 domains using a wide variety of binding surfaces. Unfortunately, the binding surface for the vast majority of ZF-mediated PPIs has not been determined. Table 4 presents a survey of over 100 PPIs, in which C2H2 domains are ostensiblely involved in the interface. In many cases, the interactions were not localized to a specific finger since clusters of multiple fingers were frequently treated as a single binding unit. In other cases, the interaction was found in a high throughput assay and not verified, or the protein fragment tested contained one or more C2H2 domains in conjunction with a large flanking sequence and the binding interface was not assigned to one region. These examples provide strong evidence that C2H2 ZFs are frequently involved in PPIs.

Engineering Zinc Finger PPIs

The DNA-binding properties of C2H2 ZF domains have been extensively studied and it is now possible to engineer ZFP that will bind to almost any desired DNA sequence

[3, 152]. This has led to the generation of artificial proteins that can be used as research tools and therapeutics [153–155]. Synthetic proteins can be generated by altering specific residues within the framework of a standard C2H2 domain, typically that of either Zif268 or Sp1. Alternatively, engineered proteins can be constructed by mixing and matching naturally occurring C2H2 domains to create a protein with novel DNA-binding properties [156].

McCarty et al. [83] reported the first successful attempt to create novel interactions by mixing together C2H2 domains from separate proteins. Working with Hunchback, a *Drosophila* protein that forms homodimers with a C-terminal pair of C2H2 domains, they examined the ability of both Ikaros and Hunchback to form heterodimers. They also examined chimeric domains containing residues from both proteins. Neither Ikaros nor Hunchback was able to heterodimerize. However, of the 12 chimeras tested, seven were able to interact with either Ikaros or Hunchback. In addition, of the three chimeras that did not interact with Ikaros, two were able to homodimerize.

Going one step further, Giesecke et al. [157] created an artificial gene network able to activate transcription from an endogenous gene in human cells [157]. Initially, they created novel PPIs by shuffling the ZFs from the Ikaros family. Treating the two PPI C-terminal fingers as individual domains, they mixed and matched fingers from human Eos, Ikaros, Pegasus, and Trps1, as well as Hunchback from *Caenorhabditis elegans*, *Drosophila melanogaster*, *Helobdella triserialis*, and *Locusta migratoria*, to generate an assortment of two-domain chimeric binding surfaces. Using a bacterial two-hybrid system, they were able to detect combinations of fingers yielding unique PPIs, as well as linking together the synthetic two-domain regions to create extended four-finger binding domains [157]. Finally, by linking one synthetic PPI domain to a synthetic DNA-binding domain and another synthetic PPI domain to an RNA Pol II activator (p65), they were able to stimulate VEGF-A expression in HEK293 cells.

Predicting Function

It had been previously observed that certain amino acid linker sequences connecting tandem repeats of C2H2 were highly correlated with ZF-DNA binding [158, 159]. In mammals, tandem ZFs are frequently separated by linkers of five amino acids, with roughly 50% of these having the sequence TGEKP (3]. The conserved linker plays an important role in DNA binding, with each residue playing an identifiable role in stabilizing the interaction. Although some known DNA-binding ZFs do not have this linker (for example, Tramtrack ZF1-2 [160]), and some that do not bind DNA do have it (for example, Gli ZF2-3 [31]), the presence of a TGEKP-like linker is currently the best predictor of DNA-binding. Tables 2 and 3 of this review list the best-characterized ZF-mediated PPIs. A survey of the linkers from these ZFs shows that many of them also have TGEKP-like linkers (Table 5). Specifically, linkers between Gli ZF2-3, Bcl6 ZF2-4, PLZF ZF5-8, Sp1 ZF1-3, Zac1 ZF6-7, and YY1 ZF2-4 contain close variations of this motif. Interestingly, with the possible exception of Gli ZF2-3, all of these ZF are involved in DNA binding as well [57, 66, 127, 134, 161]. This observation leads to several conclusions. First, while a TGEKP linker may be a useful predictor of DNA-binding function, its inclusion or exclusion does not appear to have prognostic value for ZF PPIs. Second, not only do C2H2 ZF domains deserve appropriate recognition as mediators of PPIs, it must also be appreciated that several of these domains appear to mediate both DNA and protein recognition. Indeed, some ZF may be capable of binding to both DNA and protein at the same time, such as those in Zac1 [57].

These conclusions suggest that one cannot hope distinguish ZFs that bind DNA from those that bind protein, because some ZF actually do both. Nor is it likely that a "protein-binding signature" can be identified that could be used to predict protein binding, as the TGEKP linker predicts DNA binding. ZF-PPIs are far more diverse in their modes of interaction than are the comparatively restricted ZF-DNA interactions, and are thus less likely to leave such an obvious calling card. This assertion is supported by the study that adjoins this review, in which we performed a bioinformatics-based search for such a protein-binding signature among known protein-binding ZFs and even clusters of partially related protein-binding ZFs [162]. We were unable to identify any pattern that was obviously distinct from that of known DNA-binding ZFs. It is also not possible to predict protein binding as a default of *not* having a TGEKP linker. Some ZF may have structural roles or no biological role, and it has also been known for a long time that some ZFs bind to RNA [21, 146]. The most cited example is the 9-finger TFIIIA, which contains two clusters of three DNA-binding ZFs, three or four RNA-binding ZFs, and some fingers that appear to be involved in both DNA- and protein-binding [21, 163–165]. Unfortunately, our knowledge of the prevalence and diversity of ZF-RNA interactions is comparatively even more limited than for ZF-PPIs. Only a handful of examples have been described [13, 14], and structures of the well-characterized TFIIIA-5S RNA interaction have only recently been described [15, 16]. Like ZF-PPIs, the role of ZF-RNA interactions has probably been underappreciated and deserves further investigation. Complexities such as the potential to bind DNA, RNA, or protein, and perhaps any combination of these, make it exceptionally challenging to accurately predict the function of C2H2 ZF domains.

Table 5 Linker regions for protein interacting C2H2 domains

Protein/Finger No.	Finger seq	Linker seq
Bcl6/F1	FFCNECDCRFSEEASLKRHTLQTH	SDKP
Bcl6/F2	YKCDRCQASFRYKGNLASHKTVH	TGEKP
Bcl6/F3	YRCNICGAQFNRPANLKTHTRIH	SGEKP
Bcl6/F4	YKCETCGARFVQVAHLRAHVLIH	TGEKP
EEA1	FICPQCMKSLGSADELFKHYEAVH	DAGND*
Eos/F5	FKCEHCRILFLDHVMFTIHMGCH	GFRDP
Eos/F6	FECNICGYHSQDRYEFSSHIVRGEH	KVG**
Fog1/F3	FVCLICLSAFTTKANCERHLKVH	TDTLS
Gli/F1	TDCRWDGCSQEFDSQEQLVHHINSEH	IHGERKE
Gli/F2	FVCHWGGCSRELRPFKAQYMLVVHMRRH	TGEKP
MBP1/F4	YICEECGIRCKKPSMLKKHIRTH	TDVRP
MBP1/F5	YHCTYCNFSFKTKGNLTKHMKSKAH	SKKCV
OAZ/F14	YPCNQCDLKFSNFESFQTHLKLH	LELLLRK
OAZ/F15	QACPQCKEDFDSQESLLQHLTVH	YMTTSTH
OAZ/F16	YVCESCDKQFSSVDDLQKHLLDMH	TFVL
OAZ/F17	YHCTLCQEVFDSKVSIQVHLAVKH	SNEKKM
OAZ/F18	YRCTACNWDFRKEADLQVHVKHSH	LGNPAKA
OAZ/F19	HKCIFCGETFSTEVELQCHITTH	SKK
PLZF/F1	EQCSVCGVELPDNEAVEQHRKLH	SGMKT
PLZF/F2	YGCELCGKRFLDSLRLRMHLLAH	SAGAKA
PLZF/F3	FVCDQCGAQFSKEDALETHRQTH	TGTDMA
PLZF/F4	VFCLLCGKRFQAQSALQQHMEVH	AGVRS
PLZF/F5	YICSECNRTFPSHTALKRHLRSH	TGDHP
PLZF/F6	YECEFCGSCFRDESTLKSHKRIH	TGEKP
Rag1/FA	VKCPAKECNEEVSLEKYNHHISSH	KESKEIFVHI***
Rag1/FB	YICTLCDATRLEASQNLVFHSITRSH	AENLE*
rOAZ/F29	YDCSQCPQKFFFQTELQNHTMSQH	AQ**
Sp1/F1	HICHIQGCGKVYGKTSHLRAHLRWH	TGERP
Sp1/F2	FMCTWSYCGKRFTRSDELQRHKRTH	TGEKK
Sp1/F3	FACPECPKRFMRSDHLSKHIKTH	QNKKG*
YY1/F1	IACPHKGCTKMFRDNSAMRKHLHTH	GPRV
YY1/F2	HVCAECGKAFVESSKLKRHQLVH	TGEKP
YY1/F3	FQCTFEGCGKRFSLDFNLRTHVRIH	TGDRP
YY1/F4	YVCPFDGCNKKFAQSTNLKSHILTH	AKAKN*
Zac1/F2	YKCVQPDCGKAFVSRYKLMRHMATH	SPQKS
Zac1/F6	HQCDHCERCFYTRKDVRRHLVVH	TGCKD
Zac1/F7	FLCQFCAQRFGRKDHLTRHTKKTH	SQELM*
Zap1/F1	LKCKWKECPESCSSLFDLQRHLLKDH	VSQDFKHPMEP
Zap1/F2	LACNWEDCDFLGDDTCSIVNHINCQH	GINFDIQFAN***

* Truncated at five residues, last C2H2 domain in a string

** Protein ends

*** Linker is longer than ten residues shown here

Concluding Remarks

The C2H2 protein domain is one of the simplest folds found in nature, yet it is proving to be profoundly intricate in its PPIs. Long recognized as a DNA-binding domain, an appreciation of C2H2 domains as protein-binding domains is growing. With recent advances, including structural information and more complete mutagenesis studies, the characterization of C2H2 and C2HC ZF-mediated PPIs is starting to approach our understanding of ZF-DNA interactions. However, the complexity and variety of PPIs add further challenges to this task. DNA-binding C2H2 domains rely on a binding surface comprised of a small number of amino acids invariably located in the N-terminal region of the α-helix. In contrast, protein-binding C2H2 domains utilize many different regions of the fold,

including the β-sheets, the linker regions, as well as residues in the α-helix. Different surfaces of the α-helix can be used, and the overall protein-binding surface is frequently larger than that observed for DNA binding.

Given the wider variety of interaction shapes and surfaces, one might speculate that C2H2 ZF interactions with proteins might actually be more common than interactions with DNA. In the research article that adjoins this review, we present experimental evidence that suggests this speculation may in fact be true [162]. We used an unbiased approach to investigate if clusters of ZF from the protein hOAZ could bind DNA or protein. We observed that several ZF clusters interacted with protein, including one previously known to bind DNA. However, none of the other ZF clusters were found to support DNA binding. This data suggested that DNA-binding might be a more difficult task for the ZFs to accomplish than protein binding, consistent with the more restricted interaction mode for DNA discussed in this review. Most studies attempting to characterize the functions of ZFPs have been concerned only with their DNA-binding activity. A re-examination of these proteins for potential PPI activity might provide additional and more accurate information about their biological functions.

Finally, the manipulation of DNA-binding ZFs to create diverse sets of custom DNA-binding proteins, research tools and drug therapies has resulted in a much greater understanding of how these domains interact with DNA. The recent works of Westman [30], McCarty [83], and Giesecke [157] have demonstrated that protein-binding ZFs can also be manipulated to create modified and selective protein-binding surfaces. It is hoped that continued engineering efforts will eventually produce custom protein-interaction tools as well as greater insights into the structural features underlying C2H2 ZF PPI function and specificity.

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