Structural Analyses of Zinc Finger Domains for Specific Interactions with DNA

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Introduction

One of the most well-known groups of transcriptional factors in eukaryotes is the zinc finger proteins, which coordinate zinc ions to achieve a folded structure that is crucial for interactions with its binding partners in physiological systems [20, 34, 36, 38–40, 43, 48, 51, 68]. Biochemical and biophysical studies of these metalloproteins have focused on eukaryotes owing to their critical functions in signaing cascades. The growing evidence and genomic advances proved that zinc fingers perform crucial roles in maintaining physiological processes from prokaryotes to eukaryotes, including the plant kingdom [2, 28, 31, 42, 52, 57, 68]. Early studies of zinc fingers have been limited to DNA binding ability and its transcriptional regulation, but recent applications of zinc fingers in various fields are of major interest owing to their specific interactions for cognate DNA, RNA, proteins, lipids, and small molecules through hydrogen bonds and hydrophobic interactions [32, 43, 56, 68]. The binding affinities of zinc fingers to their binding partners are significantly high, and preliminary reports proved that these proteins bind to their specific nucleic acids with sub-nanomolar ranges of dissociation constants (K_d). The elaborate control of transcriptional regulation is triggered by this tight binding, and gene expression is up-regulated through this complex generation

Zinc finger proteins are among the most extensively applied metalloproteins in the field of biotechnology owing to their unique structural and functional aspects as transcriptional and translational regulators. The classical zinc fingers are the largest family of zinc proteins and they provide critical roles in physiological systems from prokaryotes to eukaryotes. Two cysteine and two histidine residues (Cys2His2) coordinate to the zinc ion for the structural functions to generate a ββα fold, and this secondary structure supports specific interactions with their binding partners, including DNA, RNA, lipids, proteins, and small molecules. In this account, the structural similarity and differences of well-known Cys2His2-type zinc fingers such as zinc interaction factor 268 (ZIF268), transcription factor IIIA (TFIIIA), GAGA, and Ros will be explained. These proteins perform their specific roles in species from archaea to eukaryotes and they show significant structural similarity; however, their aligned amino acids present low sequence homology. These zinc finger proteins have different numbers of domains for their structural roles to maintain biological progress through transcriptional regulations from exogenous stresses. The superimposed structures of these finger domains provide interesting details when these fingers are applied to specific gene binding and editing. The structural information in this study will aid in the selection of unique types of zinc finger applications in vivo and in vitro approaches, because biophysical backgrounds including complex structures and binding affinities aid in the protein design area.

Keywords: Zinc finger proteins, metalloproteins, classical zinc finger, transcriptional regulator
Structural domains of zinc fingers have been applied to gene-editing technologies to improve low specificity in vitro and in vivo for the use of clinical trials in the last decade [20, 48, 74]. This technology has received considerable attention because zinc fingers can improve specific binding abilities to target nucleic acids as a part of nucleases. Most zinc finger proteins have more than one domain, and many biophysical reports proved that one zinc finger domain is not enough to obtain specific bindings [60, 76], although one GAGA binding domain from Drosophila has a significantly high binding affinity to its cognate sequence of DNA [53]. To understand these tight bindings at the atomic level, interaction details of zinc fingers have been widely studied using nuclear magnetic resonance (NMR) and X-ray crystallography techniques. Unfortunately, the structures of zinc fingers still need to be investigated because of the limited solubility of full sequences of zinc finger proteins and difficulties of crystallization. Many zinc fingers have limited solubility due to zinc finger domains and other parts of the proteins, and this limited solubility retards structural and functional studies.

In this account, the authors would like to discuss interaction details of Cys$_2$His$_2$-type zinc finger domains based on structural features that are widely studied and applied to recent advances in biotechnologies. There are growing reports of zinc finger domains in most kingdoms, including archaic, prokaryotic, and eukaryotic species, and their physiological and biochemical features need to be discovered for successful applications in terms of biotechnologies, including gene editing, therapeutic targets, alteration of enzymatic pathways, and improving the yields of specific metabolites. The zinc ion is a structural factor in zinc finger proteins that regulates transcriptional and translational pathways [38, 43]. These structural domains are essential in biological systems, including normal processes to maintain routine pathways and to respond to specific signals that can be artificially controlled based on the primary to quaternary structures of these domains. Cys$_2$His$_2$-type domains have been extensively and intensely investigated over the last three decades in order to understand their binding events and affinity against their cognate binding partners. This article will further compare the structural aspects of various zinc finger domains, from metal coordination to nucleic acid interactions. The structural similarity and sequence variations are reviewed to give valuable information for the selection and application of suitable candidates among zinc fingers for bioengineering.

![Fig. 1. Classical zinc finger domain (Cys$_2$His$_2$-type) coordinates with the zinc ion (cyan ball), and the secondary structures are generated rapidly.](image)

Four residues, Cys107, Cys112, His125, and His 129, coordinate to the zinc ion with tetrahedral geometry. The first zinc finger domain from synthesized ZIF268 coordinates with the zinc ion to form a ββα structure to interact with dsDNA (PDB accession: 4X9J) through an α-helix.
including zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALENs) [5, 27, 48]. Although cleavage domains have powerful activity, they should recognize specific sequences to perform their cleavages to their target DNAs. These applications of binding domains have powerful advantages in terms of safety and toxicity when they are applied to in vivo systems. The zinc ion generates tetrahedral geometry (Td) rapidly when it is applied to the apo-zinc finger domain in vitro system to recognize target DNA [19, 23, 24, 46, 51]. The side chains of Cys107 and Cys112 coordinate with zinc ion in the first and second β-strands, respectively (Fig. 1). Two histidine residues, His125 and His129 on the α-helix, coordinate to zinc ion and these finally aid in generation of proper folding of the zinc finger domain [78]. Reports have discovered that zinc finger domains show significantly tight binding affinity with their physiological ions, Zn\(^{2+}\) (femto- to picomolar dissociation constant), compared with other metal ions such as cobalt ion (Co\(^{2+}\), micromolar dissociation constant) [10, 11, 41, 49, 50, 63, 68, 69]. Several studies, however, have reported that toxic heavy metals, such as Cd\(^{2+}\), can bind tighter than the zinc ion [37, 55, 72].

The structural and functional studies of nonclassical zinc finger domains showed many interesting aspects based on the combinations of Cys and His residues such as Cys\(_5\), Cys\(_5\)His\(_3\), Cys\(_5\)His\(_5\), and Cys\(_2\)His\(_5\)(His\(_1\))Cys\(_1\) types of zinc finger domains [43, 54, 68].

**Structures of Classical Zinc Fingers**

Classical zinc fingers are the largest family among zinc finger proteins, and a single domain usually has 28–30 amino acids involved in their structural role to perform specific DNA interactions, although some of them generate tight binding with RNA, proteins, and lipids [43-45, 73]. Each zinc finger is modular and can fold independently in the presence of a zinc ion, although some cases do not achieve suitable binding affinities. The structures of ZIF268 and its cognate DNA complex have been intensively studied by X-ray crystallography, and the specific roles of each finger have been discovered [25, 62, 78]. ZIF268 regulates the memory storage and recall through specific mechanisms of neural plasticity in the human brain. Studies at the molecular and cellular levels demonstrated that activation and repression of diverse signals of brain...
behavior were generated through ZIF268, an activity-dependent transcriptional factor, and this zinc finger is considered as a central regulator of neural plasticity [3, 4]. This zinc finger is also called early growth response-1 (EGR-1), and these proteins show rapid and transient responses against extracellular stresses [21, 35, 67, 75]. ZIF268 consists of a threonine (Thr)- and serine (Ser)-rich N-terminal domain and a Thr-, Ser-, and proline (Pro)-rich C-terminal domain. ZIF268 shows high sequence similarities among these three domains, although their recognitions to their binding partners are quite specific, since each zinc finger shows different DNA binding specificity and different binding affinities [53]. The primary structures of these fingers show that Cys-X$_4$-Cys-X$_{12}$-His-X$_7$-His (X represents any amino acids) generates the appropriate secondary structures (Fig. 2). There are linker regions between each zinc finger that usually show repeated patterns in eukaryotes. These linker regions have a TGEKFQP sequence that generates loops between each domain. Three domains in ZIF268 generate crucial binding with 5'-GCGG(T)GGGCG to control transcription at promoter regions in the nucleus for the regulation of the nervous system [53]. The structural information from X-ray crystallography indicates that the Cys$_2$His$_2$ motif coordinates to the zinc ion with a stable $\beta\alpha\beta$ fold, as shown in Fig. 2B, which interact tightly with the major groove of DNA [25]. The preliminary studies showed that ZIF268, as a transcriptional regulator, binds to the major groove of DNA and wraps around the double helix. Each finger interacts with three or four bases in DNA.

The sequence Cys-X$_4$-Cys-X$_{12}$-His-X$_7$-His is a general pattern of zinc fingers in the eukaryotic kingdom. Transcription factor IIIA (TFIIIA) is the first identified zinc finger domain, which was discovered from oocytes of African frogs, and this zinc finger has a sequence pattern of zinc fingers in the eukaryotic kingdom. TFIIIA is the first identified zinc finger domain, which was discovered from oocytes of African frogs, and this zinc finger has a sequence pattern like that of ZIF268, as shown in Fig. 2A [7, 8, 18, 30, 33, 34, 56, 70]. The overall secondary structures of both ZIF268 and TFIIIA are a $\beta\alpha\beta$ fold, but the sequences of these two zinc fingers show low sequence homology. TFIIIA has nine zinc fingers, and initial studies proved that these modular fingers have approximately 30 amino acids and a zinc ion in each finger. This protein, TFIIIA, consists of a zinc finger with an N-terminal region containing 280 amino acids for DNA or RNA binding regions and 65 amino acids that interact with other transcriptional factors in the C-terminus. The folding mechanism of classical zinc fingers proposed that coordination is initiated by two cysteines in the $\beta$-strands with Zn$^{2+}$ through the support of the hydrophobic core (Fig. 2B). Computational studies proposed a metal-coordinating mechanism. The first His in the $\alpha$-helix participates in this coordination, and the second His completes a stable $\beta\alpha\beta$ fold in the presence of a zinc ion [26, 42, 58, 77].

In most organisms, TFIIIA has been widely studied and detected owing to the structural and functional importance of the cellular system, as it requires complex generation with 5S ribosomal RNA (5S rRNA) for transcriptional activity. Among different organisms, interestingly, all TFIIIA has nine consecutive zinc fingers except that of Schizosaccharomyces pombe, which has a tenth domain in the C-terminus with a significant space [42]. The general sequence of TFIIIA includes phenylalanine (Phe), isoleucine (Ile), and/or leucine (Leu), and these residues have specific interactions with specific sites in an internal control region (ICR) of 5S rRNA genes. The mutational studies proved that all zinc finger domains of TFIIIA contribute to DNA interactions, although they have different binding affinities to cognate DNAs [22]. TFIIIA has the same general sequence as ZIF268 (C-X$_4$-C-X$_{12}$-H-X$_7$-H), but the last zinc finger has one more amino acid between the first and second zinc-coordinating histidine residues. The overall $\alpha$-helix structure is not affected owing to one more amino acid, but the last turn toward the C-terminus shows a wider turn compared with those of the first and second zinc fingers.

**Specific Interaction with Single Zinc Finger**

Most zinc finger proteins require two or more zinc finger domains for specific interactions, but Pedone et al. [59, 64, 65] proposed that the structural motif from GAGA (Fig. 2B), a single Cys$_2$His$_2$-type zinc finger isolated from Drosophila, has sufficient affinity to bind DNA in the GA(CT)-rich region of the promoter. The GAGA finger has 82 residues at the end of the N-terminal region that generate 5'-GAGAGAG near transcription start sites. The minimal DNA binding domain shows a very low dissociation constant ($K_d \sim 5$ nM) to (GA)n sequences. In the N-terminus of the GAGA zinc finger domain, there are two basic regions called basic region 1 (BR1) and basic region 2 (BR2). Although single zinc finger domains show tight binding to DNA, these basic residues are critical for complex formation. BR1 and BR2 have highly conserved arginine (Arg) and lysine (Lys) residues, and BR2 residues generate an $\alpha$-helix. This N-terminal extension is unique in its binding compared with other zinc fingers. The structural studies show that the GAGA DNA binding domain generates hydrogen bonds through the major and minor grooves [59, 64]. As shown in Fig. 2A, GAGA has a general X$_3$-C-X$_7$-C-X$_{12}$-H-X$_7$-H-X$_7$ sequence. Compared with other eukaryotic zinc finger
domains, GAGA has one less amino acid between two zinc-coordinating Cys residues (C-X_2-C) and one more amino acid between two His residues (C-X_4-C). These additional interactions with basic residues are an interesting primary structure and a main reason that GAGA binds specifically to GAGA-rich sequences with one zinc finger domain.

The basic residues, including Lys13, Arg14, and Lys23, generate contacts with the minor groove. There is an extended α-helix (Arg27-Ser32) and secondary structure of these amino acids that participate in interactions with major grooves. Arg27, Ser28, and Ser30 interact with the bases, phosphate, and deoxyribose sugar ring in double-stranded DNA [59]. The well-elaborated sequences of primary structures for the functional activity as a transcriptional regulator with an extended N-terminus are crucial for the complex generation of protein-DNA. The GAGA zinc finger domain has one structural motif, and it can be limited to the βαβ secondary structures, but a single domain of GAGA cannot generate satisfactory interactions without the help of extended regions of the N-terminus. Hydrogen bonds from basic residues and structural features from the α-helix aid in specific and tight binding with one single zinc finger domain to its binding partner.

Classical Zinc Fingers in Prokaryotes

The first identified zinc finger domain in prokaryotes is Ros from Agrobacterium tumefaciens and this 15.5 kDa protein, with a coordinating zinc ion, serves as a transcriptional regulator. Ros mutational studies proved that the zinc finger domain negatively controls the virC and virD operons that regulate the oncogene-bearing region in the T-DNA of the T1 plasmid [1, 14]. This signaling cascade finally suppresses ipt in the plant. Ros affects a large number of plants, and it was proposed that horizontal gene transfer occurred from bacteria to plants, although there is a second opinion for this proposal. The primary sequence of this zinc finger domain shows that only nine amino acids are placed between the second Cys and the first His residue (Fig. 2A).

This classical and prokaryotic zinc finger shows significant differences in structural and functional aspects compared with those of eukaryotic zinc fingers. The apo-domain, which is the nonmetal-coordinating state, of Ros cannot generate typical secondary structures like the other zinc fingers, including the classical and the nonclassical fingers. NMR studies revealed that two β-strand regions show high structural similarity compared with those of eukaryotes, although the α-helix structures of zinc fingers from eukaryotes demonstrate different patterns [47]. The α-helical structures from ZIF268, TFIIIA, and GAGA have more than three turns, but the same regions of Ros have two turns due to a lower number of amino acids (Fig. 2). The α-helix looks more tilted owing to the second His residue. For the generation of tetrahedral (T_d) geometry around the zinc ion with ε-nitrogen, the His residue requires more space, which ultimately causes the different angles of the α-helix to the β-sheet (Fig. 2B). Although different numbers of amino acids are placed between eukaryotic and prokaryotic zinc fingers, the overall pattern of His residues is almost similar [47]. The front view of the zinc fingers shows that the side chains of the first and second His residues are positioned almost perpendicular to each other for the generation of T_d geometry.

Structural Similarity among Cys d His d-Type Zinc Fingers

Zinc Finger Domain as a Transcriptional Regulator

A single domain in zinc finger proteins usually has 20–30 amino acids that compose the structural motif critical for its biological functions [9, 11, 12]. These domains, in many cases, show sequence homology and structural similarity in zinc finger proteins. X-Ray crystallography provides structural details of ZIF268 when it is bound to its interaction partner, although most structural information about the zinc finger motif was obtained by NMR studies owing to instabilities when this structural motif did not bind to its interaction partners [25, 62, 67, 76]. There are three domains that bind to the major groove of dsDNA, and these show sequence and structural similarities in ZIF268 (Figs. 2A and 3A). Their interactions through hydrogen bonds and hydrophobic stacking induce diverse interactions in the finger domains, although their sequence homologies in each domain are highly conserved. All three domains from the first (gray) to the third (magenta) zinc fingers show high structural similarity, more than those of their sequence homologs (Fig. 3A). Two β-strands and a single α-helix show high structural similarity because the root mean square deviation (r.m.s.d.) values are less than 0.6 when they are superimposed upon each other. The calculated r.m.s.d. values when the first and second, first and third, and second and third zinc fingers were superimposed were 0.529, 0.364, and 0.404, respectively (Fig. 3A). The linker region between the two β-strands has six amino acids in the first zinc finger in ZIF268, because the first β-strand (Tyr5, Ala6, and Cys6) and the second β-strand (Arg14, Arg 15, and Phe 16) have two more amino acids compared with those of the second zinc finger in

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December 2016 | Vol. 26 | No. 12
ZIF268 (Fig. 2A). The sequences of the first, second, and third β-strands show high homologies. The first β-strand starts with aromatic amino acid residues (Tyr5, Phe35, and Phe63), and the first residues of the second β-strand of each zinc finger starts with Arg residues (Arg14, Arg41, Arg70). Aromatic and hydrophobic residues are crucial for the folding of zinc fingers, and polar and hydrophilic residues are crucial for the folding for the generation of specific interactions through the α-helix [25, 35].

TFIIIA, the first identified zinc finger, is one of the most well-known and investigated structural motifs from different organisms and species. This zinc finger protein includes several interesting aspects owing to its extraordinarily strong binding affinities to DNAs and RNAs. In addition, this zinc finger protein has a large number of domains and species. The first three zinc fingers are critical for the interactions, and these residues are overlapped and show high structural similarity, as shown in Fig. 3B. Preliminary studies support that this N-terminus has three zinc fingers that are necessary for the specificity of binding. The preliminary studies proved that the first and third fingers interact with 5′-TGGATGGGAGACC in Box C at ICR. Nine zinc fingers were considered to bind to BOX A, intermediate element (IE), and Box C at ICR [58, 71]. The superimposed structures of the N-terminus three zinc fingers show high structural similarity, as demonstrated by the alpha carbon (Cα) aligned r.m.s.d. value. The superimposed structures with Cα aligned structures of the first–second, first–third, and second–third finger domains of TFIIIA have r.m.s.d. values of 1.082, 0.634, and 0.780, respectively. These values are slightly high compared with those of ZIF268, although they have the same sequence patterns (C-X4-C-X12-H-X4-H). The slightly high values of r.m.s.d. were generated from the β-sheet and α-helix in this domain. The two β-strands of ZIF268 were well matched in space, although first zinc finger has one more amino acid compared with those of the second and third zinc fingers (Figs. 2A and 3B). In addition, structures of the helical turns from the three zinc fingers of ZIF268 are well superimposed, and these will accelerate the interactions to the major groove of dsDNA [15, 25, 62]. The β-strands of the second zinc finger of TFIIIA are not positioned in the same space compared with those of first and third zinc fingers, and this is observed by slightly higher r.m.s.d. values compared with those of ZIF268 (Fig. 3B). In addition, the α-helix of the second zinc finger is positioned in a different space. These factors made the differences of r.m.s.d. of these three zinc fingers. Subtle differences from structural motifs generate specific interactions with strong binding affinities with their binding partners, and it proved that slight differences in structure distinguishes their cognate binding partners.

**Structural Similarity in a Single Zinc Finger**

The structural similarity is important for generating specific interactions in this case. They recognize different sequences from different nucleic acids, but structural similarities are monitored in zinc fingers. The first three fingers from TFIIIA show high structural similarity as described, although the r.m.s.d. values of these are slightly lower than those of ZIF268 [42, 58, 76, 77]. The significant changes are not monitored, but the position of the second β-strand of the second zinc finger is slightly different.

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**Fig. 3.** Superimposed structures of classical zinc finger domains.

Each zinc finger domain of ZIF268 (A) and TFIIIA (B) shows high structural similarity. The first (gray), second (green), and third (magenta) zinc fingers are aligned with the backbone (Cα). (C) The superimposed structure of the first zinc finger motif of ZIF268 (purple) and TFIIIA (pale green). The two structures show structural homology.

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compared with those of the first and the third. In addition, the turns of the α-helix show different lengths of pitches among zinc fingers. These subtle changes in structures can generate specific interactions among different fingers in the same proteins.

The structures of zinc fingers are usually characterized through NMR studies, but the structures of ZIF268-DNA and TFIIIA-DNA complexes were discovered by X-ray crystallography owing to the stability from the zinc finger domains and the DNA complexes. The first domains of these zinc fingers were studied to understand the structural similarity, although their sequences show differences (Fig. 3C). The numbers of amino acids between the Cys and His residues that coordinate the zinc ion show the same patterns between these two zinc fingers. The Cα-aligned structure has an r.m.s.d. value of 0.584, and the overall structures are well superimposed between these two zinc fingers, although low sequences are monitored except for the linker regions. These results demonstrated that the structures are more conserved compared with that of sequences, but the specificity of the sequences may be a critical factor for the recognition of its binding partners. Zinc fingers as transcriptional and translational regulators require specific binding affinity when signals are triggered, and these events activate enzyme activation immediately in our biological systems [43].

Protein and DNA Interactions

Classical zinc fingers naturally acquire zinc ions to recognize DNAs through folding processes in physiological systems [6, 9, 12, 13, 33]. Among these various complexes, ZIF268 is one of the most intensively investigated to understand the interactions between protein and nucleic acids because the high-resolution crystal structures have been reported for more than decades. Pabo and co-workers have made significant progress to achieve elaborate DNA-ZIF268 structures [25, 60, 62, 76]. These improvements enhance the understanding of the biological implications of transcriptional factors. The initial crystal structure proposed that the finger domains of ZIF268 wrap up specific sequences of DNA, which fit nicely into the major groove [62]. Each finger holds tightly to three base pairs, and high-resolution X-ray crystallography proved the details of the interactions [25]. Water molecules between ZIF268 and DNA generate hydrogen bonds, and this successfully explained how these fine bindings are generated between certain sequences of DNA and other proteins. There are two conserved Arg residues in each ZIF268 domain among the three domains that generate complexes with its cognate sequence of DNA. The first conserved Arg residues are located in the second β-strand, and they point in the same directions in space; these residues are Arg114, Arg142, and Arg170 (Fig. 4A). The second set of interesting Arg residues include Arg118, Arg146, and Arg174, positioned at the starting N-terminus of the α-helix, and these residues play pivotal roles in generating specific interactions (Fig. 4B). These residues face the same directions when they are superimposed, demonstrating that these Arg residues in each finger domain perform similar interactions.

The first conserved set of Arg residues (Arg114, Arg142, and Arg177) positioned at the second β-strand of the domains, and these amino acids generate hydrogen bonds, not with bases of DNA but through the phosphate backbone or ribose atoms within a 2.5–3.5 Å distance (Fig. 4A). This second β-strand faces the major groove DNA and supports strong interactions with the α-helix. The linker placed between finger 1-2 and finger 2-3 twist for the fitting, and its flexibility is necessary to obtain the high association constant of this protein-DNA complex. The superimposed structures of side chains of the first conserved set of Arg residues appear slightly different on space, but this is required for the suitable hydrogen bonds.

The second conserved set of Arg residues (Arg118, Arg146, and Arg174) located at the starting point of the α-helix are critical for overall complex generation with hydrogen bonds in ZIF268 (Fig. 4B). All three of these residues generate hydrogen bonds with purine or pyrimidine atoms. Arg residues generate more than one hydrogen bond
through nitrogen atoms in its side chains. These aspects proved that repeated zinc fingers from ZIF268 interact with 3 bp DNA, and the functional consequences are quite repeated. The major contacts between ZIF268 and DNA depend on the bases and a few hydrogen bonds with the DNA backbone and are not critical for interactions. The complex structure demonstrated that the bases of DNA regulate the orientation of each zinc finger. These binding events control the partial structures in zinc finger proteins, and the secondary structures are tightly controlled in an interaction-based manner.

Conclusions and Perspectives

The binding affinity of the three zinc fingers of ZIF268 to its DNA show quite strong interactions \( K_d = 1.7 \times 10^{-10} \text{ M} \) with its target sequence of DNA [25]. Three zinc fingers show repeated fits into the major groove of DNA, and these interactions are through mostly one strand (Fig. 5A). The GAGA zinc finger protein has one zinc finger domain, and it undergoes specific interactions with its binding partner \( K_d = 5.3 \pm 0.7 \times 10^{-9} \text{ M} \), although it has extended regions, including BR1 and BR2 as described in Fig. 5B [64]. The superimpose studies in this account \( \text{vide supra} \) summarized that the binding affinities of classical zinc fingers are quite different although their structures are almost the same. Similar structures of zinc finger domains with different amino acids can recognize their binding partners for specific interactions. These binding events are well explained through the complex of GAGA and DNA [59, 64, 65]. One zinc finger usually does not have strong enough binding for DNA, and two or more zinc fingers are usually included in zinc finger proteins. For this reason, the extended N-terminal regions increase binding affinity through interactions from side chains and the base of DNA to achieve specific interactions.

For applications in the diverse fields of bioengineering technology, the zinc finger proteins, especially classical zinc fingers, have been widely studied to understand their biological implications and biochemical details [11, 43, 68]. Classical zinc finger proteins, the second largest family of all proteins, participate in a wide spectrum of cellular activities, including differentiation, development, and suppression of tumors. The metal binding aspect and their specific folding process show totally different structural patterns compared with other metalloproteins, since the

![Fig. 5. The complex structures of the zinc finger domains and DNA.](image)

(A) ZIF268 binds with its target DNA through the major groove. The first (gray) to third (magenta) zinc fingers fold independently with the zinc ion (yellow sphere). The \( \alpha \)-helices face the nucleic acids for the generation of hydrogen bonds. (B) Single zinc finger domain of GAGA with an extended region of the \( \alpha \)-helix clamped efficiently through the major and minor grooves of DNA. This N-terminal extension includes highly basic regions (BR1 and BR2).
zinc finger domains can fold only in the presence of metal ions. In addition, extraordinarily strong binding affinities to their cognate partners are promising for applications in the fields of engineered zinc fingers. There are some applications for the inhibition and activation of specific genes, including HIV-1, herpes simplex virus, VEGF-A, and the regulation of small molecules [68]. Scientific achievement in the realm of zinc fingers is still needed, specifically to discover the huge number of zinc finger proteins and to understand the transcriptional progress against diverse responses. The small region of domains from these large proteins is a powerful candidate to provide a key for gene regulation in terms of biotechnology.

Acknowledgments

This research was supported by the Yuyu Pharma, Inc. and Chonbuk National University research program in 2016.

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