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Neural Zinc Finger Factor/Myelin Transcription Factor Proteins: Metal Binding, Fold, and Function

Angelique N. Besold and Sarah L. J. Michel*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland 21201-1180, United States

ABSTRACT: Zinc finger (ZF) proteins make up a large family of metalloproteins that contain discrete domains with amino acid ligands (cysteine and histidine) that serve to coordinate zinc in a tetrahedral geometry. Upon zinc coordination, the domains adopt three-dimensional structure. The most well-studied ZFs are the "classical" ZFs, which use a Cys_2His_2 motif to bind zinc and adopt an antiparallel β sheet/α helical fold. In addition to the classical ZF class, at least 13 other ZF classes, collectively termed nonclassical ZFs, have been identified. These other classes are distinguished by the combination and order of the cysteine and histidine ligands within each domain, the spacing between each ligand (i.e., number and type of amino acid), and the structural architecture that the domain adopts in the presence of zinc. One class of nonclassical ZFs, the neural zinc finger/myelin transcription factor (NZF/MyT) class, contains ZF domains with a Cys_2His_2Cys ligand set, adopts a fold that consists of a series of loops in the presence of zinc, and functions as transcription factors by binding to and regulating genes that are critical for the development of the central nervous system. This Current Topic focuses on our understanding of the NZF/MyT class of nonclassical ZFs and presents current hypotheses regarding this class' unique mechanism of metal-mediated folding and function.

Metal ions, which are required for a myriad of biological processes, are found as cofactors in more than a third of structurally characterized proteins.[1−6] Zinc, in particular, plays diverse roles in the body, with functions ranging from serving as a cofactor in up to 10% of all proteins to acting as a signaling molecule.[7−12] Zinc finger (ZF) proteins make up a large family of zinc cofactored proteins that comprise 3−5% of the human genome.[7,13−17] More than 14 different classes of ZF proteins have been identified, all of which coordinate zinc ions in a tetrahedral geometry using a combination of four cysteine and/or histidine residues.[12,14−25] The classes of ZF proteins are delineated on the basis of the exact composition of the metal-coordinating ligand set (e.g., the number and sequence of cysteine and histidine residues), the spacing between these ligands, and the fold that the protein adopts once metal is bound.[15,17] In the absence of metal ions, these domains lack secondary structure but adopt structure and thus function when zinc ions are coordinated (Figure 1).[24] These ubiquitous proteins have various functions, including transcriptional and translational control.[7,15,17,24] Of particular interest is their ability to interact with DNA, as more than half of all human transcription factors contain ZF domains.[26,27] The most well-studied DNA binding ZFs are the "classical" ZF proteins, which have Cys_2His_2 domains (CCHH motif) and adopt a $\beta\beta\alpha$ fold upon zinc coordination.[14,15,17] The DNA binding properties of these classical ZFs are so well understood that they are the focus of many protein design efforts to engineer artificial agents for gene therapy.[26,28−31] The "nonclassical" ZF proteins are less well studied, and the data obtained to date have revealed that these proteins adopt a large range of secondary structures upon zinc coordination.[16,17] The focus of this Current Topic is the neural zinc finger/myelin transcription factor (NZF/MyT) family of nonclassical ZF proteins, which are essential to the development of the central nervous system (CNS). These proteins contain unique sequence and structural elements that are critical for their function.[17,32−34]

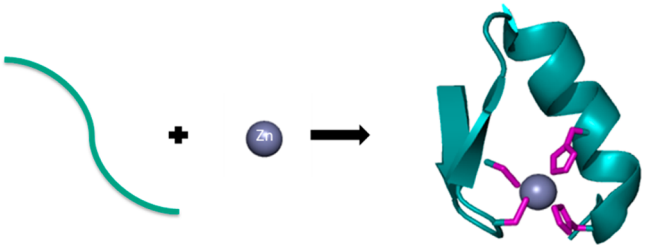

Figure 1. Cartoon depicting the role of Zn(II) in promoting the structure of ZFs. In the absence of Zn(II), the ZF domain is unstructured. Upon zinc coordination, the domain adopts distinct secondary strucutre. In the case of classical ZFs (CCHH), such as XFIN (PDB entry 1ZNF), this involves a $\beta\beta\alpha$ fold.

The NZF/MyT family of ZF proteins is a small but critically important family of ZFs. Three members have been identified: myelin transcription factor 1 (MyT1 or NZF-2), neural zinc finger factor-1 (NZF-1 or MyTl-like or png-1), and suppression of tumorigenicity 18 (ST18 or NZF-3).[32−34] These proteins

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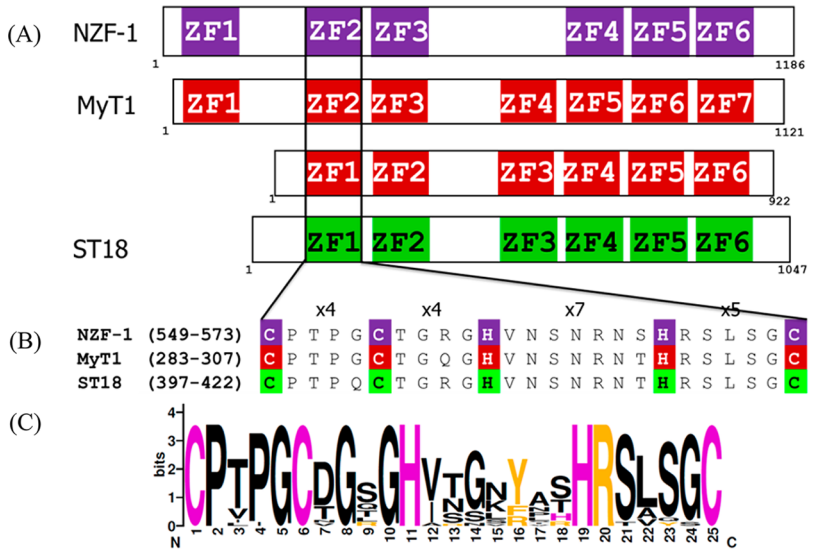

Figure 2. Alignment and sequences of the ZF domains of the NZF/Myt family. (A) Cartoon of ZF clusters in each NZF/MyT protein from *Homo sapiens*. Note that MyT1 contains two isoforms, both of which are shown here. (B) Alignment of ZF2 from each *H. sapiens* protein. (C) Sequence logo depicting conservation of amino acids. The height corresponds to the degree of conservation, with four bits being 100% conserved.[110]

contain multiple ZF domains that are found in clusters of one, two, three, or four (Figure 2A). Each of these domains contains five absolutely conserved cysteine and histidine residues with the sequence **C**PXPG**C**XGXG**H**X_7**H**RX_4**C** (Figure 2B,C), where X is any amino acid (CCHHC motif). There is little variability in the amino acid sequence in these zinc sites, with domains between proteins being ∼100% identical (Figure 2C).[17] This is unusual as classical ZF domains typically have high conservation only in the metal-coordinating residues as well as a few additional amino acids that are important for protein structure ($CX_{2-5}CX_{12-13}HX_{3-5}H$).[35] MyT1 was the first CCHHC ZF to be identified.[33] Identification was achieved in a study aimed at discovering proteins that control the differentiation of glial cells, such as oligodendrocytes and astrocytes, from progenitor cells. Specifically, a lambda phage cDNA expression library derived from human fetal brain was screened for transcription factors that bound to a cis-regulatory element in the proteolipid protein (PLP) promoter. PLP is the main myelin-forming protein in the CNS and is one of the final targets of regulation in oligodendrocytes, serving as a good target gene to use to identify essential transcription factors in glial cell development.[33,36] This study identified MyT1 as the transcription factor responsible for binding to the PLP promoter. Subsequently, NZF-1 was discovered using the same approach, but with the goal of identifying proteins that bound to retinoic response elements. Specifically, these studies aimed to identify proteins that bound to the promoter of *β*-retinoic acid receptor (*β*RAR), a transcription factor responsible for regulating genes of various functions such as those essential for the control of cell growth and differentiation, and pituitary-specific positive transcription factor 1 (Pit-1), which is essential for the development of the pituitary gland and for hormone production.[32,37−39] Given the emerging importance of this family of proteins, two years after the discovery of NZF-1, an attempt to find additional members of this family was made. Using primers corresponding to conserved regions of NZF-1 and MyT1, similar DNA sequences from various human tissues was isolated using the polymerase chain reaction. From these studies, ST18 was cloned and isolated and found to be enriched in the brain.[34]

BIOLOGICAL ROLE

All members of the NZF/MyT family of proteins are found primarily in the CNS where they control the differentiation of neuronal cells, including neurons and oligodendrocytes.[32−34,40] NZF-1 is found predominantly in neurons and is essential for full maturation of neuronal cells.[41−46] NZF-1 promotes neuronal development by activating the expression of *β*RAR[32,37,39] and is also present in the pituitary gland, where it can bind to the *Pit-1* gene.[32,38] In the CNS, the level of NZF-1 expression is highest during development and decreases in adulthood.[32,47] The opposite is true in the pituitary gland, where the level of expression is highest in adults. Of note, an alternatively spliced form of NZF-1 is found in the testes, but the role of NZF-1 in this organ has not been explored.[32] Thus, NZF-1 plays essential roles throughout life by regulating different genes that are important for the development and function of the CNS and pituitary gland.

Unlike NZF-1, MyT1 is found predominantly in oligodendrocytes, where it activates PLP expression, which forms myelin.[33] In *Xenopus laevis*, MyT1 has been shown to be essential in both retinal and neural differentiation.[48] In the retina, low levels of MyT1 are needed to activate the protein Xath5, which is essential for the development of neurons located near the inner surface of the retina. After initial activation of Xath5, this protein further activates the expression of MyT1 leading to retinal growth. In neurons, MyT1 promotes growth by acting as a repressor. Specifically, MyT1 is part of a complex with lysine-specific demethylase 1 (LSD1), which demethylates lysines of histones to control transcription, CoREST, which acts as a corepressor with LSD1, and histone deacetylase 1/2 (HDAC1/2), which controls transcription by deacetylating lysines of histones. This complex negatively regulates the expression of Pten, which is a protein responsible for decreasing the extent of cell proliferation. This negative regulation counteracts the effect of Pten and enhances neuronal growth.[49] MyT1 has also been identified as being important in the development of the peripheral nervous system of the invertebrate chordate *Ciona intestinalis*.[50] MyT1 is also strongly expressed in the pancreas where it is involved in islet cell

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formation.[51−53] Interestingly, if MyT1 is deleted in this organ, there is an increase in the level of expression of the other homologues of this family, suggesting possible cross-talk in their functions.[52] Thus, despite the dual roles of MyT1 as an activator and a repressor of transcription, this CCHHC protein always works to promote cell growth and differentiation.

The third member of this family of proteins, ST18, is not well understood in terms of its biological function. It is highly expressed in the brain but is also present in several other tissues, including the heart, kidney, liver, eye, testis, ovaries, prostate, thyroid, aorta, and stomach.[54] Downregulation of ST18 expression results in a change in the mRNA levels of pro- and anti-inflammatory as well as pro- and anti-apoptosis genes in fibroblast cells, suggesting ST18 plays a role in these pathways.[55] The majority of these genes are pro-inflammatory and pro-apoptotic, and these effects are potentially a result of ST18's regulation of tumor necrosis factor α (TNFα).[55] Studies have shown that nuclear extracts that contain ST18 are able to bind to the promoter region of *TNF*α and ST18 upregulates TNFα mRNA in fibroblast cells. ST18 is also expressed in the pancreas, where it mediates lipotoxicity and apoptosis of β-cells.[56] Thus, ST18 appears to play a variety of roles in the body, but many of its biological functions have not yet been defined.

All members of the CCHHC ZF family are present in the nucleus of cells when active.[34,47,57] The level of nuclear expression of NZF-1 and MyT1 is highest during development, with low levels of the proteins typically found in adults, with the exception of NZF-1, which is found in high levels in the adult pancreas.[32,58] As PLP accumulates in oligodendrocytes, MyT1 is transported to the cytoplasm, presumably as a means of quenching its activity, where levels of this protein eventually become undetectable.[58] When present in the cytoplasm, NZF-1 and MyT1 can interact with the plasma membrane protein Lingo-1, which likely holds these proteins in the cytoplasm to render them inactive.[59] Recently, Lingo-1 expression has been associated with a decreased level of myelination, potentially by detrimentally regulating MyT1 activity.[60] Less is known about the localization of ST18 in the nervous system, but this protein is present in the cytoplasm of the pancreas and shifts to the nucleus only when it is induced by factors such as fatty acids.[56]

REGULATION OF CCHHC ZF PROTEINS

In addition to the regulation of NZF-1 and MyT1 by Lingo-1, other factors have also been shown to control the expression and activity of the NZF/MyT family. Regulation of MyT1 in the CNS is mostly widely studied in *X. leavis* development. In this organism, pro-neural genes NGN-1 and NGNR-1 activate the transcription of MyT1.[61,62] Low levels of MyT1 are required for Xath5 expression, which then further enhances MyT1 expression.[48] In mammals, growth factor bFGF enhances MyT1 expression while Sin3B, which negatively regulates transcription through the recruitment of HDACs, decreases MyT1 activity and potentially the activity of NZF-1, as well.[63,64] Expression of NZF-1 is upgregulated in retinoic acid-induced cells, which is interesting given that NZF-1 regulates the expression of βRAR, which requires retinoic acid to function.[65]

Less is known about how these proteins are regulated in other organs, but in the pancreas NKX6, a transcription factor required for β-cell development, regulates MyT1 expression.[66] Another pancreatic protein, manic fringe, increases the level of expression of both MyT1 and ST18.[67] More research needs to be done to fully understand how these proteins are regulated in the CNS, which is essential given that misregulation of these proteins has been linked to a number of disorders.

DISEASE STATES LINKED TO NZF/MYT PROTEINS

Table 1 summarizes the diseases and disorders that occur when NZF/MyT proteins are either misregulated or deleted.[54,57,68−90] Typically, misregulation or deletion is detrimental. For example, deletion of the MyT1 or NZF-1 genes is linked to intellectual disability, although how the brain develops in these patients as a result of these deletions is unclear.[73,80,90] Upregulation of NZF-1 and ST18 has been linked to oligodendroglioma and acute myloid leukemia (AML), respectively. The link to oligodendroglioma is particularly interesting as this type of brain cancer originates from oligodendrocytes, where NZF-1 is not normally expressed. There are a few documented cases in which misregulation of these proteins is beneficial. For example, a single-nucleotide polymorphism (SNP) of NZF-1 is associated with a better outcome in gastric cancer.[75] Upregulation of MyT1 has been observed in areas of multiple sclerosis lesions associated with remyelination.[79] Interestingly, upregulation of MyT1 expression has also been associated with a decreased cocaine self-administration level, suggesting that induction of MyT1 may be a beneficial means of treating addiction.[81,82] MyT1 is also upregulated in the brain of alcoholics, while ST18 is downregulated; however, the consequences of these changes in transcript levels are not known.[84] ST18 misregulation has various consequences as this protein can act as an oncogene as

Table 1. Diseases and Disorders Associated with the NZF/MyT Family of Proteins

protein	disease or disorder	association	ref
NZF-1	oligodendroglioma	upregulation	68
	ADHD	SNP rs2241685	69
	schizophrenia	duplication	70
		alternate gene regulation	71
	depression	SNP rs3748989	72
		SNP rs1617213	72
		SNP rs6759709	72
	intellectual disability	gene deletion	73
		deletion or duplication	90
	multiple sclerosis	SNP rs2053906	74
	gastric cancer (beneficial)	SNP rs17039396	75
MyT1	spinal injury (beneficial)	upregulation	57
	perventricular leukomalacia	upregulation	76
	schizophrenia	alternate gene regulation	77, 78
	multiple sclerosis (beneficial)	upregulation	79
	intellectual disability	gene deletion	80
	cocaine addiction (beneficial)	upregulation	81, 82
	Alzheimer's	alternate gene regulation	83
	alcoholism	upregulation	84
ST18	breast cancer	gene deletion	54
	acute myloid leukemia	upregulation	85
	Down syndrome-megakaryoblastic leukemia	downregulation	86
	alcoholism	downregulation	84
	glaucoma	SNP rs1015213	87−89

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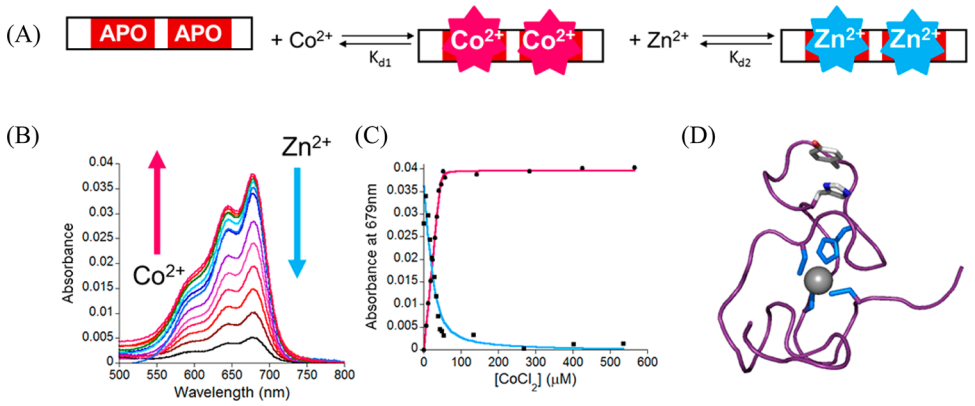

Figure 3. (A) Schematic representation of the experimental procedure for UV−visible-monitored metal titrations. Apo-ZF is titrated with Co(II), and once saturated, Co(II)-ZF is titrated with Zn(II). (B) Example of d−d transition bands that form when Co(II) binds to the CCHHC ZF domains during a titration, in this case F2 and F3 of MyT1. (C) An example of the titration data that have been fit to a 1:1 binding model: pink for the Co(II) fit and blue for the Zn(II) fit. (D) NMR solution structure of F2 of NZF-1 (PDB entry 1PXE).

well as tumor suppressor.[85,86] ST18 is most often down-regulated in disease states, which can be a detriment given the tumor suppression activity of ST18, as is the case with Down syndrome-megakaryoblastic leukemia and breast cancer.[54,86] However, ST18 can also be detrimental when this proteins acts as an oncogene, as is the case of AML.[85] The roles played by these proteins in this myriad of diseases have not yet been fully delineated; however, proper regulation of these proteins is clearly important.

METAL ION SITE: COORDINATING LIGANDS

Metal ions are the required functional cofactors for the NZF/MyT family of proteins. Five potential metal-coordinating ligands are present in the ZF domains of this family, arranged in a CCHHC motif (Figure 2B). This motif is unusual as ZFs typically require only four coordinating ligands. To determine which of the five potential Zn(II) ligands are involved in metal ion coordination, two approaches have been taken: UV−visible spectroscopy and nuclear magnetic resonance (NMR). Zn(II) binding cannot be directly assessed using UV−visible spectroscopy as Zn(II) is spectroscopically silent because to its d^{10} electron count. Thus, Co(II) is often used as a spectroscopic probe because Co(II) coordinates ZF proteins in a manner similar to that of Zn(II) but has a d^7 electron count resulting in rich spectroscopic properties.[91−93] Typically, when Co(II) is coordinated to ZF sites, d−d transitions between 550 and 750 nm, indicative of tetrahedral geometry, are observed.[94] These d−d transitions are very sensitive to the environment at the Co(II) center and can provide information regarding coordination number and ligand set.[95] Zn(II) binds more tightly to these sites than Co(II) because there is no ligand field stabilization energy penalty for Zn(II) in tetrahedral versus octahedral geometries (Figure 3A−C).[18] Thus, Zn(II) binding can be monitored by following the disappearance of d−d bands as Zn(II) is titrated with Co(II)-ZF [Zn(II) displaces the Co(II)].[17,18] This approach allows one to determine upper limit dissociation constants (K_ds) for both Co(II) and Zn(II), and it has been utilized for several ZF domains within the NZF/MyT family of proteins.[22,96−98] The d−d transitions that appear upon Co(II) binding for the CCHHC ZF domains display absorbance maxima at 593, 646, and 679 nm, indicative of tetrahedral geometry with a CCHC ligand set. Mutational analyses to determine which of the conserved histidine residues coordinates metal have been reported by our group and others.[98,99] When the second histidine residue is mutated to a glutamine or a phenylalanine, the d−d transitions indicate retention of tetrahedral geometry with a CCHC ligand set (Figure 4), but the shapes of the d−d transitions differ,

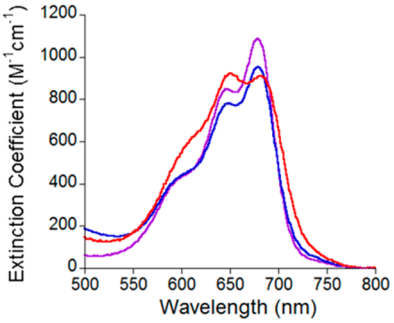

Figure 4. Effects of alternate histidine coordination on the d−d transition envelope in the optical spectra: purple for wild type CCHHC NZF-1, blue for CCFHC NZF-1, and red for CCHFC NZF-1.

indicating the environment at the metal center has been altered.[98,99] This is not observed when the first histidine is mutated to a glutamine or phenylalanine; thus, the mutagenesis/UV−visible spectroscopy studies revealed that the second histidine coordinates metal ions. These results have been validated via the three NMR solution structures: F2 of NZF-1, F5 of MyT1, and F4+F5 of ST18 (Figure 3D).[96,98−101] In all three structures, the second histidine is shown to coordinate Zn(II) along with the three cysteine residues.

AFFINITIES OF CO(II) AND ZN(II) FOR THE CCHHC DOMAINS OF NZF-1 AND MYT1

The affinity of the CCHHC ZF domains for metal ions has been assessed using both UV−visible spectroscopy, as described above, and isothermal titration calorimetry (ITC).[22,96,98] ITC, unlike UV−visible spectroscopy, does not require the use of Co(II) as a spectroscopic probe. Using this method, Wilcox and co-workers determined a dissociation constant in the low nanomolar regime for finger 2 (F2) of MyT1, and this dissociation constant was validated via the Co(II) displacement method.[22] This is in contrast to the midpicomolar regime that was determined using the Co(II)

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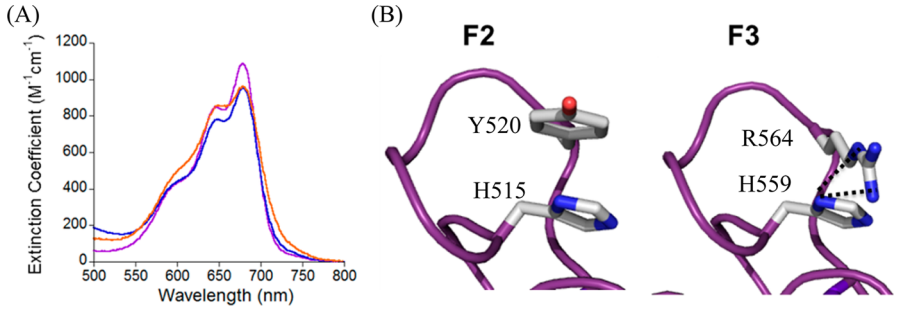

Figure 5. Hydrogen bond analysis of NZF-1. (A) d−d transition envelope of WT CCHHC NZF-1 (purple), CCFHC NZF-1 (blue), and CCQHC (orange). (B) Analysis of possible hydrogen bonds, represented by a dotted line, to or from the non-metal-coordinating histidine residues in F3.

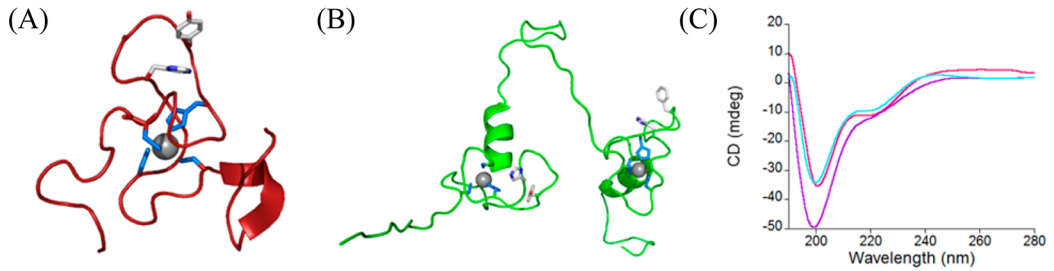

Figure 6. Structural analysis of the NZF/MyT family. (A) NMR solution structure of F5 of MyT1 (PDB entry 2JYD). (B) NMR solution structure of F4+F5 of ST18 (PDB entry 2CS8). (C) CD spectra of apo F2+F3 of NZF-1 (purple), Co(II)-bound F2+F3 of NZF-1 (pink), and Zn(II)-bound F2+F3 of NZF-1 (blue).

displacement method for a construct comprised of F2 and F3 of MyT1.[98] The tighter binding seen for the two-finger construct of MyT1 suggests that there may be cooperative metal binding for these multiple ZF domains. Cooperative metal binding is possible in ZF domains; for example, Seneque and Latour report this behavior in the treble clef CCCC type ZFs.[102] In addition to dissociation constants, ITC also revealed that zinc binding is entropically favored, which contrasts the classical ZFs for which an equal contribution of entropy and enthalpy has been reported.[22]

ROLE OF NONCOORDINATING HISTIDINE IN CCHHC DOMAINS

The ubiquity of the noncoordinating histidine in each ZF domain of the NZF/MyT family suggests that it plays an important structural and/or functional role. From the NMR structure of F2 of NZF-1, it was predicted that the non-metal-coordinating histidine participates in a stacking interaction with a tyrosine residue, helping to stabilize one of the loops present in the folded protein, which then allows the protein to function (Figure 3D).[99] However, we reported that when this conserved non-metal-coordinating histidine is mutated to phenylalanine to retain stacking in a two-domain construct of NZF-1 (F2+F3), DNA recognition is abolished, indicating that the role of this histidine is not simply to be involved in π stacking, as equivalent DNA binding should have been observed in the mutant case.[98] Further evidence of a larger role for this non-metal-coordinating histidine came from sequence analysis. The tyrosine residue thought to be important for π stacking is not conserved in all CCHHC ZF domains. For instance, in F3 of NZF-1, this residue is an arginine (Figure 5B). The presence of an arginine suggests hydrogen bonding may be a key interaction for this amino acid position instead of π stacking.[103] This hypothesis was borne out in studies by our laboratory in which the noncoordinating histidine was mutated to a glutamine residue.[104] Mutation of histidine to glutamine in a single ZF domain of the two-domain construct of NZF-1 (F2+F3) results in a peptide that still binds DNA with sequence specificity, albeit with a weaker K_d. This decrease in affinity cannot be attributed to single-finger binding by the intact ZF domain as a single ZF construct of NZF-1 cannot interact with DNA and instead suggests that hydrogen bonding is important for the structure and/or function of NZF-1. Current work in the Michel laboratory is focused on defining additional factors that are important for DNA recognition.[104]

FOLD OF THE CCHHC ZF DOMAINS

The available structural data for the NZF/MyT family of proteins (F2 of NZF-1, F5 of MyT1, and F4+F5 of ST18) reveal that the folded protein is composed of a series of loops centered around the zinc ion (Figures 3D and 6A,B).[99,100,105] This contrasts with the behavior of classical ZF proteins, in which the zinc-bound form contains significant β sheet and α helix content (Figure 1).[24] The lack of α helix and β sheet content observed in the NMR structures of the CCHHC family has been confirmed in circular dichroism (CD) studies of F2+F3 of NZF-1, F2 of MyT1, and F2+F3 of MyT1.[22,98] In the apo form, the CD is representative of random coil, as evidenced by the large negative signal at 195 nm. Upon addition of Co(II) or Zn(II), this signal becomes less negative and an additional feature appears around 225 nm, indicating the protein has folded but remains largely coiled (Figure 6). These domains bind DNA only when they are bound to metal; thus, the predominantly "loop" type structure that is adopted upon metal coordination is clearly important for function.[98,106]

DNA PARTNERS

Although there is an unusually high degree of sequence similarity among the CCHHC ZF family members, each member recognizes and regulates different genes.[32,33,55] MyT1

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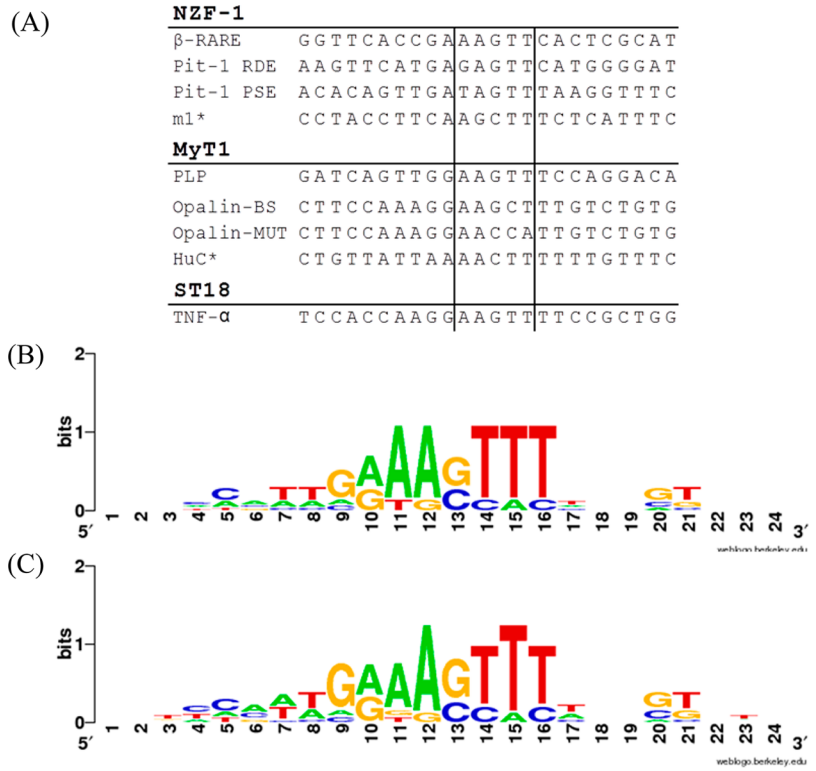

Figure 7. DNA targets of the NZF/MyT family. (A) Alignment of DNA targets identified in the literature. The conserved AAGTT region is boxed. (B) Sequence logo of DNA targets for which there are published binding data. Height corresponds to degree of conservation with two bits being 100% conserved. (C) Sequence logo of DNA targets for all identified DNA targets, including putative targets. Height corresponds to degree of conservation with two bits being 100% conserved.

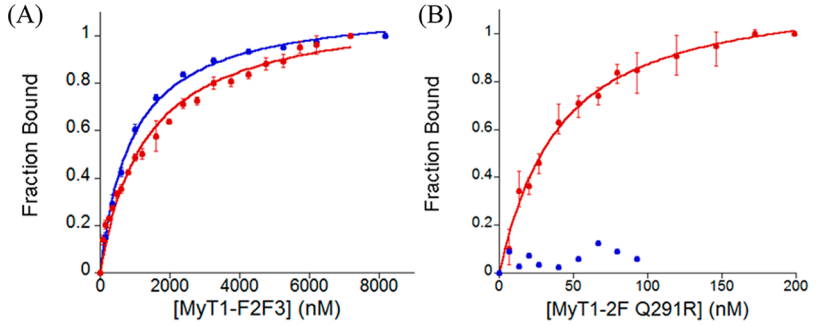

Figure 8. DNA binding studies of MyT1. (A) Fluorescence anisotropy of wild type F2+F3 of MyT1 interacting with *βRAR* (red) and a random DNA segment (blue). (B) Fluorescence anisotropy data of Q291R F2+F3 of MyT1 interacting with *βRAR* (red) and a random DNA segment (blue).

was discovered because of its ability to recognize a specific sequence of the PLP promoter, termed "site 4", which has the sequence AAGGATCAGTTGG**AAGTT**TCCAGGACATC-TTC.[33] Likewise, NZF-1 was identified by its ability to bind a sequence in the *β*RAR promoter, AATTGGGTTCACCGA-**AAGTT**CAC.[32] The DNA sequences used to identify these proteins each contain a common "AAGTT" sequence (bolded above). Given the high degree of sequence similarity in these ZF domains and the repeated appearance of this AAGTT sequence, it was proposed that all the clusters within the NZF/MyT proteins recognize this DNA target.[32,33,55,62,96] Examination of the DNA sequences for which binding data have been published supports this: the AAGTT motif is largely conserved, albeit with some variation (Figure 7).[32,33,55,107−109] Interestingly, the AAGTT sequence is not present in every DNA to which this family binds. For instance, MyT1 binds specifically to the "Opalin-MUT" sequence, which has an "AACCA" sequence in place of the "AAGTT" sequence, suggesting other factors outside of these five bases are important for sequence-specific DNA interactions.[108]

Exactly how members of this family of proteins recognize DNA remains unresolved. To better understand DNA recognition by the CCHHC family, we have recently examined the DNA binding specificity of F2+F3 of NZF-1 and F2+F3 of MyT1. Using the *βRAR* sequence in fluorescence anisotropy studies, we have demonstrated that F2+F3 of NZF-1 binds to this target sequence specifically with a K_d in the low nanomolar regime, while F2+F3 of MyT1 binds to this sequence nonspecifically.[98,106] The nonspecific DNA binding of F2+F3 of MyT1 was striking as this construct is 92% identical to F2+F3 of NZF-1. Mutation of just one amino acid in this MyT1 construct, a glutamine, to the amino acid present in that

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position of NZF-1, an arginine, resulted in MyT1 binding to the *βRAR* sequence specifically with low nanomolar binding affinity (Figure 8).[98] The amino acid in this important position is not conserved in this family of proteins (Figure 2C, position 9 in Figure 2B), suggesting a model of DNA recognition in which the few nonconserved amino acids in the protein sequence drive sequence-specific DNA recognition.

To gain more insight into the DNA binding properties of this family, MacKay and co-workers reported a HADDOCK modeling approach coupled with NMR that allowed them to propose a mechanism for binding of MyT1 to *βRAR* (Figure 9).[101] While the *βRAR* gene is not the physiological target of MyT1, it does contain the AAGTT sequence thought to be important for DNA recognition, and thus, these studies may provide insight into how this family recognizes this important motif. The main feature of the Mackay model is that the ZF domains of MyT1 fit into the major groove of DNA, making contact with the AAGTT sequence. This binding is driven by the conserved amino acids in the ZF domain, which are likely important for the conserved fold of the protein.[35] This model does not take into account the sequence selectively we have observed as a result of the nonconserved amino acids with our DNA binding studies of NZF-1 and MyT1. Thus, additional structural studies are needed to delineate the mode of protein/DNA recognition to test the model proposed by Mackay and co-workers.

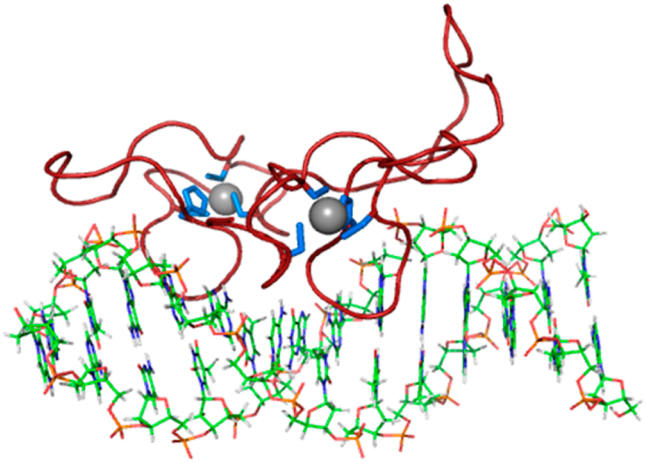

Figure 9. Published model of interaction of MyT1 F4+F5 with *β*RAR DNA (PDB entry 2MF8).

CONCLUSION

The NZF/MyT family of proteins contains several features that are unique to this class of nonclassical ZFs. These include the presence of five potential metal binding ligands in each ZF domain and a high degree of sequence similarity within these domains. Moreover, the DNA binding specificity of the domains appears to be modulated by a handful of amino acids. The full mechanism of zinc-mediated DNA recognition remains unresolved, and it is likely that additional unique features will be identified. A clearer understanding of this mechanism not only will contribute to our fundamental knowledge of metal-mediated transcriptional regulation but also has the potential to help us better understand the biological role of these proteins in neuronal development.

AUTHOR INFORMATION

Corresponding Author

*E-mail: smichel@rx.umaryland.edu. Fax: (410) 706-5017. Telephone: (410) 706-7038.

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Notes

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ABBREVIATIONS

ZF, zinc finger; NZF-1, neural zinc finger factor-1; MyT1, myelin transcription factor 1; ST18, suppression of tumorigenicity 18; *β*RAR, *β* retinoic acid receptor; PLP, proteolipid protein; Pit-1, pituitary-specific positive transcription factor 1; LSD1, lysine-specific demethylase 1; HDAC, histone deacetylase; AML, acute myloid leukemia; SNP, single-nucleotide polymorphism; NMR, nuclear magnetic resonance; K_d, dissociation constant; ITC, isothermal titration calorimetry; CD, circular dichroism; PDB, Protein Data Bank.

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