



The multiple roles of zinc finger domains

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Declaration

The work described in this Thesis was performed between March 2001 and March 2004 in the School of Molecular and Microbial Biosciences (formerly the Department of Biochemistry) at the University of Sydney. The experiments were carried out by the author unless stated otherwise. This work has not been submitted, in part or in full, for a higher degree at any other institution.

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Abstract

Zinc finger (ZnF) domains are prevalent in eukaryotes and play crucial roles in mediating protein-DNA and protein-protein interactions. This Thesis focuses on the molecular details underlying interactions mediated by two ZnF domains.

The GATA-1 protein is vital for the development of erythrocytes and megakaryocytes. Pertinent to the protein function is the N-terminal ZnF. In particular, this domain mediates interaction with DNA containing GATC motifs and the coactivator protein FOG. The importance of these interactions was illustrated by the findings in Chapter 3 that naturally occurring mutations identified in patients suffering from blood disorders affect the interaction of the N-terminal ZnF with either DNA (R216Q mutation) or FOG (V205M and G208S mutations).

In addition to the interaction FOG makes with GATA-1, it also interacts with the centrosomal protein TACC3. In Chapter 4, this interaction is characterised in detail. The solution structure of the region of FOG responsible for the interaction is determined using NMR spectroscopy, revealing that it is a true classical zinc finger, and characterisation of the interaction domain of TACC3 showed that the region is a dimeric coiled-coil. The FOG:TACC3 interaction appears to be mediated by α -helices from the two proteins. The data presented here represent some of the first described molecular details of how a classical ZnF can contact a *protein* partner. Interestingly, the α -helix used by the FOG finger to bind TACC3 is the same region utilised by DNA-binding classical zinc fingers to contact DNA.

In addition to the multiple roles played by ZnFs, this domain is also known for its robustness and versatility. In Chapter 5, incomplete ZnF sequences were assessed for its ability to form functional zinc-binding domains. Remarkably, CCHX sequences (in the context of BKLF finger 3) were able to form discrete zinc-binding domains and also, mediate both protein-DNA and protein-protein interactions. This result not only illustrates the robust nature of ZnFs, it highlights the need for expanding ZnF sequence criteria when searching for functional zinc-binding modules.

Together, the data presented here help further our understanding of zinc finger domains. Similar to the use of DNA-binding ZnFs in designer proteins, these data may start us on the path of designing novel protein-binding ZnFs.

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List of abbreviations

1D	one-dimensional
$A_{600\text{nm}}$	absorbance at 600 nm
AAS	atomic absorption spectrometry
BKLF	Basic Krüppel-like factor protein
BF3	BKLF zinc finger 3 (residues 316–344)
BSA	bovine serum albumin
bZIP	basic leucine zipper
CCHH	zinc ligation topology of Cys-Cys:His-His
CCHX	zinc ligation topology of Cys-Cys:His-X, where X is any amino acid except Cys or His
cCF or cC-finger	chicken GATA-1 C-terminal zinc finger
CD	circular dichroism
CV	column volume
D218G	NF54 with D218G mutation
DTT	dithiothreitol
dNTPs	deoxyribonucleoside triphosphates
dsDNA	double-stranded DNA
DQF-COSY	double quantum filtered <i>J</i> -correlated spectroscopy
D218Y	NF54 with D218Y mutation
ϵ	molar extinction coefficient
EMSA(s)	electrophoretic mobility shift assay(s)
FOG	Friend of GATA protein
FOG-F3	FOG zinc finger 3 (residues 328–360)
FOG-F3 _{KRA}	FOG-F3 with E330K, L336K and E349A mutations
G208S	NF54 with G208S mutation
GSH	glutathione (reduced form)
GST	glutathione <i>S</i> -transferase
H $^{\alpha}$	protons attached to the α -carbon
HSQC	heteronuclear single quantum coherence
IPTG	isopropyl β -D-thiogalactopyranoside
ITC	isothermal titration calorimetry

LB	Luria-Bertoni medium
MALLS	multiangle laser light scattering
MBP	maltose binding protein
MRE	mean residue ellipticity
MQW	Milli-Q [®] water
MWCO	molecular weight cut-off
NF or N-finger	N-terminal zinc finger of GATA-1
NF48, NF54	NF domain encompassing residues 200–248 and 200–254
NMR	nuclear magnetic resonance
NOE	peak in NOESY spectrum resulting from dipolar connectivity
NOESY	nuclear Overhauser enhancement spectroscopy
PCR	polymerase chain reaction
PDB	Protein Data Bank
PMSF	phenylmethylsulfonyl fluoride
rpHPLC	reverse-phase high performance liquid chromatography
RT	room temperature
R216Q	NF54 with R216Q mutation
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
ssDNA	single-stranded DNA
TACC3	Transforming Acidic Coiled-coil protein 3
TACC103, 77, 47	TACC3 constructs encompassing residues 535–637, 561–637 and 591–637, respectively
TCEP	tris(2-carboxyethyl)phosphine
TFA	trifluoroacetic acid
TFE	trifluoroethanol
TOCSY	total correlation spectroscopy
Tris	tris(hydroxymethyl)aminomethane
Ush-F1	Ushaped zinc finger 1
V205M	NF54 with V205M mutation
ZnF(s)	zinc finger(s)

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