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Characterisation of the Conserved Protein IMPACT from Yeast (Yih1)

A thesis presented in partial fulfilment of the requirements for the degree of

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New Zealand.

Natalie Sarah Burr

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Abstract

Regulation of translation under conditions of amino acid starvation is an important survival mechanism to ensure the continued viability of an organism. The accumulation of uncharged tRNA under amino acid starvation conditions triggers the activation of Gcn2, a kinase that phosphorylates the translation initiation factor eIF2α, inhibiting translation initiation. The protein IMPACT has been shown to inhibit Gcn2 by sequestering Gcn1, a protein that binds Gcn2 and is required for its function *in vivo*. IMPACT is a highly conserved protein, but despite its conservation, little is known about the role(s) it plays in the cell.

The initial aim of this study was to investigate the three dimensional structure of Yeast IMPACT Homologue 1 (Yih1) using X-ray diffraction, in the hope that knowledge of the structure would inform further understanding of its many and varied complex biological functions. Because of the difficulties in obtaining diffraction quality crystals, a number of different techniques were employed that resulted in the production of a number of different plasmids for protein expression. These included surface entropy engineering, the use of folding and stability tags, and co-crystallisation with known binding partners.

Further investigation into why the protein refused to crystallise revealed an innate heterogeneity that included a propensity to bind nucleic acids. Efforts were made to determine if this was related to function without success.

Acknowledgements

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Mum and Dad for being the best parents ever, allowing me to find my own way in life and supporting me no matter what direction I went in. Thank you for all the discussions we had about my work and the effort to understand what the hell it was that I was doing.

Last but not least I want to thank my partner Ben. Your love and support gave me the drive to follow my passion.

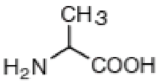
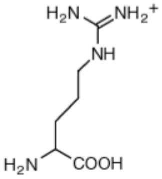
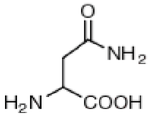
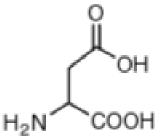
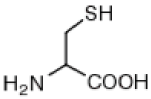
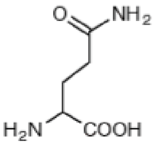
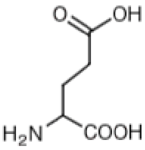
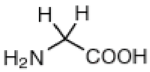
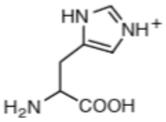
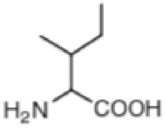
Abbreviations

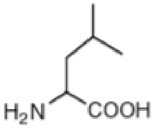
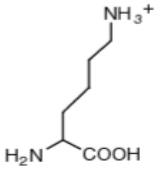
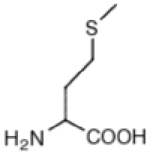
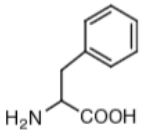
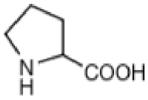
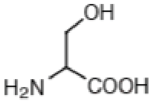
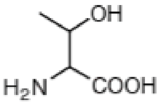
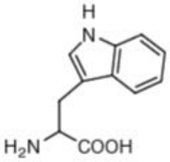
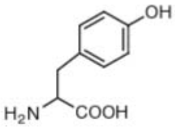
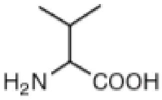
× g	Multiples of gravitational force
°C	Degrees Celsius
µL	Microlitre
µm	Micrometre
3D	Three-dimensional
Å	Angstrom (10^{-10}m)
A_{260}	Absorbance at 260 nm
A_{280}	Absorbance at 280 nm
AEX	Anion Exchange Chromatography
ATF4	Activating transcription factor 4
ATP	Adenosine-5'-triphosphate
CD	Circular Dichroism
CV	Column Volumes
Da	Dalton
DEPC	Diethylpyrocarbonate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide Triphosphate
dsDNA	Double-stranded Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
eIF	Eukaryotic Initiation Factor
EtBr	Ethidium Bromide
g	Gram
Gcn	General Control Non-derepressible
$Gcn1_{frag}$	Gcn1 fragment consisting of residues 2050-2428
GDP	Guanosine-5'-diphosphate
GST	Glutathione S-transferase
GTP	Guanosine-5'-triphosphate
H	Hydrogen
His_6	Hexa-histidine tag

His_6-$Yih1_{cys-}$	His_6-Yih1 C67A, C99A
HTS	High-throughput Screening
IDD	Intrinsically disordered domain
IDO	Indoleamine 2,3-dioxygenase
IMPACT	Imprinted and Ancient
IPTG	Isopropyl-β-D-thio-galactoside
IUP	Intrinsically unfolded protein
kB	Kilobases
kBp	Kilobase-pair
kDa	Kilodalton
kPa	Kilopascal
L	Litre
LB	Luria Bertani media
LB-AB	Luria Bertani media with antibiotics
M	Mole
mAU	Milli absorbance units
mg	Milligram
Min	Minute
mJ	Millijoule
mL	Millilitre
mM	Millimole
mRNA	Messenger ribonucleic acid
MW	Molecular Weight
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
NZ	New Zealand
OD_{600}	Optical density at 600 nm
Pa	Pascal
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction

pH	Negative decadal logarithm of proton concentration
pKa	Negative decadal logarithm of acid dissociation constant
RBS	Ribosome binding site
RNA	Ribonucleic acid
RPM	Revolutions per minute
SAXS	Small-angle X-ray scattering
SD	Selective dropout media
SDM	Site-directed Mutagenesis
SDS	Sodium dodecyl sulfate
SEC	Size exclusion chromatography
SER	Surface entropy reduction
TAE	Tris-acetate-EDTA buffer
TCEP	Tris(2-carboxyethyl)phosphine
TE	Tris-EDTA buffer
TEMED	Tetramethylethylenediamine
tRNA	Transfer ribonucleic acid
USA	United States of America
UV	Ultra violet
V	Volt
Yih1	Yeast IMPACT homologue 1
Yih1-56	Yih1 E56A, E57A
Yih1-116	Yih1 E116A, E117A, E118A

Amino Acid Abbreviations

Name	3-letter code	1-letter code	Structure
Alanine	Ala	A	
Arginine	Arg	R	
Asparagine	Asn	N	
Aspartic Acid	Asp	D	
Cysteine	Cys	C	
Glutamine	Gln	Q	
Glutamic Acid	Glu	E	
Glycine	Gly	G	
Histidine	His	H	
Isoleucine	Ile	I	

Leucine	Leu	L	
Lysine	Lys	K	
Methionine	Met	M	
Phenylalanine	Phe	F	
Proline	Pro	P	
Serine	Ser	S	
Threonine	Thr	T	
Tryptophan	Trp	W	
Tyrosine	Tyr	Y	
Valine	Val	V	

Nucleic Acid Abbreviations

Base	One-letter Code
Adenosine	A
Thymidine	T
Guanine	G
Cytosine	C
Uracil	U

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