

The Aryl Hydrocarbon Receptor: Structural Analysis and Activation Mechanisms

This thesis is submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in the School of Molecular and Biomedical Sciences (Biochemistry), The University of Adelaide, Australia

Fiona Whelan, B.Sc. (Hons)
2009

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Thesis Summary

The Aryl-hydrocarbon Receptor (AhR) is a basic Helix-Loop-Helix Per-ARNT-Sim (bHLH.PAS) transcription factor (TF) which binds partner protein Aryl hydrocarbon Receptor Nuclear Translocator (ARNT), in order to activate target genes in response to environmental or endogenous stimuli. The PAS region of these TFs consists of two adjacent repeats of the PAS domain, where the PAS repeat defines dimerization specificity and also serves as a primary sensor in exogenous ligand activation of AhR. Active AhR/ARNT heterodimer binds specific DNA sequences, termed Xenobiotic Response Elements (XRE). The molecular detail of interactions that dictate dimerization and DNA binding specificity are unknown for this TF family. In addition, active AhR has recently been shown to function as a recognition component of an E3 ubiquitin ligase. Reports that AhR null mice have poor fertility and defects in liver vasculature are indicative of the potential for a number of endogenous roles. Research into activation of AhR has highlighted that post-translational modifications may affect function by regulating subcellular localization.

The complex regulatory outcomes of AhR expression and activation require a number of approaches to elucidate mechanistic information. In this thesis, a structural investigation of heterodimerization and DNA binding has been used to propose a molecular mechanism for target gene recognition and activation following XRE binding. Crystallographic approaches have yielded crystals of bHLH.PAS-A regions of AhR/ARNT heterodimer bound to DNA. Atomic force microscopy and small angle x-ray scattering analyses have illustrated an XRE binding mechanism whereby the DNA is bent, and the PAS-A region of the dimer flattens around the DNA. A targeted mutagenesis screen of the AhR ligand binding domain (LBD) was performed to investigate polycyclic aromatic hydrocarbon (PAH) and atypical ligand binding specificity. In parallel, mutant AhR proteins were assessed for inducibility by non-exogenous ligand modes of activation, including cell suspension and application of shear stressed serum. This process identified an LBD mutant selectively activated by novel ligand YH439, and completely inactive following PAH, cell suspension and shear stressed serum treatments, inferring the potential for differential ligand binding pocket access by YH439. Finally, given the complex output following expression and activation of AhR, regulation by post-translational modification was investigated as a potential means of subtle regulation of signalling fate. A thorough analysis of untreated AhR has revealed a concert of modifications occurring on functionally relevant regions of the protein that are implicated in regulating subcellular localization, protein:protein interactions, and potentially, protein stability. Preliminary analyses of YH439 and cell suspension treated AhR has additionally indicated the possibility of activation state specific modification patterns. In summary, this thesis describes: Novel approaches to structural characterisation of a bHLH.PAS protein dimer bound to DNA; atypical ligand binding to a novel site of AhR; and an analysis of a proposed AhR PTM code.

Declaration

This work contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University library, being available for loan and photocopying.

Fiona Whelan

Publications Arising From This Thesis

Whelan F., Hao N., Chapman-Smith A. and Whitelaw M.L. (2009) Atypical Aryl hydrocarbon receptor ligand YH439 has a novel binding mode with interesting implications for endogenous activation. Manuscript in preparation.

Chapman-Smith A., Whelan F., Mechler A., Ng S.M., Shearwin K., Martin L.L., and Whitelaw M.L. (2009) PAS domains mediate protein-DNA interactions and alter DNA conformation. Journal of Biological Chemistry. Manuscript in preparation.

Dave K.A., Furness S.G.B., Goswami H., Whelan F., Bindloss C., Chapman-Smith A., Whitelaw M.L. and Gorman J.J. (2009) Comprehensive phosphoproteomic analysis reveals that the latent Dioxin Receptor is highly post-translationally modified. Molecular and Cellular Proteomics. Manuscript submitted.

Dave K.A., Whelan F., Bindloss C., Furness S.G., Chapman-Smith A., Whitelaw M.L. and Gorman J.J. (2008) Sulfonation and phosphorylation of regions of the dioxin receptor susceptible to methionine modifications. Molecular and Cellular Proteomics. Electronic publication December, 2008.

Presentations

53rd Annual Conference of the Australian Society for Biochemistry and Molecular Biology (Combio)

Presentation: "PAS repeats of the Dioxin Receptor: Coconspirators in Detection and Response"

F. Whelan, C. Bindloss, K. Dave, A. Mechler, S. Ng, L. Martin, J. Gorman, M.L. Whitelaw and A. Chapman-Smith. September 2008

Poster Prize

"Ligand Activation, Post-Translational Modification and DNA Binding: Understanding Differential Gene Activation by the Dioxin Receptor."

F. Whelan, S. Berry, M.C.J. Wilce, M.L. Whitelaw and A. Chapman-Smith

33rd Lorne Protein Structure and Function Conference

February 2008

Poster Prize

"A Novel Developmental Switch for the Dioxin Receptor?"

F. Whelan, S. Furness, K. Dave, J. Gorman, A. Chapman-Smith and M. Whitelaw

28th Lorne Genome Conference

February 2007

Poster Prize

"The role of the PAS domain in DNA Binding by Dioxin Receptor and ARNT: Biochemical and Structural Analysis"

F. Whelan, J.A. Wilce, M. Wilce, M. Whitelaw and A. Chapman-Smith

School of Molecular and Biomedical Sciences

School Research Symposium, 2005

Acknowledgements

I've seen a lot of PhD students come and go over the years, and I've enough experience of acknowledgements to know it's the only bit anyone really wants to read. They're hoping primarily that they get a mention, and, if they do get a mention, that they don't have to go through too much waffle, too many quotes, and too many nauseating clichés. So my acknowledgements are a bit of a stream of consciousness, which will attempt to avoid the aforementioned acknowledgement horrors. I should start at the beginning, with Velta, whose understanding and confidence instilled bravery in a terrified first year. Then onto Lynn and Tony – they have to be thanked together for patience and kindness in teaching us little second year students. Separately, Prof. Lynn, you've been such a fantastic matriarch, role model and friend over the years... and years of study. John Wallace hosted me as a summer scholarship student and honours student and, in collaboration with Steve and Briony, taught me a lot about the masochistic side of protein chemistry. There I met Bec, who introduced me to the darker side of biochemistry to be found on the 3rd floor, and teased me mercilessly for about the last 10 years. Luckily, the Wallace group also introduced me to a great protein chemist, whose rigorous and encouraging teaching have taught me a lot about mentorship and science. So, thanks to Anne for encouragement all the way from a summer scholarship when I was a little undergrad, through tons of advice during my 'fd' and finally with bashing the fi'sis into shape at the end. Anne, it's had it's highs and of course its aggregation, but most of all it's been FUN! Murray has been Murray for as long as I've known him, and deserves a special mention for urging me on to utilise more techniques than anyone thought humanly possible to squeeze into a PhD. You're still the boss! To the lab, it's morphed over the years, and I've managed to pick up lifelong (confidently) friends along the way. From the days when I shared a bay with the famous Dan Peet (as an aside, I used to think Danpeet was one name because, for some reason, most people referred to him with both names, just for clarity one assumes), who was always ready with a joke, or at least ready to listen to my awful jokes and just about always groaned... to the big split where all my Hiffy buddies left me (well, moved across the corridor)... I think I pined for Cameron Bracken and our 'Power'base of two for at least the next two years... Choppy saw me through the worst of the protein expressions Cam! Sarah Linke also took off (was it something I said?) and took all her awful music with her. I've been studying alongside Slinky since first year uni, and as a peer, there couldn't have been anyone more capable of making me laugh, comforting me and pushing me all at once – I'll always cherish you mate. Anthony Fedele – what can you say really, except... *ehhhhh, we had a very good race*. Always good to catch up with you Amf, and James in cool stance corner – see you back there in a few years I'm sure. Then the Whitelaws started disappearing too – my gorgeouses Susi and Jo – I miss you both so much! Hopefully we can catch up one of these days over a few bottles of red, a couple of games of cards, and that all night dancing we were famous for. I guess the waffle is

hard to avoid, so I'll press on. Alix joined a short time after I started and is the hardest working biochemist I know. Always an inspiration and ready with answers just when you need em, and a sympathetic ear... oh, and my first and possibly only opera experience, thanks Alix (and Pels)! Much as I'd like to include everyone, the list is getting ridiculous, so to all the relative newbies – thanks for trying your best to keep me young, you failed miserably, but bless your hearts, you all tried (Anne, Colleen, Dave, Andrew, Sam, Sarah W, Rach, Adrienne and Dale). I haven't known you a long time, but I'm glad we got to know each other Edwina – it's been a wonderful experience to watch you become a gorgeous young mum. Rock on physics man. Scott, it was a great to see you learn about crystallography with the same energy and enthusiasm that I have for the practice (alchemy) – the memory of those synchrotron trips still brings a tear to my eye! Thanks to Matthew Wilce, for teaching me some of what you need to know to crystallize stuff! Thanks also to Nathan Cowieson for all the help with SAXS, and for being the most fun collaborator! Thanks most recently to Keith Shearwin for keeping me gainfully employed and engaged in the work that I so enjoy – and to the lab for being so welcoming and helpful – Ian, Julian and my dear friend Alex. Thanks to all the service staff over the years who've made EVERYTHING simpler – from the store and administration (Shelley, Soki, Serge, Jan and Genny), to the central services guys (Judy, Ros, Shirley, Adrianna and John Mac) – your patience and kindness over the years has been truly remarkable and more help than you'll ever know.

To all my patient friends who put up with every late arrival, and invitation refusal – thanks especially to lovely Suzy who helped me through some of the worst mid PhD blues; and for introducing me to a heap of new mates – foxy Jade, Mark, Andrew and gorgeous Kate. Also to the old friends; Alana Banana, John, Cec, Sam, Hannah, Christine, Karen and Aurelia. All the martinis, food, wine and great company kept me cheery through most of the study, so thank you all! Thanks also to Les, for welcoming me into your life and honouring me with inclusion as a member of the Porter family – your generosity and warmth continue to enrich my life. I can't really tell you how much my Mum and Dad have encouraged and supported my study over all these years. When I was a little secretary on K.I., then repeating a bit of high school, all the way through undergrad – they never doubted me for a second (well, not that they told me anyway) and provided all the emotional and financial support that kept me going, and of course, countless hot dinners waiting for me in the middle of the night. It's your PhD too I reckon! Thanks also to my sister Siobhan for all the late night pickups in the city after missing the last bus at the end of a long purification, and all the little kindnesses that cheered up a pitiful biochemist whose protein just kept aggregating! Finally a huge thank you to Nate – for keeping my spirits up through the worst of the process, supporting my academic obsessions, embracing all my eccentricities, making me laugh, and sharing any little successes along the way. Suffice to say, (cliché warning) I think you saved me from myself.

Abbreviations

°C - Degrees Celsius
3D – Three Dimensional
3MC – 3-Methylcholanthrene
3M4NF – 3'-methoxy-4'-nitroflavone
53BP1 – p53 binding protein 1
ACH – Active Chromatin Hub
Adr - Adricamycin
AEC – Anion Exchange Chromatography
AFM – Atomic Force Microscopy
AhR – Aryl hydrocarbon Receptor
AhRR – AhR Repressor
AP1 – Activating Protein 1
APBS – Adaptive Poisson-Boltzmann Server
APS - Ammonium Persulphate
AR – Androgen Receptor
ARD1 – N-Acetyltransferase
ARNT – Aryl hydrocarbon Receptor Nuclear Translocator
ATP - Adenosine Triphosphate
B[a]P – Benzo[a]Pyrene
Bcl-2 – B-cell CLL/Lymphoma 2
bHLH – basic Helix-Loop-Helix Domain
bHLH.O – bHLH.Orange Domain
BMAL – Brain Muscle ARNT like
BNF – Beta-Naphthoflavone
bp – Base Pair
BPDE – B[a]P diol epoxide
Brg-1 – Brahma/SWI2-related gene product 1
BSA - Bovine Serum Albumin
C – Carbenicillin
CCD – Charged Coupled Device
cdk – cyclin dependent kinase
cDNA - Coding Deoxyribonucleic Acid
ChIP – Chromatin Immunoprecipitation
CME – Central Midline Element
CNS – Central Nervous System
CRM-1 – Chromosome Region Maintenance 1
CTCF – CCCTC-binding factor
CV-1 – African green monkey kidney cells
Cyp – Cytochrome P450
DHT – Dihydrotestosterone
DIC – Differential Interference Contrast
 D_{Max} – Maximum Dimension
DMBA – 9,10-dimethylbenz[a]anthracene
DMEM - Dulbecco's Modified Eagle's Medium
DMSO - Dimethylsulphoxide
DNA – Deoxyribonucleic Acid
DNMT – DNA Methyltransferases
dNTP - Deoxynucleotide Triphosphate
dpi – Dots per square inch
ds – Double Stranded
DTT – Dithiothreitol
DV – Ductus Venosus
E# - Embryonic Day #

EAE – Experimental Autoimmune Encephalitis
E-cad – E-Cadherin
ED – Essential Dynamics
EDTA - Ethylene Diamine Tetra-acetic Acid
EGF-R – Epidermal Growth Factor Receptor
EMT – Epithelial Mesenchymal Transition
EMSA – Electrophoretic Mobility Shift Assay
EPO - Erythropoietin
ER – Estrogen Receptor
EROD – Ethoxyresorufin-O-deethylase
EtBr - Ethidium Bromide
FAD – Flavine Adenine Dinucleotide
FAM – carboxyfluorescein
FCS – Foetal Calf Serum
FICZ – 6-formylindolo(3,2b)carbazole
FITC – Fluorescein isothiocyanate
FMN – Flavine mononucleotide
FOXO1 – Forkhead Box O-1
FPLC – Fast Protein Liquid Chromatography
FRET – Fluorescence Resonance Energy Transfer
G₁ – Gap Phase 1
GFP – Green Fluorescent Protein
gsc - goosecoid
HaCaT – Human Keratinocyte Cell line
HAH – Halogenated Aromatic Hydrocarbon
HAS – HIF ancillary sequence
HAT – Histone Acetyl Transferase
HDAC – Histone Deacetylase
HEK293T – Human Embryonic Kidney Transformed 293T Cells
HEPES - 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HERG – Human Ether-a-go-go
HES-1 – Hairy and Enhancer of Split Homolog 1
HIF – Hypoxia Inducible Factor
HNF4 – Hepatic Nuclear Factor Receptor 4
H-NOXA – Heme-Nitric Oxide/Oxygen binding domain
hOGG1 – human 8-Oxoguanine DNA Glycosylase 1
hPASK – Human PAS Kinase
HPLC – High Performance Liquid Chromatography
HRE – Hypoxia Response Element
HRP – Horseradish Peroxidase
HSE – Heat Shock Element
HSF2 – Heat shock transcription factor 2
Hsp90 – Heat shock protein 90
Hz – Hertz
ICZ – Indolo[3,2-*b*]carbazole
IDA – Iminodiacetic Acid
IDPs – Intrinsically disordered proteins
IGEPAL - IGEPAL CA-360
IGFBP-1 – Insulin-like Growth Factor Binding Protein 1
IgG – Immunoglobulin
IL – Interleukin
ITE – 2-(1H-indole-3'-carbonyl)thiazole-4-carboxylic acid methyl ester
IMAC – Immobilized Metal Affinity Chromatography
iNOS – Inducible Nitric-oxide Synthetase
IPTG – Isopropyl-beta-D-thiogalactopyranoside
K - Kanamycin

KCs – Keratinocytes
K_D – Dissociation Constant
KDa – Kilodaltons
KinA – Histidine Protein Kinase
K/O - Knockout
L - Litre
LB – Luria Broth
LBD – Ligand Binding Domain
LCR – Locus Control Region
LDL – Low Density Lipoprotein
LIMK1 – LIM (Lin-11, Isl-1 and Mec-3) Kinase 1
LM – Left Median
LOV – Light Oxygen or Voltage Domain
LPS – Lipopolysaccharide
LSD1 – Lysine demethylase 1
LXA₄ – Lipoxin A4
LZ – Leucine Zipper Domain
M – Molar
mA – Milliampere
MALDI – Matrix Assisted Laser Desorption/Ionization
μg – Microgram
mg – Milligram
min – Minute
μL – Microlitre
mL – Millilitre
ML – Mother Liquor
mM – Millimolar
MMP – Matrix Metalloprotease
MOF – Males absent on first
MPa – Megapascals
MQ – MilliQ Water
MS – Mass Spectrometry
MT – Melting Temperature
MW – Molecular Weight
MWCO – Molecular Weight Cut-Off
MybBP2a – Myb Binding Protein 2a
NES – Nuclear Export Sequence
NFκB – nuclear factor kappa-light-chain-enhancer of activated B cells
ng - nanogram
Ni - Nickel
ng - Nanograms
NLS – Nuclear Localization Sequence
nm – nanometres
nM - nanomolar
NMR – Nuclear Magnetic Resonance
NMT2 – N-myristoyltransferase-2
NP40 – Tergitol
NPAS – Neuronal PAS domain Protein
NPC – Nuclear Pore Complex
NQO-1 – NAD(P)H:quinone oxidoreductase 1
NXF – Neuronal X Factor
OD₆₀₀ – Optical Density 600nm
ODDD – Oxygen Dependent Degradation Domain
O/N – Overnight
PAGE - Polyacrylamide Gel Electrophoresis
PAH – Polycyclic Aromatic Hydrocarbon

PAI-2 – Plasminogen Activator Inhibitor-2
 PAS – Per-ARNT-Sim Homology Domain
 PBS – Phosphate Buffered Saline
 PBS/T – Phosphate Buffered Saline with 1% Tween-20
 PCAF – p300/CBP Associated Factor
 PCB – Polychlorinated biphenyl
 p/CIP₂ – protein kinase PAM C-terminal Interacting Protein 2
 PCR – Polymerase Chain Reaction
 PCDD – Polychlorinated Dibenzo[*p*]dioxins
 PDBID – Protein Data Bank Identification
 PEG – Polyethylene Glycol
 pg – picogram
 PG - Prostaglandin
 Phyre – Protein Homology/Analogy Recognition Engine
 PIC – Polymerase Preinitiation Complex
 PKA – Protein Kinase A
 pM – picomolar
 PMA – phorbol-12-myristate-13-acetate
 PMSF - Phenylmethyl Sulphonyl Fluoride
 Polκ - DNA polymerase kappa
 PONDR – Predictor of Natural Disordered Regions
 P(r) – Distance Distribution Function
 PRH – Proline Rich Homeodomain
 PRMT – Protein arginine Methyltransferase
 PSGL-1 – P-selectin Glycoprotein Ligand 1
 PTM – Post Translational Modification
 puro - Puromycin
 PYP – Photoactive Yellow Protein
 q-PCR – quantitative PCR
 Q-rich – Glutamine Rich
 RA – Retinoic Acid
 RB – Retinoblastoma Protein
 RD – Regulatory Domain
 Rg – Radius of Gyration
 RIP140 – Receptor Interacting Protein 40
 RL-TK – Renilla Luciferase Thymidine Kinase
 RM – Right Median
 rpm - revolutions per minute
 ROR2 – Human Orphan Receptor Tyrosine Kinase 2
 RT – Room Temperature (~21°C)
 SANS – Small Angle Neutron Scattering
 SAXS – Small Angle X-ray Scattering
 SBE – Smad Binding Element
 SC – Stratum Corneum
 SDS - sodium dodecyl sulphate
 S.E. – Standard Error
 sec - seconds
 SEC – Size Exclusion Chromatography
 SET – Su(var)3-9, Enhancer-of-Zeste, Trithorax
 sGC – Soluble Guanylyl Cyclase
 SILAC – Stable Isotope Labelling of Amino Acids in Cell Culture
 Sim – Single Minded
 Sima – Similar
 siRNA – Small interfering RNA
 Smyd2 – SET and MYND domain-2
 S-phase – Synthesis Phase

SRC1 – Steroid Receptor Coactivator 1
ss – Single Stranded
STAS – Sulphate Transporter and AntiSigma Factor antagonist domain
STAT1 – Signal Transducer and Activator of Transcription 1
Su(HW) – suppressor of Hairy Wing
TAD – Transactivation Domain
TAF10 – TBP Associated Factor 10
TBP – TATA-binding protein
TCDD – 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin
T-cell - Thymocyte
TE - Tris.HCl/EDTA
TEA – Triethylamine
TEAAc – Triethylammonium Acetate
TEMED - N,N,N',N'-tetramethyl-ethylenediamine
TEV – Tobacco Etch Virus
TF – Transcription Factor
TGF β - Transforming Growth Factor Beta
TGN – Trans-Golgi Network
T_H17 – Thymocyte Helper Cell 17
Tip60 – HIV Tat interacting protein of 60kDa
TOF – Time of Flight
TPM – Tethered Particle Motion
TPST – Tyrosylprotein sulfotransferase
TR – Thyroid Hormone Receptor
T_{REG} – Regulatory Thymocyte Cell
Trh – Trachealess
Tris.HCl - Tris(hydroxymethyl)aminomethane
Trityl - Triphenylmethane
Trx – Thioredoxin
TSA – Trichostatin A
Tween-20 - Polyoxyethylene-sorbitan Monolaurate
USF – Upstream Stimulatory Factor
UV – Ultraviolet Light
VEGF – Vascular Endothelial Growth Factor
VHL – Von Hippel Lindau Protein
VP16 – Viral Protein 16
VVD – Vivid blue-light photosensor
WCE - whole cell extract
wt – Wild Type
X1X1 – Duplexed XRE
XAP2 – hepatitis B virus X associated protein 2
X-gal – 5-bromo-4-chloro-3-indolyl-b-D-galactopyranoside
XRE – Xenobiotic Response Element