The Role of the Histone Variant H2A.Z in Early *Xenopus laevis* Development

Karl David Brown

December 2007

A thesis submitted for the degree of Master of Philosophy
of the Australian National University

College of Medicine and Health Sciences Graduate Program

John Curtin School of Medical Research

Australian National University

Canberra, Australia
Statement

The research described in this thesis was solely and entirely conducted by the author unless acknowledgement is made in the text. It has not presented for any other degree.

Karl David Brown

December 2007
Acknowledgements

I would like to thank my supervisory panel for their support. Dr Patricia Ridgway was my primary supervisor whose guidance throughout this project was invaluable, likewise for Dr David Tremethick, leader of the Chromatin and Transcriptional Regulation group. Eldon Ball taught me the nuances of in situ hybridisation, and Sudha Rao provided valuable guidance relating to RNA-based techniques.

In addition to my panel, the wider scientific community at the ANU should be thanked. Among this community I would like to specifically thank: Danny Rangasamy and Torsten Juerlich for explaining the arcana of real-time PCR; Dave Hayward for showing me the minutiae of the molecular aspects of in situ hybridisation; and members of the Chromatin and Transcriptional regulation, and Gene Expression and Epigenomics Groups for their input and companionship over the period. Electrophoresis of sequencing reaction products and automated sequence data collection was performed by the staff of the Australian Cancer Research Foundation (ACRF) Biomolecular Resource Facility (BRF). The ANU and JCSMR provided generous scholarships and funding that made this work possible.

On a more personal note I would like to thank Jun Fan and Stephen Olms for taking a stray into their home.

I would like to thank my families for their support; my mother, father, my brother Nathan, Rose and Richard Bowman, Kylie, Damon, Sten, and Carolyn Jakobsen. Finally, my patient partner Elizabeth deserves special gratitude.

This document is dedicated to the memory of Dennis Woodhouse. He will remain an example to live up to.
Abstract

The genome of eukaryotes is packaged into the small volume of the nucleus in an organised manner. This structure of DNA and associated proteins is called chromatin. The basic unit of chromatin is the nucleosome; an octomer of core histone proteins and associated DNA. Other proteins such as linker histones can also associate with the DNA or the core histones. The modular structure of chromatin allows for structural variation with functional consequences including activation or repression of transcription. Alterations can include post-translational modifications to histones, remodelling by multi-protein complexes, DNA methylation, and non-allelic variants of the canonical histones. Changes to chromatin structure have an important impact on all DNA processing events.

This thesis investigated the histone variant H2A.Z, a variant of the canonical core histone H2A. H2A.Z is highly conserved and essential in a number of species suggesting it has a critical function. Preliminary work using the *Xenopus laevis* developmental model system had revealed that disruption of H2A.Z function resulted in defective embryo morphology consistent with disrupted gastrulation and mesoderm development (Ridgway et al., 2004a). This led to the following hypothesis: H2A.Z is important to gastrulation and mesodermal development in *X laevis* because it plays a developmental role.

Temporal and spatial expression patterns of *H2A.Z* mRNA demonstrated in this study are consistent with a role in mesoderm development. Peak *H2A.Z* mRNA expression levels occur during gastrulation. *H2A.Z* mRNA is enriched in the marginal zone of the late blastula, involuting tissue in the gastrula and in notochord (a mesodermal tissue) in tailbud embryos. Significantly, maternal *H2A.Z* mRNA is enriched asymmetrically in
dorsal cells of the early blastula before zygotic transcription, indicating that H2A.Z may play a role in determining polarity of the dorsal ventral axis.

Two important processes for development were examined in this thesis: cell fate and cell movement. Determination of mRNA levels and localisations for a selection of mesodermal marker genes indicates that cell fate programs progress normally in embryos where H2A.Z function is disrupted. However, the localisation of mesoderm derived cells is perturbed suggesting cell movement is perturbed. Taken together these studies suggest the H2A.Z histone variant has a specific role in regulating cell mobility during early *Xenopus laevis* development.
Publication arising in part from this research

# Table of contents

THE ROLE OF THE HISTONE VARIANT H2A.Z IN EARLY XENOPUS LAEVIS DEVELOPMENT ........................... I

STATEMENT .............................................................................................................................................. II

ACKNOWLEDGEMENTS ............................................................................................................................ III

ABSTRACT ................................................................................................................................................ IV

PUBLICATION ARISING IN PART FROM THIS RESEARCH ....................................................................... VI

TABLE OF CONTENTS ............................................................................................................................... VII

LIST OF ABBREVIATIONS ........................................................................................................................ XI

1 INTRODUCTION .................................................................................................................................. 1

1.1 Chromatin structure .......................................................................................................................... 1

1.1.1 Nucleosome assembly and structure ............................................................................................ 1

1.1.2 Higher order chromatin structures ............................................................................................... 3

1.1.3 Alterations to chromatin structure ............................................................................................... 7

1.1.3.1 Chromatin remodelling machines ............................................................................................ 8

1.1.3.2 Post translational modification ............................................................................................... 11

1.1.3.3 DNA methylation ................................................................................................................... 15

1.1.3.4 Histone variants .................................................................................................................... 15

1.2 The histone variant H2A.Z .............................................................................................................. 16

1.2.1 Gene and transcript ....................................................................................................................... 16

1.2.2 Protein ......................................................................................................................................... 17

1.2.3 Incorporation of H2A.Z into chromatin ....................................................................................... 19

1.2.4 H2A.Z and transcription ............................................................................................................. 21

1.3 Chromatin and transcriptional regulation during metazoan development .................................... 24

1.4 H2A.Z is essential for metazoan development ................................................................................. 26

1.4.1 H2A.Z is targeted to a selection of foregut genes during C. elegans development ................. 26

1.4.2 Essential regions of H2Av during fly development .................................................................... 27

1.4.3 H2A.Z localisation in early mouse development ....................................................................... 28

1.4.2 H2A.Z is essential for correct mesoderm development in X. laevis ...................................... 29

1.4.2.1 Morphological observations indicate defective mesoderm formation .............................. 29
1.4.2.2 A histidine motif on the H2A.Z-containing nucleosome surface is important for vertebrate development.

1.5 Scope of thesis

2 MATERIALS AND METHODS

2.1 Production of plasmids

2.1.1 Plasmids constructed for production of mRNA for microinjection

2.1.2 Plasmids constructed for production of RNA probes for in situ hybridisation

2.1.3 Polymerase chain reaction

2.1.4 Molecular cloning

2.1.4.1 Restriction Digests

2.1.4.2 DNA Ligation

2.1.4.3 Gel extraction for plasmid purification

2.1.4.4 Agarose gel electrophoresis

2.1.4.5 Agarose gel extraction

2.1.4.6 DNA Sequencing

2.1.4.7 Preparation of electrocompetent E. coli bacterial cells

2.1.4.8 Transformation of electrocompetent E. coli bacterial cells

2.1.4.9 Plasmid amplification

2.2 In vitro transcription

2.2.1 Determination of nucleic acid concentration

2.3 X. laevis as a model animal

2.3.1 In vitro fertilization and culture of X. laevis embryos

2.3.2 mRNA microinjection

2.4 Whole mount in situ hybridisation

2.5 Gene expression analysis

2.5.1 Total RNA extraction from whole embryos

2.5.2 Reverse transcription

2.5.3 Densitometry of PCR products

2.5.3.1 Calibration of RT-PCR

2.5.4 RT real-time PCR

2.5.4.1 Real-time PCR conditions and primers

2.5.4.2 Analysis of real-time PCR data

2.5.5 Statistical Analysis
3 TEMPORAL AND SPATIAL EXPRESSION OF H2A.Z mRNA DURING EARLY X. LAEVIS DEVELOPMENT

3.1 Introduction

3.1.1 Overview of X. laevis development to gastrulation

3.1.1.1 Early cleavage divisions

3.1.1.2 Mid-blastula transition

3.1.1.3 Gastrulation and mesoderm formation

3.1.2 Expression and localisation of H2A.Z mRNA in early X. laevis development

3.1.3 Experimental approach

3.2 Results

3.2.1 H2A.Z mRNA levels in the early embryo

3.2.2 Determining H2A.Z mRNA levels by RT real-time PCR

3.2.2.1 Real-time PCR optimisation

3.2.2.2 Endogenous H2A.Z mRNA levels peak at gastrulation

3.2.3 H2A.Z mRNA localisation during development

3.2.3.1 H2A.Z mRNA is enriched in a subset of blastomeres at stage 5

3.3 H2A.Z mRNA is enriched in the marginal zone and some mesodermal tissues

3.4 Discussion

4 H2A.Z AND CELL FATE IN MESODERMAL LINEAGES

4.1 Introduction

4.1.1 A possible role for H2A.Z in the blastula

4.1.2 A possible role for H2A.Z in the gastrula

4.1.3 Marker genes for mesoderm

4.1.4 Experimental approach

4.2 Results

4.2.1 Effect of perturbing H2A.Z function on the expression levels of mesodermal marker genes

4.2.2 Impaired H2A.Z function and the localisation of mesodermal mRNA

4.3 Discussion

5 H2A.Z HAS A ROLE IN REGULATING CELL MOVEMENT DURING EARLY DEVELOPMENT
List of abbreviations

*A. thaliana: Arabidopsis thaliana*

Ab: antibody

Abs: Absorbance

Ac: acetylation

ACF: ATP-utilising chromatin-assembly and –remodelling factor

ACRF: Australian Cancer Research Foundation

ACT: Australian Capital Territory

AEEC: Animal Experimentation Ethic Committee

ANU: The Australian National University

Arp: Actin related protein

AQIS: Australian Quarantine Inspection Service

AS: anti-sense

ATP: adenosine triphosphate

BAF: BRG associated factor

BAP: Brahma associated protein

BB/BA: Benzyl-benzoate / benzyl alcohol

Bdf1: Bromo-domain factor one

bp: base pairs

BRF: Biomolecular Resource Facility

BRG: Brahma related gene

Brm: Brahma

*C. elegans: Caenorhabditis elegans*

CBP: CREB binding protein
cDNA: complementary DNA
CENP-A: centromere protein A
CHD: chromodomain helicase DNA-binding protein
CHRAC: chromatin-accessibility complex
Chz1: chaperone for H2A.Z-H2B dimers
CRE: Core enhancer region
Ct: cycles at threshold
CTP: cytosine triphosphate
C-terminal: carboxy-terminal of an amino acid chain
DEPC: diethyl pyrocarbonate
DIG: digoxigenin
DMSO: dimethyl sulfoxide
DNA: deoxynucleic acid
dNTP: deoxyribonucleotide triphosphate
D. melanogaster: Drosophila melanogaster
DOM: Domino
ds: double stranded
DSB: double strand break (in DNA)
Dsh: named for the null mutants effect on fly hair orientation (Wallingford et al., 2002; Wallingford et al., 2000).
E. coli: Escherichia coli
EDTA: ethylene diamine tetraacetic acid
EGFP: enhanced green fluorescent protein
EGTA: ethylene glycol tetraacetic acid
FGF: fibroblast growth factor
Fz: Frizzled

GAPDH: Glyceraldehyde 3-phosphate dehydrogenase

Gsc: Gooscoid

gDNA: genomic DNA

GTP: guanine triphosphate

hBRM: human Brahma

_H. sapiens: Homo sapiens_

H1: histone 1

H2A: histone 2A

H2A-Bbd: histone 2A Barr-body deficient

H2A.Zdn defect: The defect arising from the injection of mRNA encoding the dominant negatives H2A.ZNQ and H2A.ZCS into _Xenopus laevis_ embryos at the two cell stage.

H2B: histone 2B

H3: histone 3

H4: histone 4

HAT: histone acetyltransferase

HDAC: histone deacetylase

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HP1: heterochromatin protein 1

HPRI: human placental RNase inhibitor

INCENP: inner centromere protein

INO80: complex named for the gene product ino80 first identified from a mutation causing inositol auxotrophy in yeast

ISWI: Imitation switch
K: lysine

kb: kilobase pairs

LB: Luria broth

LBA: Luria broth with ampicillin.

MBT: mid-blastula transition.

MBSH: modified Barth’s saline with HEPES

me: methylation

MEMFA: MOPS, EDTA, MgSO₄, formaldehyde

Mi-2: human dermatomyositis-specific antigen recognised by patient Mitchell’s autoimmune antibodies

MMR: Marc’s Modified Ringer’s Solution

MOR: Moira

mRNA: messenger ribonucleic acid

myo-2: Myosin-2

N-terminal: amino-terminal

NHMRC: National Health and Medical Research Council

Nhp: non-histone protein

NQdn: H2A.ZNQ dominant negative mRNA injected embryos

nt: nucleotides

NTD: neural tube defect.

NTP: nucleotide triphosphate

NuRD: nucleosome-remodelling histone-deacetylation

NuRF: nucleosome remodelling factor

OD: optical density

p: probability value
PBAF: polybromo BRG associated factor
PBAP: polybromo Brahma associated protein
PBS: phosphate buffered saline
PBT: Phosphate buffered saline with 0.1% v/v Tween 20
PCR: polymerase chain reaction.
PEH: paired ends of helices
RbAp: retino-blastoma associated protein
RIPA: radio-immuno-precipitation assay (buffer)
RNA: Ribonucleic acid
RNAi: RNA interference
RSC: remodel structure of chromatin
RT: reverse transcription
Rvb: RuvB-like protein
S: serine
S. cerevisiae: Saccharomyces cerevisiae
SD: standard deviation
SEM: standard error of the mean
siRNA: short interfering RNA
Snf: sucrose non-fermenting
Spp.: species
ss: single stranded
Stbm: Stabismus
SUMO: small ubiquitin-like modifier
SWI/SNF: switching defective/sucrose non-fermenting
SWR1: SWI/SNF related protein
T: threonine
TAE: Tris-acetate-EDTA
TE: Tris-EDTA
Tip60: Tat interacting protein 60
Tm: melting temperature
TSA: trichostatin A
UTP: uracil triphosphate
UTR: untranslated region
UV: ultraviolet

Wnt: these genes are named for two members of the family, wingless of D. melanogaster (Nusslein-Volhard and Wieschaus, 1980) and integrated from vertebrates (Nusse and Varmus, 1982).

X. laevis: Xenopus laevis
Xbcan: Xenopus brevican
Xbra: Xenopus brachyury
XMyoD: Xenopus myogenic determinant
XVent2: Xenopus Ventral 2