Investigating the Organisation of the Platypus Sex Chromosome Chain During Meiotic Prophase I

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Aaron Edward Casey, B.Sc. (Hons.)

Discipline of Genetics

School of Molecular and Biomedical Science

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Declaration

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Aaron Casey

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Abbreviations

^{o}C: Degrees Celsius

µL: Microlitre

BAC: Bacterial Artificial Chromosome

CC: Cohesin Complex

cDNA: Complementary DNA

DAPI: 4'-6-Diamidino-2-phenylindole

DMSO: Dimethylsulfoxide

DNA: Deoxyribonucleic Acid

FISH: Flourescence *in situ* Hybridisation

GFP: Green Fluorescence Protein

HRR: Homolog Recognition Region

NE: Nuclear Envelope

NOR: Nucleolar Organising Region

PAR: Pseudoautosomal Region

PBS: Phosphate Buffered Saline

PFA: Paraformaldehyde

PI: Protease Inhibitor

SC: Synaptonemal Complex

SCC: Sister Chromatid Cohesion

SLX: SYCP3-like, X-linked

SLX2: SYCP3-like, X-linked 2

SLY: SYCP3-like, Y-linked

SMC1α: Structural Maintenance of Chromosomes Alpha

SMC1β: Structural Maintenance of Chromosomes Beta

SSC: Standard Saline Citrate

SYCP1: Synaptonemal Complex Protein One (a.k.a SCP1)

SYCP1: Synaptonemal Complex Protein Two

SYCP1: Synaptonemal Complex Protein Three

SYCE1: Synaptonemal Complex Element One

SYCE2: Synaptonemal Complex Element Two

SYCE3: Synaptonemal Complex Element Three

TEX12: Testis Expressed Twelve

TRF1/2: Telomeric Binding Factor 1/2

Abstract

Meiosis is a specialised form of cell division that occurs specifically in the gonads of sexually reproducing species. It comprises a round of DNA replication followed by two successive rounds of cell division to produce haploid gametes. Each stage is divided into four substages of prophase, metaphase, anaphase and telophase. Prophase I is the longest and most complex stage of meiosis during which homologous chromosomes pair and recombine. The evolution of heteromorphic sex chromosomes has led to a number of changes in meiotic organisation. This includes the non-pairing of sex specific parts of the heteromorphic sex chromosomes and their inactivation in many species.

The platypus has a unique set of 10 sex chromosomes with homology to bird sex chromosomes that exist as a chain during meiotic metaphase I. Questions of mode and extent of pairing and the existence of meiotic silencing remained unknown but can inform our understanding of the evolution and mechanisms of meiotic prophase I. Work presented in this thesis provides novel insights into evolution and meiotic organisation of the monotreme sex chromosome complex.

The platypus sex chromosome chain forms during zygotene in stepwise manner, with remarkable consistency beginning at the Y5 end of the chain and ending with the X1 (Chapter 1). Synapsis generally relies on 3 main proteins; SYCP1, SYCP2 and SYCP3. Surprisingly platypuses express three different copies of *SYCP3* (including a multicopy version on a Y chromosome), genes that generally exist as single isoforms in most other species. Particularly given the SYCP3Y isoform is male specific, this raises the possibility that

SYCP3 paralogs may have evolved in relation to the sex chromosome chain during prophase I (Chapter 2).

During pachytene, the asynaptic regions of the sex chromosomes adopt a state of folding, similar to that of the avian Z and W chromosomes during synaptic adjustment, albeit without the formation of a central element. During this time the cohesin complex is heavily loaded onto the axial elements of the asynaptic regions of the X and Y regions of the chain. Furthermore at mid-pachytene the asynaptic regions of the chain are pulled to a giant nucleolus at which time the cohesin appears to spread onto the chromosome loops of the asynaptic regions of the chain that are also coincident with DNA condensation (Chapter 3).

During platypus pachytene there is global transcriptional downregulation. We observe no localised phosphorylation of the histone H2AX, a hallmark of MSCI but we do observe localised patterns of H2AFY, H3K27me3 and H3K9me3 at a paranucleolar location, however the H2AFY and H3K27me3 showed some colocalisation with sex chromosomes, there was not consistent pattern and H3K9me3 was always associated with a section of chromosome 6 (Chapter 4).

Together these results provide novel insights into the meiotic organisation of the monotreme sex chromosome complex and the evolution of MSCI in mammals.