A Focus on the Essential Roles of the Musashi Family of RNA Binding Proteins during Mammalian Spermatogenesis

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Declaration

Statement of Originality

The thesis contains no material which has been accepted for the award of any other 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 the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

Statement of Authorship

I hereby certify that the work embodied in this thesis contains a published paper/s/scholarly work of which I am a joint author. I have included as part of the thesis a written statement, endorsed by my supervisor, attesting to my contribution to the joint publication/s/scholarly work.

Thesis by Publication

I hereby certify that this thesis is in the form of a series of published papers of which I am a joint author. I have included as part of the thesis a written statement from each co-author, endorsed by the Faculty Assistant Dean (Research Training), attesting to my contribution to the joint publications.

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Publications included as part of this thesis

Chapter 1

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Chapter 2

Sutherland JM, Fraser BA, Sobinoff AP, Pye VJ, Davidson T, Siddall NA, Koopman P, Hime GR, and McLaughlin EA. Developmental Expression of Musashi-1 and Musashi-2 RNA-Binding Proteins During Spermatogenesis: Analysis of the Deleterious Effects of Dysregulated Expression. Biology of Reproduction: 90(5):92. 2014 (Journal article) **Published**

Chapter 3

Sutherland JM, Sobinoff AP, Fraser BA, Redgrove KA, Siddall NA, Koopman P, Hime GR, and McLaughlin EA. RNA binding protein Musashi-1 directly targets *Msi2* and *Erh* during early testis germ cell development and interacts with IPO5 upon translocation to the nucleus. . FASEB: under review November, 2014

Chapter 4

Sutherland JM, Sobinoff AP, Fraser BA, Redgrove KA, Siddall NA, Koopman P, Hime GR, and McLaughlin EA. The RNA binding protein Musashi-2 interacts with splicing factor SFPQ and mediates the post-transcriptional regulation of *Piwil1* and *Tbx1* during spermatogenesis.. Developmental Biology: under review November, 2014

Chapter 5

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Statements of Contribution

I attest that the Research Higher Degree candidate Jessie Sutherland has contributed upward of 50% towards data collection/analysis and manuscript preparation for all the publications included in this thesis for which I am a co-author.

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Conference Proceedings relevant to this Thesis

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Sutherland JM, Siddall NA, Hime GR, McLaughlin EA. Unique and essential roles of RNA-binding proteins Musashi-1 and Musashi-2 during Spermatogenesis: Insights from a transgenic mouse model. The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology 2013, Sydney, NSW, 25 – 28 Aug 2013. (Conference)

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Sutherland JM, Fraser BA, Siddall NA, Hime GR, Davidson T-L, Koopman P, McLaughlin EA. Musashi-2: an essential regulator of DNA recombination and repair. The Annual Scientific Meeting of the Endocrine Society of Australia and the Society for Reproductive Biology 2012, Gold Coast, QLD, 26 – 29 Aug 2012. (Conference)

Sutherland JM, Fraser BA, Gunter KM, Siddall NA, Hime GR, McLaughlin EA. RNA binding protein Musashi-2 is essential to post-mitotic Spermatogenesis. Second World Congress in Reproductive Biology 2011, Cairns, Australia, 9 – 11 Oct 2011. (Conference)

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Abstract

Spermatogenesis describes the complex process male gamete development whereby spermatogonial stem cells undergo a series of mitotic amplification, meiotic divisions, and a series of profound morphological changes in order to produce physically mature spermatozoa This self-renewing process requires the appropriate translational programming of more than 700 mRNAs. It is therefore an interesting feature of spermatogenesis that despite the cells requirements for continued growth and rapid proliferation, it undergoes extended periods of transcriptional quiescence. As such, spermatogenesis is highly reliant on mechanisms of post-transcriptional regulation, driven by RNA binding proteins which remain abundantly expressed throughout this process.

The Musashi family of RNA binding proteins are historically renowned for their fundamental roles in stem cell function, cell fate determination, CNS development, cellular proliferation, and tumorigenesis. Comprising of two mammalian orthologues, Musashi-1 (MSI1) and Musashi-2 (MSI2), this family of proteins remains highly conserved across vertebrate species. Given the established importance of Musashi in stem cells, it was surprising that these proteins had not been previously explored in the stem cell niche of the testis during spermatogenesis and we hypothesised a role for Musashi in this process. From pioneering studies we discovered an essential function for Musashi as critical regulator of testis germ cell maintenance and meiosis via genetic screening in *Drosophila*, and more recently have identified the differential expression of MSI1 and MSI2 in the mammalian testis.

The purpose of this thesis was to describe and characterise the expression and function of both MSI1 and MSI2 during mammalian spermatogenesis, primarily using a mouse model. Through examining the differential expression of mammalian MSI1 and MSI2 during germ cell development, we found that MSI1 was predominately localized in mitotic gonocytes and spermatogonia, while MSI2 was detected in meiotic spermatocytes and differentiating spermatids. Examination of the role of Musashi in spermatogenesis was achieved through the use of two transgenic mouse models with germ cell specific over-expression of full-length isoforms of *Msi1* (TgMsi1) or *Msi2* (TgMsi2). These models demonstrated that aberrant expression of either *Msi1* or *Msi2*

has deleterious effects on normal spermatogenesis, with the *Msi2* over-expression resulting in male sterility.

Focussing further on the differential expression of MSI1, in particular the transition between cytoplasmic and nuclear localisation, we identified two unique RNA-binding targets of MSI1 in spermatogonia in *Msi2* and *Erh*. Again utilising our TgMsi1 animal model we demonstrated potential roles for MSI1 in translational regulation, providing evidence to suggest that nuclear import protein IPO5 facilitates nuclear translocation of MSI1 to the transcriptionally silenced XY chromatin domain in meiotic pachytene spermatocytes, resulting in the release of MSI1 RNA-binding targets.

In terms of defining the role MSI2, differential gene expression studies and comparative protein expression analyses aided in the identification key molecular networks, and essential biological processes, globally affected by overexpression of *Msi2*. Functional analysis revealed *Tbx1* and *Piwil1* as direct RNA-binding targets of MSI2-mediated translational repression. Protein-protein interaction studies revealed MSI2 acts in complex with splicing factor SFPQ in testis germ cells, suggesting a function for MSI2 in pre-mRNA processing.

Overall this body of work provides strong evidence for the essential roles and unique functions of the Musashi family of RNA binding proteins during spermatogenesis, firmly establishing clear evidence for both proteins in post-transcriptional gene regulation. For MSI1 as a master regulator of translation repression during early germ cell development; and MSI2 as a key regulator of later stage male gamete development, involved in pre-mRNA processing.