Translational Control of Mouse Folliculogenesis and Oocyte Development

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BSc (Biotechnology) (Hons) Class I

Doctor of Philosophy
December, 2012
**Declaration**

I hereby certify that the work embodied in this thesis is the result of original research and has been submitted for a higher degree to any other University or Institution.

Signed ………………………………………

Kara M. Gunter

**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.

Signed……………………………………

Kara M. Gunter
Acknowledgements

I would like to acknowledge my supervisors, Prof. Eileen McLaughlin and my Co-Supervisor, Dr. Gary Hime for their support throughout my PhD candidature. In particular Eileen, for always being so readily available, enthusiastic about my project, for sharing and instilling your vast amount of knowledge. I have been very lucky to have such supportive and knowledgeable supervisors to guide me over the past 4 years.

To all the members of the Aitken laboratory, past and present: you have all been such helpful and terrific people to work with and have made my time in the laboratory very memorable. In particular to Jessie Sutherland, Barbara Fraser, Victoria Pye, Simone Stanger, Mark Bigland and Dr. Alexander Sobinoff for always being there to share your knowledge, help and wipe away the tears. I would also like to thanks Laureate Professor John Aitken for giving me the opportunity to complete my research training within the Aitken laboratory.

Thanks also to the Australian Genome Research Facility and the Australian Proteomics Analysis Facility for conducting the microarray and LC MS/MS analysis (respectively) included in this thesis.

Last but in no ways least, I would like to thank my family and friends for all their support and encouragement over the past 4 years. To Patrick, thank you for being my rock over the final stages of my thesis write up and helping me see the brighter side of life through all the stress. Thank you to my parents, Murray and Janine, for their support, encouragement and their belief in my abilities. To my sister, Christie, and my brother, Paul, thank you for keeping me grounded and reminding me there is more to life than research.

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<tr>
<td>μl, μg, μm, μM</td>
<td>micro-litres, grams, moles or molar respectively</td>
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<tr>
<td>bp</td>
<td>base pairs</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<td>dUTP</td>
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</tr>
<tr>
<td>E.coli</td>
<td>Escherichia Coli</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediamine tetra acetic acid</td>
</tr>
<tr>
<td>FCS</td>
<td>fetal calf serum</td>
</tr>
<tr>
<td>g (in reference to weight)</td>
<td>grams</td>
</tr>
<tr>
<td>g (in reference to centrifugation)</td>
<td>gravity</td>
</tr>
<tr>
<td>hr</td>
<td>hours</td>
</tr>
<tr>
<td>H₂O</td>
<td>water</td>
</tr>
<tr>
<td>HRP</td>
<td>horseradish peroxidase</td>
</tr>
<tr>
<td>ITS-X</td>
<td>insulin/transferrin/selenium</td>
</tr>
<tr>
<td>kDa</td>
<td>kilodalton</td>
</tr>
<tr>
<td>LB</td>
<td>luria broth</td>
</tr>
<tr>
<td>Milli Q</td>
<td>ultra-pure water</td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>ml, mg, mM</td>
<td>milli-litres, grams and molar respectively</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>Msi-1</td>
<td>Musashi-1</td>
</tr>
<tr>
<td>Msi-2</td>
<td>Musashi-2</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>ng</td>
<td>nanograms</td>
</tr>
<tr>
<td>PAP</td>
<td>hydrophobic barrier pen</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PGC</td>
<td>primordial germ cell</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNAi</td>
<td>RNA interference</td>
</tr>
<tr>
<td>RT (with reference to temperature)</td>
<td>room temperature</td>
</tr>
<tr>
<td>SDS</td>
<td>sodium dodecyl sulfate</td>
</tr>
<tr>
<td>shRNA</td>
<td>short hairpin RNA</td>
</tr>
<tr>
<td>siRNA</td>
<td>short interfering RNA</td>
</tr>
<tr>
<td>TBS(T)</td>
<td>tris buffered saline (plus Tween-20)</td>
</tr>
<tr>
<td>Tween-20</td>
<td>polyoxyethylenesorbitan monolaurate</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>v/v</td>
<td>volume per volume</td>
</tr>
<tr>
<td>w/v</td>
<td>weight per volume</td>
</tr>
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Abstract

The production of healthy, euploid oocytes from a finite pool of primordial follicles is crucial to the reproductive success of the mammalian female. Central to the production of a healthy oocyte is correct expression of a number of proteins which govern processes such as cell cycle, chromosome segregation and cell differentiation. Control of the maternal mRNA pool during oocyte maturation is crucial for the correct temporal and spatial expression of protein necessary for these processes. Control of the mRNA pool is assisted through the action of sequence-specific RNA-binding proteins, including those of the Musashi (Msi) family, which has been widely described to be a family of translational repressor proteins in stem cells while studies in the mammalian germline have been limited.

Utilising both mRNA and protein expression analysis, both Msi-1 and Msi-2 were found to be expressed throughout mouse folliculogenesis, with Msi-1 primarily expressed with the cytoplasm of the mouse oocyte and granulosa cells, while Msi-2 is expressed within the nucleus of these cell types. Transgenic overexpression mice for both Msi homologs were utilised to determine the downstream mRNA targets of both homologs in the mouse ovary. Through the utilisation of Native protein-RNA immunoprecipitation techniques, coupled with mRNA and protein expression techniques such as qPCR and immunoblotting performed on WT and tg ovaries, Msi-1 was found to act as a translational control protein capable of both translational activating the c-mos transcript and translationally repressing the ccng2, robo3 and msi-2 transcript in the ovaries of 5 week old mice. Functional analysis performed through use of a Ligation-mediated poly(A) tail length (LM-PAT) assay, revealed the differences in these two functions could result from the ability of Msi-1 and/or its interacting proteins to manipulate the length of the poly(A) tail of target transcripts, which governs mRNA translation efficiency.

While Msi-2 was found to act as a translational repressor of cdkn1c in the mouse ovary, a novel function of Msi-2 as a transcriptional control and/or splicing factor of m-numb was uncovered by this study, with Msi-2 found to complex with the transcriptional repressor and splicing proteins SFPQ and Nono.

Global transcriptome analysis of ovaries excised from WT, tgMsi-1 and tgMsi-2 mice revealed the RNA-binding proteins to primarily influence the expression of genes governing processes such as cell cycle, chromosome segregation, cellular differentiation,
organism development and cell survival, with all of these processes being tightly controlled in order to produce a healthy oocyte and subsequent zygote. Therefore, the studies outlined in this thesis represent the first in depth expression and functional analysis of the RNA-binding proteins Msi-1 and Msi-2, with results highlighting the complex network of transcriptional and translational control that exists within the mouse ovary.