

**FUNCTIONAL
CHARACTERISATION OF
DYNAMIN IN MOUSE
SPERMATOZOA**

Andrew Timothy Reid, BSc (Hons.) (Class I)

*Thesis submitted to the Faculty of Science and Information
Technology, The University of Newcastle, Australia in
fulfilment of the requirement for the degree of Doctor of
Philosophy*

November, 2014

Declaration

I hereby certify that this thesis is submitted 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 and endorsement from the Faculty Assistant Dean (Research Training) attesting to my contribution to the joint publications.

(Signature)

Andrew Timothy Reid

Date: 10th November 2014

Acknowledgements

...And so it all comes to an end and with it my infinite gratitude to all of those that have helped me to achieve this incredible milestone. I really hope that I don't forget anyone and really really hope that those of you I remember to thank feel all of my appreciation toward you. First and foremost, my principal supervisor Brett thank you for taking me on as your esteemed student, you have shown me how important hard work and understanding is in establishing yourself as a great scientist and mentor. I shall do my best to do you proud in the future. To John Aitken, you are an inspiration and I hope that one day I gain as much respect from my fellow co-workers as you do within the scientific community, thank you so much for everything. Finally, to dear Shauny, you are the exact person I want to be in, what is it... 30 years? (haha). Honestly you are an incredible person and I'm so grateful for even knowing you, even more so for being able to call you a close friend; thank you for everything.

To everyone else that has helped me in my research, thank you, Eileen McLaughlin and Phil Robinson in particular. Mark Baker and Nenad Naumovski as well as Geoff and Matt Jobling it was always a blast around you guys and I'm going to miss the laughs. Mona, Neets, Haylz, Sar'z x 2, Louise, Amy N, and Kristen I guess this is the last individual hello/ goodbye, thank you for all your happiness and help. To the old school students that have previously escaped the jaws of the PhD monster, Matty Dun, Skye, Tegan, Zamira and Rose, I NOW UNDERSTAND and thank you for all your support.

To my office full of beautiful friends, Aimee K., Amy L., Ally, Sally, Taylz, Tessa and Lizzy I'm gonna miss you all and all the ear-blocking conversations that we've had and I know you'll all destroy your PhD's (well... finish). Thank you all for being my outlets this last year.

To my forever friends Bel, Mandsy, Alex (Squire), Kate and Jessie, you have all been there for me and all inspirational. Alex and Kate, ah those first years were incredible with you guys and I can't wait to catch up more in the future. Jessie, you're an awesome happy person and you've earned all my respect, I know you'll have no trouble finishing your own PhD. Mandsy you are the best, I always loved your singing and happiness, it'll be missed and I'm so thankful I got to know you. We all better keep catching up. Bel, you have totally earned the biggest thank you of all. I have never had such a high density of incredible moments with a single person before in my life. You are truly somebody that understands what it means to live. From the beginning with guitar/music to the unbelievably close friends we are now, you were always inspirational and that'll never change, I honestly believe we will be best friends forever.

To Mum, Dad, Ben and Georgia, I am who I am because of you and will be forever grateful, I love you all and this PhD is equally yours to share.

Finally, and most importantly, to Harrison, I will never be anything but 100% proud of all you do in life and though you don't understand what a PhD is right now, I know one day

you'll be able to outshine your old man with something even more amazing. Thanks for being the world's greatest son, I love you.

Publications Included as Part of the Thesis

- Reid, A. T., Redgrove, K., Aitken, R. J., Nixon, B., (2011) *Cellular mechanisms regulating sperm-zona pellucida interaction*. Asian Journal of Andrology 13: 88-96
- Reid, A. T., Lord, T., Stanger, S. J., Roman, S. D., McCluskey, A., Robinson. P. J., Aitken, R. J., Nixon, B., (2012) *Dynamin regulates specific membrane fusion events necessary for Acrosomal Exocytosis in Mouse Spermatozoa*. Journal of Biological Chemistry 287: 37659-37672
- Reid, A. T., Anderson, A. L., Roman, S. D., McLaughlin, E. A., McCluskey, A., Robinson. P. J., Aitken, R. J., Nixon, B., (2015) *Glycogen synthase kinase 3 regulates acrosomal exocytosis in mouse spermatozoa via dynamin phosphorylation*. FASEB Journal 29: 000-000 (Article in press)
- Reid, A. T., Roman, S. D., Aitken, R. J., Nixon, B., (2014) *An investigation into novel dynamin function and its impact on fertilization*. International Journal of Molecular Sciences (Article submitted)

Permission Regarding Copyright

I, Andrew Timothy Reid, warrant that I have obtained, where necessary, permission from the copyright owners to use my own published journal articles in which the copyright is held by another party (e.g. publisher).

Date: 10 November 2014

Statement of Contribution of Others

I attest that Research Higher Degree candidate, Andrew Timothy Reid, contributed to greater than 50% of the written work, generation of data, and data analysis of the publications included in this thesis.

(Signature of Co-author)

A/Prof. Brett Nixon

Date: __08/12/14__

(Signature of Co-author)

Tessa Lord

Date: __10/11/14__

(Signature of Co-author)

Dr. Shaun D. Roman

Date: __10/11/14__

(Signature of Co-author)

Prof. Eileen McLaughlin

Date: _10/11/14__

(Signature of Co-author)

Laureate Prof. R. John Aitken

Date: __9/11/14__

(Signature of Co-author)

Prof. Adam McCluskey

Date: __10/11/14__

(Signature of Co-author)

Simone J. Stanger

Date: _10/11/14__

(Signature of Co-author)

Prof. Phillip J. Robinson

Date: _10/11/14__

(Signature of Co-author)

Dr. Kate Redgrove

Date: __11/11/14_____

(Signature of Assistant Dean Research training)

(A/Prof. Jenny Cameron)

Date: __08/12/14__

(Signature of Co-author)

Amanda L. Anderson

Date: __10/11/14_____

List of Additional Publications

- A.T. Reid, S.D. Roman, R.J. Aitken, B. Nixon (2012) Dynamin regulates the acrosome reaction in murine spermatozoa. Biology, RHD Conference, University House, University of Newcastle, Australia (Oral presentation).
- A.T. Reid, S.D. Roman, R.J. Aitken, B. Nixon (2011) Functional characterisation of the GTPase dynamin in mouse spermatozoa. Proceedings of the 42nd Annual Conference of the Society for Reproductive Biology, Cairns, Australia (Oral presentation).
- A.T. Reid, S.D. Roman, R.J. Aitken, B. Nixon (2011) Functional characterisation of the GTPase dynamin in mouse spermatozoa. Biology, RHD Conference, University House, University of Newcastle, Australia (Poster presentation).

- B. Nixon, A.T. Reid, S.D. Roman, R.J. Aitken (2011) Characterization of the GTPase dynamin throughout murine sperm maturation. 44th Annual Meeting of the Society for the Study of Reproduction, Portland, OR, USA (Poster).
- A.T. Reid, S.D. Roman, R.J. Aitken, B. Nixon (2010) Investigation of the role of dynamin in sperm surface remodeling. OzBio International Conference on “The Molecules of life: from Discovery to Biotechnology” Melbourne, Australia (Poster presentation).
- A.T. Reid, S.D. Roman, R.J. Aitken, B. Nixon (2010) Characterisation of the GTPase dynamin throughout murine sperm maturation. Proceedings of the 41st Annual Conference of the Society for Reproductive Biology, Sydney, Australia (Oral Presentation).
- A.T. Reid, S.D. Roman, R.J. Aitken, B. Nixon (2010) Characterisation of the GTPase dynamin in murine spermatozoa. Biology, RHD Conference, University House, University of Newcastle, Australia (Oral presentation).
- B. Nixon, A.T. Reid, B. Skinner, R.J. Aitken (2010) Elucidation of the molecular mechanisms that underpin capacitation-associated sperm surface remodeling. 11th International Symposium on Spermatology, Okinawa, Japan. Abstract S08-03 (Oral presentation)

Table of Contents

Contents

<i>FUNCTIONAL CHARACTERISATION OF DYNAMIN IN MOUSE SPERMATOZOA</i>	<i>i</i>
Declaration.....	i
Acknowledgements.....	ii
Publications Included as Part of the Thesis	iv
Permission Regarding Copyright.....	iv
Statement of Contribution of Others.....	v
List of Additional Publications	vi
<i>Table of Contents</i>	<i>viii</i>
<i>Abstract</i>	<i>1</i>
<i>Chapter 1: Cellular Mechanisms Regulating the Sperm-zona Pellucida interaction and Acrosomal Exocytosis (Introduction)</i>	<i>3</i>
ABSTRACT.....	4
1.0 Introduction.....	5
1.1 Maturation of mammalian spermatozoa	6
1.1.1 Spermatogenesis	6
1.1.2 Epididymal maturation	7
1.1.3 Capacitation.....	9
1.2 Structure, function and biochemistry of the zona pellucida.....	13
1.3 Primary sperm-ZP interaction	16
1.4 Formation of a multimeric zona-recognition complex.....	18
1.4.1 Membrane Priming	19
1.4.2 Lipid Rafts	20
1.4.3 Molecular Chaperones.....	20
1.5 Acrosome reaction	21
1.6 Potential role of Dynamin in sperm-ZP binding and acrosomal exocytosis.....	25
1.6.1 Dynamin in spermatozoa	26
1.7 Conclusions	27
REFERENCES	29
<i>Chapter 2: Dynamin Regulates Specific Membrane Fusion Events Necessary for Acrosomal Exocytosis in Mouse Spermatozoa</i>	<i>43</i>

Chapter 2: Introduction	44
ABSTRACT/ INTRODUCTION	45
EXPERIMENTAL PROCEDURES	46
RESULTS	48
DISCUSSION	52
REFERENCES	56
<i>Chapter 3: Glycogen Synthase Kinase 3 Regulates Acrosomal Exocytosis in Mouse Spermatozoa via Dynamin Phosphorylation.....</i>	59
Chapter 3: Introduction	60
ABSTRACT.....	62
INTRODUCTION	63
MATERIALS AND METHODS.....	65
RESULTS	74
DISCUSSION	80
REFERENCES	83
SUPPLEMENTARY FIGURES.....	87
<i>Chapter 4: Characterisation of Dynamin in the Mouse Epididymis</i>	90
Chapter 4: Introduction	91
SUMMARY	92
INTRODUCTION	93
EXPERIMENTAL PROCEDURES	93
RESULTS	95
DISCUSSION	99
REFERENCES	104
<i>Chapter 5: An investigation into novel dynamin function and its impact on fertilization (Final Discussion).....</i>	107
Abstract.....	109
5.0. Introduction	110
5.1. The controversial mammalian acrosome reaction.....	110
5.2. Fusion machinery.....	113
5.3. Dynamin crystal structure.....	113
5.4. Mammalian dynamin 1, 2 and 3	117
5.5. Secretory vesicles and the role of dynamin.....	118
5.6. Novel roles for dynamin within mammalian sperm	120
5.7. Structural basis for a dynamin-dependent fusion role.....	121

<i>5.8. Regulation of acrosomal exocytosis via dynamin phosphorylation</i>	<i>122</i>
<i>5.9. Conclusions.....</i>	<i>124</i>
<i>5.10 Limitations and future directions</i>	<i>127</i>
References.....	132
<i>Status of Publications as of Thesis Submission Date.....</i>	<i>138</i>
<i>BIBLIOGRAPHY.....</i>	<i>139</i>

Abstract

World population growth has been increasing exponentially since the 1950's, highlighting the inadequacies of current methods of contraception and the urgent need for new contraceptive measurements. Conversely, although the major driver of this population growth is the large number of unplanned pregnancies in developing countries, male factor infertility is on the rise within the developed world where it is now estimated to afflict 1 in every 20 males. To address these paradoxical problems we need a far better understanding of the mechanisms behind fertilization, with particular focus on the spermatozoon. Such knowledge would enable us to identify new contraceptive targets as well improve diagnosis of the underlying causes of infertility.

Two key functions that the spermatozoon must fulfil in order to fertilize *in vivo* are sperm-zona pellucida (ZP) binding and acrosomal exocytosis. In order for spermatozoa to obtain the ability to both bind the ZP and undergo a stimulus induced acrosome reaction they must undergo three distinct phases of maturation, namely spermatogenesis, epididymal maturation and capacitation. In the studies described in this thesis we have sought to investigate the role of the large membrane manipulator, dynamin, during sperm maturation as well as investigate its role during key phases of the fertilization cascade.

We have found that dynamin is present within the developing acrosome of immature gametes within the testis and is retained in this domain throughout epididymal maturation and capacitation. Via pharmacological inhibition of dynamin we have demonstrated that the GTPase is responsible for regulation of the progesterone induced acrosome reaction and this directly influences the rates of *in vitro* fertilization. Furthermore, upon receiving this stimulus to acrosome react, dynamin becomes phosphorylated at key serine residues. Subsequent to these findings we investigated these phosphorylation events further and identified the kinase responsible for the key phosphorylation event on serine 774 of dynamin 1 as glycogen synthase kinase 3 (GSK3). We have demonstrated via pharmacological inhibition of GSK3 that its activity is pivotal in controlling the onset of acrosomal exocytosis. Remarkably, via immunoelectron microscopy we have shown the delivery of this dynamin-phosphorylating kinase to sperm within the distal corpus epididymis via novel granular structures referred to as 'dense bodies'.

Finally we have determined that dynamin 1 and dynamin 2, are positioned to play important regulatory roles within the principal cells as well as the lumen of the epididymis. We have provided evidence that these proteins play essential roles in maintaining the composition of the intraluminal milieu across the caput, corpus and cauda epididymis, and thus influence the maturation of spermatozoa.

Taken together, these data have demonstrated a previously unappreciated role for dynamin during pivotal stages of spermatogenesis as well as during the epididymal maturation of spermatozoa. Furthermore, it is clear that dynamin function is essential for fertilization via its ability to regulate the acrosome reaction. Such findings add considerably to our understanding of the molecular mechanisms that underpin the production of functionally competent spermatozoa and will likely help to guide future research into contraceptive development as well as infertility studies.