# Whole genome approaches to identify genes involved in early meiosis

by

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B. Biotech. (Honours), The University of Adelaide

A thesis submitted for the degree of

Doctor of Philosophy

at

The University of Adelaide

Faculty of Sciences

School of Agriculture, Food and Wine

The Discipline of Plant and Food Science

Waite Campus

November 2008

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#### **Abstract**

Meiosis is a process which occurs in sexually reproducing organisms to halve the genetic complement prior to fertilisation. During meiosis a single round of DNA replication is followed by two successive rounds of chromosome segregation and cell division. The meiotic pathway in plants is complex from multiple perspectives. From a mechanical view; prior to the first meiotic division the chromosomes must replicate during meiotic interphase, then while retaining sister chromatid cohesion the homologous chromosomes must align, physically synapse and also concomitantly recombine (with the majority of sites being non-randomly positioned). Further complexities arise in allopolyploids such as bread wheat, which contains three very similar genomes from slightly diverged progenitors. Despite having homoeologous chromosomes present in the same nucleus, bread wheat displays diploid-like behaviour during meiosis I. Such an involved physical process as meiosis also has complexity reflected in the transcriptome and proteome, whether the organism be a simple eukaryote such as yeast, or a more complex eukaryote such as bread wheat.

Initially, this study utilised whole genome approaches to identify novel genes that could be involved in early meiosis, focusing on bread wheat in particular. Analysis of the wheat meiotic transcriptome over seven stages of anther development identified at least 1,350 transcripts which displayed meiotic regulation. The expression profiles of a subset of selected transcripts were analysed with Q-PCR and found to correlate strongly to those obtained in the microarray. Available meiotic transcriptome data from rice was compared to the wheat data, which enabled the identification of similar sequences, many previously unidentified, which also displayed meiotic regulation. Selected candidate genes from the

microarray study were also mapped in bread wheat. This data was combined with available literature and approximately 70% of candidate meiotic loci were located on chromosome group 3 or 5, which historically has been shown to contain multiple loci involved in chromosome pairing control.

One of the candidates located on chromosome group 3, a plant-specific mismatch repair gene, *Triticum aestivum MSH7* (*TaMSH7*), has previously been speculated to suppress homoeologous chromosome associations. Independent transgenic wheat plants produced using RNA interference (RNAi) were functionally characterised to ascertain a greater understanding of the role *TaMSH7* has during early meiosis in bread wheat. Localisation of a synaptonemal complex-associated protein (*TaASY1*) displayed subtle abnormalities in these mutants when compared to wild-type. Feulgen staining of meiotic chromosomes at metaphase I in these mutants revealed some interlocking and multivalent associations. These results suggest that *TaMSH7* may be linked to the mechanism underlying the phenotype that is observed in the *ph2a/ph2b* mutant, however further research still needs to be conducted to conclusively demonstrate that this is the case.

A component of the research presented in this study was performed in the model plant *Arabidopsis thaliana* due to the limitations of bread wheat. Extensive mutant banks and a sequenced genome have aided a decade of meiotic research in Arabidopsis and the identification of close to 50 meiotic genes. One of these, *AtMER3*, has been shown to control the non-random location of well above half of the recombination events that occur in many species. *AtMER3* was localised in meiotic nuclei in wild-type Arabidopsis and found to form foci on freshly synapsed regions of chromosomes in quantities far in excess of the average number of crossovers, indicating that *AtMER3* does not localise exclusively to sites of crossovers. *AtMER3* localisation was also analysed in several mutant

backgrounds and found to act in an *At*SPO11-dependent manner. However, *At*MER3 loading onto meiotic chromosomes was not affected in *Atrad51*, *Atdmc1* or *Atmsh5* mutant backgrounds.

**Declaration** 

I declare that the work presented in this thesis contains no material which has been

accepted for the award of any other degree or diploma in any University or other tertiary

institution. To the best of my knowledge and belief, this thesis does not contain any

material previously written or published by another person, except where due reference is

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Wayne Crismani

November 2008

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#### Acknowledgements

So many people have contributed to the contents of this thesis and my education in the past. I have done my best to thank you all but I acknowledge that these are just the top few and I wish I had the space to write out all the stories about how you have helped me get to what certainly feels like the pinnacle. Thank you.

First and foremost: Jason Able my principal supervisor. You have pushed me so hard to get the absolute best out of me and I will never be able to put in words how grateful I am for your colossal contribution to my education and the quality of the rest of my life. As I have told you before, you are an excellent supervisor who truly prioritises your students and fights for them. Your students' achievements are a reflection of your virtue. Someone said that "you are a success if you get up, do what you want to do and go to bed". You are extremely successful mate. Last but certainly not least, thank you for your friendship. I look forward to many more years of good times.

Thank you also to my co-supervisors and all of the other people who have guided me or provided a service: Ute Baumann, Tim Sutton, Peter Langridge, Neil Shirley, Amanda Able and her lab members, Andy Milligan, Ursula Langridge, Carolyn Schultz, Andrew Jacobs, Melissa Pickering, the MPBCRC education team past and present but particularly, Heather Bray and Michael McLean. Very special thanks also go to Margaret Pallotta for allowing me to use her fantastic nulli-tetra membranes.

I am eternally grateful to all of the wonderful people in France who made my time there nothing short of surreal. Thank you to Mathilde Grelon and Raphaël Mercier for your supervision, taking a chance on someone you didn't know, teaching me so much about meiosis, good cytology and good science and of course the wonderful friendship you offered me. You both went above and beyond being supervisors by allowing me into your personal lives. Liudmila Chelysheva, thank you for giving me the gift of cytology and also all of the wonderful conversations. I look forward to many more of them. Thanks also go to Arnaud De Muyt for being a wonderful friend and taking me on all of the adventures we had. I hope to see you soon. Thank you also to Fabien Nogué and Nicolas Macaisne for your friendship.

Thank you to German Spangenberg for collaborating on the microarray and the transgenic wheat plant components that are presented within this thesis. I must also thank Wojtek and Teresa Pawlowski for their hospitality and all of the members of Wojtek's lab for teaching me the 3D immuno-localisation technique which has also contributed significantly to what I have learnt during my PhD.

Thank you to all of the meiosis team over the years. Chandy, Hayley Jolly, Kelvin Khoo, Andrew Lloyd, Caroline Abrahamse, Gordon Wellman, Bill Bovill and of course my amazing friend Scott Boden. After Jas, you have contributed more than anyone to my project as both an extremely intelligent scientist and an infallible friend. You have been essential to my success by not only supporting me but also giving me an excellent bench mark to aim for.

Thank you to the funding bodies who make all of the research possible. The Molecular Plant Breeding Co-operative Research Centre, the University of Adelaide, L'Institut National de la Recherche Agronomique and the Farrer Memorial Trust.

Finally I wish to thank my entire family and circle of friends for your love and affection. Mum, Dad, Dylan, Jordan, Aaron and the 'Biotechers': Steve and Tegan, Iain and Rachel, Kylie, Nic and Kat and her family.

# **Glossary of Abbreviations**

Abbreviation	Full term
3′	three prime
5'	five prime
9 mer	9 base pair nucleotide
α-dCTP	alpha-deoxycytidine triphosphate
°C	degrees Celsius
ASY1	<u>ASY</u> napsis <u>1</u>
At	Arabidopsis thaliana
BAC	bacterial artificial chromosome
bar	bialaphos resistance gene
BLAST	Basic Local Alignment and Search Tool
bp	base pair
BSA	Bovine Serum Albumin
BW26	Bob White 26 cultivar of bread wheat
CDK	<u>C</u> yclin <u>D</u> ependent <u>K</u> inase
cDNA	complimentary deoxyribonucleic acid
cv.	cultivar
CO	crossover
DABCO	diazabicyclo-[2,2,2] octane
DAPI	4',6-diamidino-2-phenylindole
DMC1	<u>D</u> isrupted <u>M</u> eiotic <u>c</u> DNA <u>1</u>
dHJ	double Holliday Junction

DNA deoxyribonucleic acid

DPSS diode-pumped solid state (laser)

dNTP deoxynucleotide triphosphate

DSBR double-strand break repair

DTT dithiothreitol

Expect value

EDTA ethylene diamine tetra-acetic acid

EFA <u>E</u>longation <u>F</u>actor 1 <u>A</u>lpha

eFP electronic fluorescent pictograph

EGTA ethylene glycol tetra-acetic acid

EST expressed sequence tag

g gram

*GAPDH GlycerAldehyde-3-PhosphateDeHydrogenase* 

gDNA genomic deoxyribonucleic acid

HOP1 <u>HO</u>mologous <u>P</u>airing <u>1</u>

Hv Hordeum vulgare

kb kilobase

LASER Light Amplification by Stimulated Emission of Radiation

M molar

mCi/mL milli Curie per millilitre

mg milligram

mM millimolar

MER3  $\underline{ME}$ iotic  $\underline{R}$ ecombination  $\underline{3}$ 

*MLH1/3* <u>*Mut L Homologue 1/3*</u>

MMR mismatch repair

mRNA messenger ribonucleic acid

MPBCRC Molecular Plant Breeding Co-operative Research Centre

MRE11 <u>Meiotic RE</u>combination <u>11</u>

*MSH2/3/4/5/6/7 Mut S Homologue 2/3/4/5/6/7* 

NCBI National Center of Biotechnology Information

NCO non-crossover

ng nanogram

nm nanometre

NT nullisomic-tetrasomic

P probability

PBS phosphate buffered saline

PCR polymerase chain reaction

Ph <u>P</u>airing <u>h</u>omoeologous

PHS1 <u>Poor H</u>omologous <u>S</u>ynapsis <u>1</u>

PMC pollen mother cell

PRD1 <u>Putative Recombination initiation Defect 1</u>

PVP polyvinyl pyrrolidone

Q-PCR quantitative real-time PCR

*RAD51* RADiation sensitive 51

RMA robust multichip analysis

RNA ribonucleic acid

RNAi RNA interference

rRNA ribosomal ribonucleic acid

SC synaptonemal complex

SDS sodium dodecyl sulphate

SNP single nucleotide polymorphism

SSC standard saline citrate

ssDNA single-stranded deoxyribonucleic acid

SPO11 <u>SPO</u>rulation-deficient <u>11</u>

Ta Triticum aestivum

Taq Thermus aquaticus

T-DNA transfer DNA

TILLING targeted induced local lesions in genomes

U units

μL microlitre

μg microgram

μm micrometre

μM micromolar

v/v volume per volume

w/v weight per volume