Whole genome approaches to identify genes involved in early meiosis

by

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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 (*Ta*ASY1) 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 *Ta*MSH7 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. *At*MER3 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 *At*MER3 does not localise exclusively to sites of crossovers. *At*MER3 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 made in the text.

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Wayne Crismani

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Glossary of Abbreviations

Abbreviation	Full term
3′	three prime
5′	five prime
9 mer	9 base pair nucleotide
α-dCTP	alpha-deoxycytidine triphosphate
ºC	degrees Celsius
ASY1	*ASYnapsis 1*
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	*Cyclin Dependent Kinase*
cDNA	complimentary deoxyribonucleic acid
cv.	cultivar
CO	crossover
DABCO	diazabicyclo-[2,2,2] octane
DAPI	4',6-diamidino-2-phenylindole
DMC1	*Disrupted Meiotic cDNA 1*
dHJ	double Holliday Junction

DNA	deoxyribonucleic acid
DPSS	diode-pumped solid state (laser)
dNTP	deoxynucleotide triphosphate
DSBR	double-strand break repair
DTT	dithiothreitol
E	Expect value
EDTA	ethylene diamine tetra-acetic acid
EFA	*Elongation Factor 1 Alpha*
eFP	electronic fluorescent pictograph
EGTA	ethylene glycol tetra-acetic acid
EST	expressed sequence tag
g	gram
GAPDH	*GlycerAldehyde-3-Phosphate DeHydrogenase*
gDNA	genomic deoxyribonucleic acid
HOP1	*HOmologous Pairing 1*
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	*MEiotic Recombination 3*
MLH1/3	*Mut L Homologue 1/3*

MMR	mismatch repair
mRNA	messenger ribonucleic acid
MPBCRC	Molecular Plant Breeding Co-operative Research Centre
MRE11	*Meiotic REcombination 11*
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	*Pairing homoeologous*
PHS1	*Poor Homologous Synapsis 1*
PMC	pollen mother cell
PRD1	*Putative Recombination initiation Defect 1*
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	*SPOrulation-deficient 11*
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