Investigating chromosome pairing in bread wheat using *ASYNAPSIS I*

by

Scott Andrew Boden
B. Science (Hons.), 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
Discipline of Plant and Food Science
Waite Campus

July 2008

Table of Contents

List of Figures

List of Tables

Abstract

Pairing and synapsis of homologous chromosomes are required for normal chromosome segregation and the exchange of genetic material during meiosis. Pairing is defined as the recognition and alignment of chromosomes that occurs either pre-meiotically or during early prophase I to ensure that associations *via* synapsis and recombination occur only between homologues. Synapsis is the intimate juxtaposition of homologous chromosomes that is complete at pachytene following formation of a tri-partite proteinaceous structure known as the synaptonemal complex (SC). In yeast, HOP1 is an essential component of the SC that localises along chromosome axes during prophase I and promotes homologous chromosome interactions. Homologues in Arabidopsis (*AtASY1*), *Brassica* (*BoASY1*) and rice (*OsPAIR2*) have been isolated through analysis of mutants that display decreased fertility due to severely reduced synapsis of homologous chromosomes. Analysis of these genes has indicated that they play a similar role to HOP1 in pairing and formation of the SC through localisation to axial/lateral elements of the SC. In this study, we have characterised the bread wheat homologue of *HOP1*, *TaASY1*, and its encoded protein.

The full length cDNA and genomic DNA clones of *TaASY1* have been isolated, sequenced and characterised. *TaASY1* is located on chromosome group 5 and the open reading frame displays significant similarity to *OsPAIR2* (84%) and *AtASY1* (63%). In addition to *Os*PAIR2 and *At*ASY1, the deduced amino acid sequence also displays sequence similarity to *Sc*HOP1, with all four proteins containing a HORMA domain. Transcript and protein analysis showed that expression is largely restricted to meiotic tissue, with elevated levels during the stages of prophase I when pairing and synapsis of homologous chromosomes occurs.

Antibodies specific to *TaASY1* were used in immuno-fluorescence microscopy and immuno-gold transmission electron microscopy to investigate the localisation of *Ta*ASY1 in

meiotic cells. Immuno-fluorescence analysis initially detected ASY1 in pollen mother cells (PMCs) during meiotic interphase as foci randomly distributed over the chromatin. The ASY1 signal became increasingly continuous during leptotene, reflecting the changes occurring in chromosome morphology. Throughout zygotene, the signal became progressively more continuous, localising along the entire length of the axial elements as chromosomes synapsed. This signal appeared to persist until pachytene, before disappearing from the chromatin as the SC disassociated through late pachytene and early diplotene. The immuno-gold based electron microscopy displayed that *Ta*ASY1 localises to chromatin that is associated with both axial elements before SC formation as well as chromatin of lateral elements within formed SCs.

Analysis of RNAi *Taasy1* mutants was performed to further define the role of ASY1 in bread wheat meiosis. ASY1 localisation was disrupted in these mutants, with a diffuse and non-continuous signal observed through leptotene and zygotene. Feulgen staining of meiotic chromosomes displayed reduced synapsis during prophase I, as well as multivalents at metaphase I and abnormal chromosome segregation during anaphase I. These observations are consistent with the presence of homoeologous chromosome interactions. *Ta*ASY1 expression and localisation was also investigated in the bread wheat pairing mutant, *ph1b*. Quantitative real-time PCR (Q-PCR) revealed that *TaASY1* is significantly up-regulated in *ph1b*, with greater then 20-fold expression compared to wild-type Chinese Spring, while maintaining the same pattern of expression as wild-type through progressive stages of meiosis. ASY1 localisation was significantly disrupted in *ph1b*, with irregular loading on axial elements during mid to late zygotene, indicative of abnormal chromatin remodelling and multiple axial element associations that have previously been reported in *ph1b*. Taken together, these results indicate that *Ta*ASY1 is essential for promoting homologous chromosome interactions during meiosis, and that impairment of ASY1 function in bread wheat meiosis results in reduced restriction of chromosome associations to homologues.

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.

I give consent to this copy of my thesis when deposited in the University Library, being available for loan and photocopying, subject to the provisions of the copyright Act 1968.

The author acknowledges that copyright of the published works contained within this thesis resides with the copyright holder(s) of those works.

Scott Boden

July 2008

Acknowledgements

Firstly, I would like to thank my principal supervisor and dear friend, Jason Able, who became the mentor that every student should have. Thank you for your enthusiasm for science, your constant guidance throughout this project, and your genuine willingness to do everything in your power to make my project as successful and enjoyable as possible. I will be eternally grateful.

Secondly, thank you to my co-supervisors and the many people along the way that have provided me with crucial technical support, without which the results of my project would not have been achieved. These people include: Andrew Milligan, Chunyuan Huang, Peter Langridge, Neil Shirley, Nadim Shadiac, Gwen Mayo, Margie Pallotta, Marilyn Henderson, Lyn Waterhouse, Ursula Langridge, Ian Dundas, Dawn Verlin, Alfio Comis, Chris Bagley and Juan Juttner. Thank you sincerely for your fantastic expertise at various stages of my project.

I would like to give special thanks to three groups who have provided significant help during the last three years. Firstly, to Professor Takashi R. Endo and his group at Kyoto University. I am extremely grateful to you all, especially Prof. Endo and Taiji Nomura, for making my stay in Kyoto so enjoyable and rewarding. Secondly, I would like to thank Dr Wojtek Pawlowski and his group at Cornell University for teaching me the wonders of fluorescent microscopy. Thank you to you all for making my dream of seeing chromatin within a meiotic nucleus come true, and for being so generous with your knowledge about this difficult technique. And finally, to Professor German Spangenberg and his group from Molecular Plant Breeding CRC, who provided me with the wonderful gift of the *Taasy1* mutants.

Many thanks also to my current and past lab members; Hayley Jolly, Kelvin Khoo, Andrew Lloyd, Caroline Abrahamse, Bill Bovill, Ryan Walker, Tim Sutton, Elise Tucker, Tim March, Annette Boettcher, James Chand and especially, Wayne Crismani, who have provided ever-useful technical knowledge, plenty of laughs and a greatly appreciated release from the pressures of a PhD.

I would also like to thank Heather Bray, Amanda Able and Michael McLean, who have provided amazing educational support throughout this project, especially at the student retreats. The success and enjoyment of the students has a lot to do with your efforts, and I for one am truly grateful.

Thank you to the Grains Research and Development Corporation and Molecular Plant Breeding CRC for your generous funding of this project. Also, thank you to the Department of Primary Industries N.S.W., the Australian Society of Plant Scientists, the former Department of Education, Science and Technology and Cheung Kong Holdings for the funding that you generously provided for travel at various stages of my PhD.

On a personal note, thank you to my family, Andrew, Anthea, Justin and James for your love, support and constant source of happiness that you have provided throughout my education. Thank you also to Anna's parents, Don and Gail, who have provided great support during the last stages of my PhD. And finally, thank you Anna, for being the loving person who makes the great days even more enjoyable, and who puts a smile on my face even after a crappy day.

Glossary of Abbreviations

Abbreviation	Full term
3'	three prime
5'	five prime
9mer	9 base pair nucleotide
α-dCTP	alpha-deoxycytidine triphosphate
ºC	degrees Celcius
Amp	*Ampicillin*
ASY1	*ASYnapsis 1*
At	*Arabidopsis thaliana*
bar	bialaphos resistance gene
BCIP/NBT	5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium
BLAST	Basic Local Alignment and Search Tool
Bo	*Brassica oleracea*
bp	base pair
BSA	Bovine Serum Albumin
BW26	Bob White 26 cultivar of bread wheat
Cdk	*Cyclin Dependent Kinase*
cDNA	complimentary deoxyribonucleic acid
Ce	*Caenorhabditis elegans*
CT	cycle threshold
cv.	cultivar
DABCO	diazabicyclo-[2,2,2] octane

DAPI	4',6-diamidino-2-phenylindole
DMC1	*Disrupted Meiotic cDNA 1*
DNA	deoxyribonucleic acid
DPSS	diode-pumped solid state (laser)
dNTP	deoxynucleoside triphosphate
DTT	dithiothreitol
EDTA	ethylene diamine tetra-acetic acid
EFA	*Elongation Factor 1 Alpha*
EGTA	ethylene glycol tetra-acetic acid
EST	expressed sequence tag
FISH	fluorescent *in situ* hybridisation
g	gram
g	relative centrifugal force
GAPdH	*GlycerAldehyde-3-Phosphate DeHydrogenase*
h	hour(s)
HIM-3	*High Incidence of Males 3*
His	histidine
HOP1	*HOmologous Pairing 1*
HORMA	Hop1p, Rev7p, MAd2
IgG	immunoglobulin G
IPTG	isopropyl-1-thio-β-D-galactoside
kb	kilobase
kDa	kilo Dalton
KLH	keyhole limpet hemocyanin
LASER	Light Amplification by Stimulated Emission of Radiation

LB	Luria Bertani
M	molar
mg	milli gram
mM	milli molar
mCi mL^{-1}	milli Curie per milli litre
MEC1	*Mitosis Entry Checkpoint 1*
min	minute(s)
MLH1/3	*Mut L Homologue 1/3*
mRNA	messenger ribonucleic acid
MPB CRC	Molecular Plant Breeding Co-operative Research Centre
MRE11	*Meiotic REcombination 11*
MSH4/5	*Mut S Homologue 4/5*
MS/MS	tandem mass spectrometry
N/A	not applicable
NCBI	National Center of Biotechnology Information
n.d.	not determined
ng	nano gram
nm	nano metre
NT	nullisomic-tetrasomic
ORF	open reading frame
Os	*Oryza sativa*
P	probability
PAIR2	*homologous Pairing Aberration In Rice meiosis 2*
PBS	phosphate buffered saline
PCR	polymerase chain reaction

PLACE	PLAnt *Cis*-acting Regulatory DNA Elements
PMC	pollen mother cell
PVDF	polyvinylidene difluoride
PVP	polyvinyl pyrrolidone
PVPP	polyvinyl polypyrrolidone
Q-PCR	quantitative real-time PCR
Q-TOF^2	quadruple time of flight squared
R40	40 μg μL^{-1} RNAse in 1X TE
RAD51	*RADiation sensitive 51*
REC8	*RECombination 8*
RED1	*REDuctional division 1*
RNA	ribonucleic acid
RNAse	ribonuclease
RNAi	RNA interference
rpm	revolutions per minute
Sc	*Saccharomyces cerevisiae*
SC	synaptonemal complex
sec	second(s)
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS - polyacrylamide gel electrophoresis
SNP	single nucleotide polymorphism
SSC	standard saline citrate
SPO11	*SPOrulation-deficient 11*
Ta	*Triticum aestivum*
Taq	*Thermus aquaticus*

TBS	tris-buffered saline
T-DNA	transfer DNA
TE	Tris EDTA solution
TEL1	*TELomere 1*
TEM	transmission electron microscopy
T_m	melting temperature
Tris	tris(hydroxymethyl)aminomethane
U	units
µL	micro litre
µg	micro gram
µM	micro molar
UV	ultra-violet
V	volts
v/v	volume/volume
w/v	weight/volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyrano-side