

**Investigating chromosome pairing in bread
wheat using *ASYNAPSIS I***

by

Scott Andrew Boden

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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 *OsPAIR2* and *AtASY1*, the deduced amino acid sequence also displays sequence similarity to *ScHOP1*, 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 *TaASY1* 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 *TaASY1* 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. *TaASY1* 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 than 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 *TaASY1* 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.

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Glossary of Abbreviations

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

DAPI	4',6-diamidino-2-phenylindole
<i>DMC1</i>	<i>Disrupted Meiotic cDNA 1</i>
DNA	deoxyribonucleic acid
DPSS	diode-pumped solid state (laser)
dNTP	deoxynucleoside triphosphate
DTT	dithiothreitol
EDTA	ethylene diamine tetra-acetic acid
<i>EFA</i>	<i>Elongation Factor 1 Alpha</i>
EGTA	ethylene glycol tetra-acetic acid
EST	expressed sequence tag
FISH	fluorescent <i>in situ</i> hybridisation
g	gram
<i>g</i>	relative centrifugal force
<i>GAPdH</i>	<i>GlycerAldehyde-3-Phosphate DeHydrogenase</i>
h	hour(s)
<i>HIM-3</i>	<i>High Incidence of Males 3</i>
<i>His</i>	histidine
HOP1	<i>HOmologous P<i>airing</i> 1</i>
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
<i>MEC1</i>	<i><u>Mitosis Entry Checkpoint 1</u></i>
min	minute(s)
<i>MLH1/3</i>	<i><u>Mut L Homologue 1/3</u></i>
mRNA	messenger ribonucleic acid
MPB CRC	Molecular Plant Breeding Co-operative Research Centre
<i>MRE11</i>	<i><u>Meiotic REcombination 11</u></i>
<i>MSH4/5</i>	<i><u>Mut S Homologue 4/5</u></i>
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
<i>Os</i>	<i>Oryza sativa</i>
<i>P</i>	probability
<i>PAIR2</i>	<i><u>homologous Pairing Aberration In Rice meiosis 2</u></i>
PBS	phosphate buffered saline
PCR	polymerase chain reaction

PLACE	PLAnt <i>Cis</i> -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 $\mu\text{g } \mu\text{L}^{-1}$ RNase in 1X TE
<i>RAD51</i>	<u><i>RAD</i>iation sensitive <i>51</i></u>
<i>REC8</i>	<u><i>RE</i>Combination <i>8</i></u>
<i>RED1</i>	<u><i>RE</i>Ductional division <i>1</i></u>
RNA	ribonucleic acid
RNase	ribonuclease
RNAi	RNA interference
rpm	revolutions per minute
<i>Sc</i>	<i>Saccharomyces cerevisiae</i>
SC	synaptonemal complex
sec	second(s)
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS - polyacrylamide gel electrophoresis
SNP	single nucleotide polymorphism
SSC	standard saline citrate
<i>SPO11</i>	<u><i>SP</i>Orulation-deficient <i>11</i></u>
<i>Ta</i>	<i>Triticum aestivum</i>
<i>Taq</i>	<i>Thermus aquaticus</i>

TBS	tris-buffered saline
T-DNA	transfer DNA
TE	Tris EDTA solution
<i>TEL1</i>	<u><i>TELomere 1</i></u>
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