An investigation of bread wheat meiosis via proteomics and gene-targeted approaches: the isolation and characterisation of four meiotic proteins

by

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Abstract

During the early stages of meiosis, three key processes occur: chromosome pairing, synapsis and DNA recombination. Chromosomes are first replicated during interphase, after which they are aligned together in a non-random fashion to enable the installation of the synaptonemal complex (SC) along the chromosome axes leading to synapsis. Recombination machinery then enables strand invasion to occur, which then leads to the formation of chiasmata and ultimately, genetic recombination. Meiosis is further complicated in organisms with multiple genomes such as allohexaploid bread wheat (*Triticum aestivum* L.) which has three genomes (inherited from similar yet distinct progenitors), each with seven chromosomes. Thus a large number of proteins are likely to be required for the successful execution of this biological process.

The first approach in this study used proteomics to identify proteins that have possible roles during the early stages of wheat meiosis. Total protein samples isolated from staged meiocytes (specifically from pooled stages of pre-meiotic interphase to pachytene and from telophase I to telophase II) of wild-type Chinese Spring and the *Pairing homoeologous* deletion mutants, *ph1b* and *ph2a*, were analysed by 2-dimensional gel electrophoresis (2DGE). This resulted in identifying six differentially expressed protein spots (designated KK01 to KK06); from which three full-length coding sequences and one partial coding sequence of the candidate genes encoding these proteins were isolated (a putative speckle-type POZ protein, a pollen-specific SF21-like protein, a putative HSP70-like protein, as well as a partial hexose transporter peptide). Southern blot analysis revealed that these genes were spread across four different chromosome groups (2, 7, 5 and

1 respectively) with a copy on each of the three genomes (A, B and D). Q-PCR analysis of these four genes across the two pooled meiotic stages and various genotypes suggests that both *KK01* and *KK06* have roles during the early stages of meiosis and that they may be directly/indirectly regulated by a combination of elements within the *Ph1* and *Ph2* loci. The high level of *KK03* mRNA transcript detected in the later stages of meiosis is consistent with its role as a pollen-specific protein-encoding gene. In contrast, *KK04* expression suggests that it is post-transcriptionally regulated resulting in *KK04* being translated in the *ph2a* mutant. Both the speckle-type POZ protein and putative dnaK/HSP70 protein were also shown to interact with DNA *in vitro*.

The second approach of this study focused on isolating and characterising wheat homologues of two known meiotic proteins, namely *PHS1* and *ZYP1*. In the maize *PHS1* mutant *Zmphs1-0*, homologous chromosome pairing and synapsis are significantly affected, with homoeologous chromosome interactions occurring between multiple partners. More recently, co-immunolocalisation assays using anti-PHS1 and anti-RAD50 antibodies showed that both proteins had similar localisation patterns in the wild-type maize plants and that RAD50 localisation into the nucleus was affected by the absence of PHS1 thus implicating PHS1 as a regulator of RAD50 nuclear transport. In this study, the full-length coding transcript of wheat *PHS1* (*TaPHS1*) was isolated, sequenced and characterised. *TaPHS1* is located on chromosome group 7 with copies on the A, B and D genomes. Expression profiling of *TaPHS1* in both wild-type and the *ph1b* mutant during and post-meiosis show elevated levels of *TaPHS1* expression in the *ph1b* background. The *Ta*PHS1 protein has sequence similarity to other plant PHS1/PHS1-like proteins but also possesses a unique region of oligopeptide

repeat units. DNA-binding assays using both full-length and partial peptides of *Ta*PHS1 show conclusively that *Ta*PHS1 is able to interact with both single- and double-stranded DNA *in vitro*, even though no known conserved DNA-binding domain was identified within the *Ta*PHS1 sequence, indicating *Ta*PHS1 possesses a novel uncharacterised DNA-binding domain. Immunolocalisation data from assays conducted using an antibody raised against *Ta*PHS1 demonstrates that *Ta*PHS1 associates with chromatin during early meiosis, with the signal persisting beyond chromosome synapsis. Furthermore, *Ta*PHS1 does not appear to co-localise with the asynapsis protein – *Ta*ASY1 – possibly suggesting that these proteins are independently coordinated. Combined, these results provide new insight into the potential functions of PHS1 during early meiosis in bread wheat.

Similar to PHS1, Arabidopsis knock-down mutants of ZYP1 also display non-homologous chromosome interactions. ZYP1 has previously been characterised as a SC protein required for holding homologous chromosomes together in other species. In this study, the full-length coding sequence of the wheat *ZYP1* (*TaZYP1*) homologue was isolated, sequenced and characterised. Expression of *TaZYP1* analysed by Q-PCR across wild-type, *ph1b* and multiple *Taasy1* mutants during meiosis showed an approximate 1.3-fold increase in the *ph1b* mutant. In addition, DNA-binding assays demonstrate that *Ta*ZYP1 interacts with dsDNA under *in vitro* conditions while immunolocalisation (using an anti-*Ta*ZYP1 antibody) across wild-type, *ph1b* and *Taasy1* revealed the spatial and temporal localisation pattern of *Ta*ZYP1. Taken together, these results show that *Ta*ZYP1 plays an identical role to its homologues in other species as a SC protein and is affected by reduced levels of *Ta*ASY1 in wheat.

This body of work utilised a two-pronged approach to investigate meiosis in wheat with the overall outcome of identifying new meiotic proteins as well as characterising the wheat equivalents of two known meiotic proteins previously reported in other organisms. To this end, two previously uncharacterised wheat proteins with possible roles (involving interactions with chromatin) during meiosis have been successfully identified using the proteomics approach while both *Ta*PHS1 and *Ta*ZYP1 have been characterised with antibodies raised against both these proteins. The characterisation of *Ta*PHS1 and its DNA-binding capabilities, both *in vitro* and *in planta*, has shed light on a previously unknown function of the PHS1 protein while the localisation profile of *Ta*ZYP1 in *Taasy1* mutant lines has contributed to our understanding of how ASY1 levels can affect chromosome pairing in wheat.

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 Kelvin Khoo Han Ping, and 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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* **Kelvin H.P. Khoo, Amanda J. Able & Jason A. Able** (2011) Poor Homologous Synapsis 1 (PHS1) interacts with chromatin but does not co-localise with ASYnapsis 1 (ASY1) during early meiosis in bread wheat. *BMC Plant Biology*, (*submitted*).

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Glossary of abbreviations

Abbreviation	Full term
2DGE	2-dimensional gel electrophoresis
3'	three prime
5'	five prime
9mer	9 base pair nucleotide
α-dCTP	alpha-deoxycytidine triphosphate
ºC	degrees Celsius
AFD1	*Absence of First Division 1*
Amp	ampicillin
At	*Arabidopsis thaliana*
ASY1	*ASYnapsis 1*
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
BTB	Bric-a-Brac, Tramtrack, Broad domain
BW26	Bob White 26 cultivar of bread wheat
CDK	*Cyclin Dependent Kinase*
cDNA	complimentary deoxyribonucleic acid
Ce	*Caenorhabdatis elegans*
CHAPS	3-[(3-Cholanidopropyl)Dimethylammonio]-1

CL	cell lysate
CT	cycle threshold
cv.	cultivar
D-A	diplotene to anaphase I pooled stage
Da	Dalton
DABCO	diazabicyclo-[2,2,2] octane
DAPI	4',6-diamidino-2-phenylindole
DIGE	2-dimensonal fluorescence difference gel electrophoresis
DMC1	*Disrupted Meiotic cDNA 1*
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
ds	double-stranded
DSB	double-stranded break
DTT	dithiothreitol
E	Expect value
EBT	eriochrome black T
EDTA	ethylene diamine tetra-acetic acid
ELP1	*Elongator Complex Protein 1*
EST	expressed sequence tag
FISH	fluorescent *in situ* hybridisation
FT	flow-through
g	gram
GAPDH	*GlycerAldehyde-3-Phosphate DeHydrogenase*
Ha	*Helianthus annuus*

His	histidine
hr	hour(s)
Hs	*Homo sapiens*
HSP70/70-2	Heat Shock Protein 70/70-2
Hv	*Hordeum vulgare*
HYP6	*Hypothetical 6*
IgG	immunoglobulin G
IEF	isoelectric focusing
IPTG	isopropyl-1-thio-P-D-galactoside
kb	kilobase
KCl	potassium chloride
kD	kilo Dalton
L	ladder/molecular weight marker
LB	Luria Bertani
µL	microlitre
µg	microgram
µM	micromolar
M	molar
MATH	Mephrin and TRAF homology domain
MALDI-TOF/TOF	Matrix-Assisted Laser Desorption Ionisation Time-of-Flight tandem mass-spectrometry
Mb	megabase
MCS	maleimidocaproyl-N-hydroxysuccinimide
MES	2-(N-morpholino)-ethane sulphonic acid
MFS	Major Facilitator Superfamily

mg	milligram
Mm	*Mus musculus*
mM	millimolar
min	minute(s)
MLH3	*Mut L Homologue 3*
MND1	*Meiotic Nuclear Divisions 1*
mRNA	messenger ribonucleic acid
MRE11	*Meiotic REcombination 11*
MRN	MND1-RAD50-NBS1 protein complex
MSH4/5	*MutS Homologue 4/5*
MS/MS	tandem mass spectrometry
MW	molecular weight
NaCl	sodium chloride
NBS1	*Nijmegen Break Syndrome 1*
NCBI	National Center of Biotechnology Information
ng	nanogram
Ni-NTA	nickel-nitrilotriacetic acid
nm	nanometre
NMR	nuclear magnetic resonance
NT	nullisomic-tetrasomic
ORF	open reading frame
Os	*Oryza sativa*
P	probability
PBS	phosphate buffered saline
PCR	polymerase chain reaction

Ph1/2	*Pairing homeoelogous 1/2*
PHS1	*Poor Homologous Synapsis 1*
pI	isoelectric potential
PM-LP	pre-meiotic interphase to pachytene pooled stage
PVP	polyvinyl pyrrolidone
PVPP	polyvinyl polypyrrolidone
Q-PCR	quantitative real-time PCR
r	correlation coefficient
R40	40 µg µL^{-1} RNAse in 1× TE
RAD50/51	*RADiation sensitive 50/51*
Rc	*Ricinus communis*
RNA	ribonucleic acid
RNAi	RNA interference
RNAse	ribonuclease
Rr	*Rattus rattus*
rpm	revolutions per minute
s	second(s)
Sb	*Sorghum bicolor*
Sc	*Saccharomyces cerevisiae*
SC	synaptonemal complex
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS - polyacrylamide gel electrophoresis
SF21/21C1	*Sunflower 21/21 variant C1*
SMC	Structural Maintenance of Chromosomes domain
ss	single-stranded

SSC	standard saline citrate
SPO11	*SPOrulation-deficient 11*
TI-TII	telophase I to telophase II pooled stage
Ta	*Triticum aestivum*
Taq	*Thermus aquaticus*
TCA	trichloroacetic acid
T-DNA	transfer DNA
TE	Tris EDTA solution
T-IP	tetrad to immature pollen pooled stage
T_m	melting temperature
Tris	tris(hydroxymethyl)aminomethane
TUC	thiourea-urea-CHAPS
U	units
UV	ultra-violet
V	volts
Vhr	volt hours
Vv	*Vitus vinifera*
v/v	volume per volume
w/v	weight per volume
X-gal	5-bromo-4-chloro-3-indolyl-P-D-galactopyranoside
Y2H	yeast-2-hybrid
Zm	*Zea mays*
ZIP1/ZYP1/ZEP1	*Molecular Zipper 1*