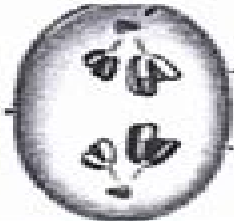


Phases of Meiosis

Name of Phase	Description
1. Prophase I	Homologous chromosomes pair up and form tetrad
2. Anaphase I	Spindle fibers move homologous chromosomes to opposite sides
3. Telophase I	Nuclear membrane reforms, cytoplasm divides, 4 daughter cells formed
4. Metaphase I	Chromosomes line up along equator, not in homologous pairs
5. Prophase II	Crossing-over occurs
6. Anaphase II	Chromatids separate
7. Metaphase II	Homologs line up along equator
8. Telophase II	Cytoplasm divides, 2 daughter cells are formed



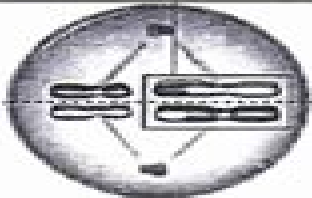
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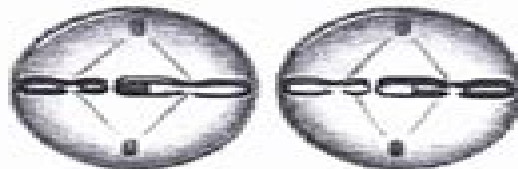
2. Anaphase II



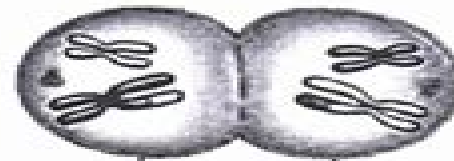
3. Telophase I



4. Metaphase I



5. Metaphase II



6. Prophase



7. Prophase



8. Telophase II

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Mitosis and Meiosis Gary Parker, W. Ann Reynolds, Rex Reynolds, 1968 *An Investigation of Bread Wheat Meiosis Via Proteomics and Gene-targeted Approaches* Kelvin Khoo Han Ping, 2011

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 TaPHS1 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 TaPHS1 show conclusively that TaPHS1 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 TaPHS1 sequence indicating TaPHS1 possesses a novel uncharacterised DNA binding domain Immunolocalisation data from assays conducted using an antibody raised against TaPHS1 demonstrates that TaPHS1 associates with chromatin during early meiosis with the signal persisting beyond chromosome synapsis Furthermore TaPHS1 does not appear to co localise with the asynapsis protein TaASY1 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 TaZYP1 interacts with dsDNA under in vitro conditions while immunolocalisation using an anti TaZYP1 antibody across wild type ph1b and Taasy1 revealed the spatial and temporal localisation pattern of TaZYP1 Taken together these results show that TaZYP1 plays an identical role to its homologues in other species as a SC protein and is affected by reduced levels of TaASY1 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 TaPHS1 and TaZYP1 have been characterised with antibodies raised against both these proteins The characterisation of TaPHS1 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 TaZYP1 in Taasy1 mutant lines has contributed to our understanding of how ASY1 levels can affect chromosome pairing in wheat

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