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Review

Highlights of meiotic genes in *Arabidopsis thaliana*

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Meiosis is a fascinating and complex phenomenon and, despite its central role in sexual plant reproduction, little is known on the molecular mechanisms involved in this process. We review the progress made in recent years using *Arabidopsis thaliana* mutants for isolating meiotic genes. In particular, emphasis is given on the description of mutants affecting either the regular commitment to meiosis, or the mechanisms of synapsis, recombination, and cytokinesis. We believe that the isolation of genes affecting some crucial meiotic events may represent the first step towards the practical use of meiotic genes in plant breeding. The introduction of deviations in the meiotic pathway into sexual crops will have important implications for the exploitation of apomixis and sexual polyploidization.

Key words: *Arabidopsis*, meiosis, mutants, fertility.

INTRODUCTION

Meiosis is a particularly significant process in sexual plant reproduction. The complexity of the events involved, such as DNA replication, chromosome pairing and synaptonemal complex formation, suggests that many tightly regulated genes are involved in this multistep process. Many meiotic genes have been reported in yeast, *Drosophila*, *Caenorhabditis elegans*, humans (Schwarzacher, 2003). As far as plants are concerned, species like maize, tobacco, and pea have provided good models to understand the cytological events and the genetic control of meiosis. However, these species still represent difficult systems for the molecular characterization of the genes involved in meiosis, mainly for the difficulty of using functional genetic approaches.

Recently, *Arabidopsis thaliana* has become the model plant to study meiosis. The availability of a large number of T-DNA and transposon mutated lines represents a powerful tool to isolate meiotic mutants and to identify the corresponding mutated genes. The scientific approach used recentely in *Arabidopsis*, as in yeast and other eucaryotic organisms, is to dissect meiosis into single events through the isolation of mutants specific for each meiotic step.

This review focuses on the progress made in the recent years in the identification of premeiotic and meiotic mutants in *A. thaliana* (Ath) through innovative

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Abbreviations. Ath: *Arabidopsis thaliana*. SC: synaptonemal complex. DSBs: Double Strand Breaks.

Table 1. Pre-meiotic and meiotic mutants in *Arabidopsis thaliana*: their effect on meiosis and homology to genes characterized in other organisms are reported.

Mutant	Gene homology	Effect on meiosis	Authors
Spl/nzz	MADS box transcription factors	Archeosporial cells unable to differentiate into mega- and microsporocytes.	Yang et al., 1999 Schiefthaler et al., 1999
swi1/dyad	none	Megasporogenesis: loss of synapsis and loss of centromere cohesion. Microsporogenesis: release of chromatid arms and centromere cohesion.	Motamayor et al., 2000 Mercier et al., 2001 Agashe et al., 2002
syn1/dif1	*REC8/RAD21*	Irregular chromosome condensation and extensive chromosome fragmentation.	Bai et al., 1999 Bhatt et al., 1999
asy1	*HOP1*	Defect in homologous chromosome synapsis.	Ross et al., 1997 Caryl et al., 2000
dmc1	*RECA*	Absence of crossing over and synapsis.	Klimyuk and Jones, 1997 Couteau et al., 1999
spo11	*SPO11*	Absence of crossing over and synapsis.	Grelon et al., 2001
sds	cyclin-like	Defect in homolog synapsis and bivalent formation.	Azumi et al., 2002
ask1	*SKP1*	Failure in separation of homologous chromosome.	Yang et al., 1999
pollenless3/tmd1	CDC23P, RAD3	Additional division without DNA replication.	Ross et al., 1997 Sanders et al., 1999
tam	nr[a]	Alteration in cell cycle progression. Cytokinesis occurring at the end of the first nuclear division.	Magnard et al., 2001
std/tes	kinesin	Lacking of cytokinesis	Hulskamp et al., 1997 Spielman et al., 1997

[a]= corresponding gene has not been cloned.

approaches. We believe that in the near future this will contribute to a full understanding of meiosis in plants and will open new perspectives for the biotechnological manipulation of plant reproduction.

PREMEIOTIC MUTANTS

The discovery of premeiotic mutants and the related genes could provide an insight into the mechanisms involved in the early step of sporogenesis for meiocyte differentiation and meiosis commitment. This is very important in that, despite decades of research in plant reproduction, there is only limited knowledge of how sporocytes differentiate and how meiosis is initiated and regulated in plants.

One premeiotic mutant defective in the specification of reproductive cell type has been discovered in Ath by Yang et al. (1999) and Schiefthaler et al. (1999). They identified *sporocyteless*/*nozzle* (*spl*/*nzz*) mutant characterized by archesporial cells unable to differentiate into both megasporocytes and microsporocytes. Molecular cloning of the *SPL* gene by both research teams provided evidence that it encodes a novel nuclear protein related to MADS box transcription factors. The sequence of *SPL* suggests that the gene product functions as a transcriptional regulator essential for sporocyte development.

MEIOTIC MUTANTS

Several Ath mutants defective in chromosome pairing and segregation, and in cytokinesis have been identified (Table 1). Their phenotypes are always associated with a specific stage of the meiotic cycle (Figure 1), and in most

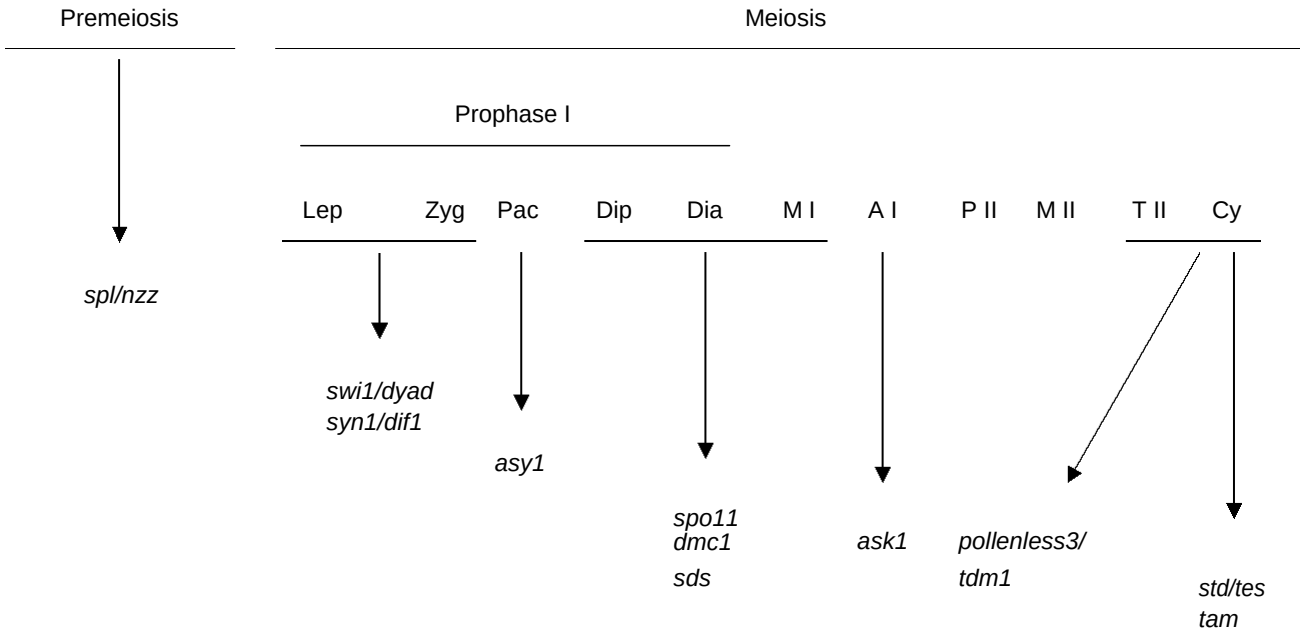

Figure 1. Time of action of Ath meiotic mutants discussed in this paper.

cases either microsporogenesis or macrosporogenesis are affected. Given the difference in form and function between male and female gametes, it is expected that the two pathways of reproductive developments are under the control of overlapping but distinct genetic programmes. From genetic evidence it is known that different genes are involved in male (Hulskamp et al., 1997; Yang et al., 1999) and female meiosis (Byzova et al., 1999; Siddiqi et al., 2000), and only few of them control both processes (Couteau et al., 1999; Grelon et al., 2001).

Prophase I

Most of Ath meiotic mutants recently isolated are defective in prophase I, a crucial meiotic stage. Indeed, synapsis, synaptonemal complex (SC), crossing-over, and chiasmata formation that lead to recombination events between homologous chromosomes and subsequently to a proper segregation, occur during this stage. At least two Ath mutants are affected in sister chromatid cohesion; *switch1*/*dyad* and *syn1*/*dif1*.

Three alleles have been isolated with mutation in the *SWI1*/*DYAD* locus: *switch1-1* (Motamayor et al., 2000), *switch1-2* (Mercier et al., 2001) and *dyad* (Agashe et al., 2002). All the three alleles cause similar defects in the female meiosis pathway. By contrast, only *swi1-2* causes aberrations in microsporogenesis. The majority of megaspore mother cells (MMCs) perform a mitotic- like division instead of meiosis I, exiting meiosis with an unreduced chromosome number. However, the MMCs are not committed to a mitotic programme but to a meiotic cycle, and the two daughter cells in the dyad perform a second cell cycle, which is either a novel mitosis-like division or an aberrant one, with unequal chromosome segregation. At the end, degenerated cells are observed instead of the embryo sac. During microsporogenesis, *swi1-2* mutant allele causes male sterility with severe defects in chromosome behaviour. The *SWI1* gene has been cloned and the putative protein does not show any similarity to known proteins. It is proposed that SWI1 protein promotes the establishment of sister chromatid cohesion, but it is not responsible for its manteinance. Mercier et al. (2003) reported that SWI1 is also required for the formation of SC, and for recombination. *Syn1*/*dif1* causes both male and female sterility (Bhatt et al., 1999; Bai et al., 1999). During early leptotene and zygotene of microsporogenesis, it causes irregular chromosome condensation and extensive chromosome fragmentation. Cytological analysis showed that sister chromatid cohesion is lacking and homologous pairing does not occur (Cai et al., 2003). The *SYN1*/*DIF1* gene has been cloned, and it shows homology to *RAD21* /*REC8* cohesin family. Also the asynaptic Ath mutant (*asy1*) exhibits defects in homologous chromosome synapsis (Ross et al., 1997; Caryl et al., 2000). This mutation leads to reduced male and female fertility. *ASY1* gene encodes a homologous of yeast HOP1 protein, which is essential for SC assembly and normal synapsis.

In plants, few molecular data are available on genes involved in meiotic recombination. Functional data come from the analysis of the Ath *dmc1* and *spo11* mutants, that show poor bivalent formation during male and female meiosis (Couteau et al., 1999; Grelon et al., 2001). The

absence of association between homologous chromosomes can be explained by an absence of crossing over. In budding yeast, where meiotic recombination is initiated by DNA Double Strand Breaks (DSBs) (Sun et al., 1989; Cao et al., 1990) and follows the DSB repair model (Szostak et al., 1983; Sun et al., 1991), there is evidence that SPO11 protein is the endonuclease responsible for the induction of DSBs (Keeney et al., 1997). There is also indication that DMC1 is involved in the first step of DSB repair (Bishop et al., 1992). So far, the generation of DSBs in micro- and macrosporocytes has not been demonstrated in plant meiosis. However, the presence in Ath genome of yeast homologous *SPO11* and *DMC1* as well as of other genes involved in meiotic recombination (Klimyuk and Jones 1997; Doutriaux et al., 1998; Hartung and Puchta, 2000; Gallego and White, 2001) strongly suggests that the essential features of meiotic recombination in yeast could be conserved in plants. In addition, the Ath *spo11* and dmc1 mutant phenotypes have contributed to better understand the link between synapsis and meiotic recombination in plants. Historically, it has been proposed that homologous chromosomes synapse before they recombine. However, recent data provided evidence that in yeast, synapsis is not required for meiotic recombination and that it may depend on DSB processing. In *zip1* and *red1* strains, for example, despite the absence of SC, recombination occurs (Rockmill and Roeder, 1990; Sym et al., 1993; 1994). In addition, none of the many yeast mutants characterized so far has been found to make SC in the absence of DSBs. Plants may have a similar control mechanism, and thus synapsis is affected when *SPO11* and *DMC1* functions are absent. Recently, another ath mutant - *solo dancers* (*sds*) isolated by Azumi et al. (2002) - showed defects similar to *spo11* and *dmc1* in meiotic recombination, SC and bivalent formation. It could provide a new and important entry point to understand the regulation of homolog synapsis and recombination in plant meiosis.

Anaphase I

In other Ath mutants, the earlier meiotic events proceed normally without any visible anomaly in synapsis and recombination, but the subsequent stages are affected. Yang et al. (1999) reported on the male sterile *ask1* mutant that exhibits a failure in separation of homologous chromosomes during anaphase I. The *ASK1* gene has homology to the yeast *SKP1*, essential to regulate the mitotic cell cycle by targeting specific proteins for ubiquitin-mediated proteolysis. Until now, it was unknown that *SKP1* is also required for meiosis. *ASK1* may play a role in the control of homologous separation by degrading/removing a protein that is required for the homologue association in prophase I.

Tetrad stage

Mutations have been detected also in the last stage of meiosis. Ath *pollenless3*/*tmd1* male-sterile mutant produces microspores in excess of four during the tetrad stage, usually 8 +/-1 or +/- 2 (Ross et al., 1997; Sanders et al., 1999). Meiosis I and II proceed normally and the production of the 'octads', with unbalanced chromosome number, is caused by an additional division without DNA replication. It has been found that the *POLLENLESS3* gene encodes a protein with limited homology to CDC23P and RAD3 proteins, involved in cell cycle of *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, respectively.

Cytokinesis

Meiosis ends with cytokinesis, in which the four haploid nuclei formed after the two meiotic divisions are partitioned by a cell wall into four spores. *Stud* (*std*)/*tetraspore* (*tes*) mutant show normal meiotic divisions but it lacks male meiosis cytokinesis (Hulskamp et al., 1997; Spielman et al., 1997). This mutation leads to pollen of bigger size than wild type due to tetranucleate microspores. In *std*/*tes* mutant, the post-meiotic development of micropores proceed relatively normally. In fact, the nuclei are capable of independently undergoing the complete mitotic cell division leading to pollen grains with variable number of sperm nuclei. Some microspore nuclei can fuse before the first mitotic division, leading to polyploid sperm nuclei instead of the normal haploid nuclei.

Defects in male cytokinesis were also observed in the tardy asynchronous meiosis (*tam*) mutant. However, in this case the abnormality seems a consequence of alteration in cell cycle progression during meiosis (Magnard et al., 2001). In *tam*, cytokinesis occurs at the end of the first nuclear division, giving a dyad with two daughter cells. In this mutant, Ath cytokinesis (that is normally simultaneous), mimics a successive type cytokinesis, typical of monocots. *TAM* gene maps to chromosome 1, but it has not been isolated yet. It is proposed that TAM protein positively regulates the cell cycle progression, perhaps promoting the G2/M transition, and that TAM has a role in coupling the normal pace of cell cycle progression with the synchrony of cell division during male meiosis.

CONCLUSIONS

Meiosis is a fascinating phenomenon with intriguing biotechnological potential. Given the high frequency with which new meiotic mutants are being isolated in Ath, this plant probably is the best model system for elucidating the molecular mechanisms of meiosis. Moreover,

progress in meiotic studies in Ath will stimulate research on meiotic genes in cultivated species. The possibility to isolate genes altering the normal meiotic pathway and to engineer them in crops has important implications for plant biotechnology and plant breeding. Indeed, meiotic mutants can be used to exploit the advantages of apomixis in F_1 hybrid production, in sexual polyploidization crossing schemes to generate polyploid genotypes, and in promoting genetic recombination in hybrids between genetically distant parents.

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