Mechanisms of unreduced gamete formation in *Arabidopsis thaliana*

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Cover: Anther of *Arabidopsis thaliana* at meiosis stage
(photo: JY. Jun Yi)

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Abstract

Polyploidy is a widespread phenomenon in plants, which is commonly believed to arise through the production of unreduced (2n) gametes due to meiotic failure. Despite the importance of unreduced gamete formation for plant polyploidization, the mechanisms leading to their formation are not well understood. Previous work of our group indicated that JASON (JAS) regulates chromosome segregation through affecting the position of organelles, which behave as a physical barrier between the two spindles.

In my thesis, I aimed at understanding how JAS affects positioning of the organelles during meiosis in *Arabidopsis thaliana*. I revealed that during meiosis, JAS was co-localized with markers for the Golgi and the plasma membrane in the organelle band at metaphase II, indicating that a subset of the Golgi apparatus and endomembrane vesicles are components of the organelle band. Maintaining the organelle band relies on the function of the JAS protein.

JAS can encode two versions of proteins, a long version including an N-terminal mitochondrial targeting signal (*JAS.1*) and a short version lacking this sequence (*JAS.2*). To investigate which version of JAS is functional during meiosis, I tested both versions for their ability to complement the *jas* mutant. I found that only *JAS.2* could complement the *jas* mutant phenotype. Consistent with the genetic data, localization of JAS.2-GFP under control of a constitutive promoter was the same as JAS-GFP expressed under control of the native promoter that also contained the N-terminal extension, suggesting that most likely JAS.2 is the protein translated in wild type during meiosis.

To further characterize the mechanism of JAS function, we performed a suppressor screen with the aim to find mutants that form reduced gametes in the presence of the *jas* mutation. In this screen, *telamon* (*tel*) was isolated as a strong suppressor of *jas* that can produce many haploid pollen in the *jas* background. While the organelle band was not restored in *jas;tel* in meiosis II, meiotic cells were enlarged in the *jas;tel* mutant. Importantly, tetraploidization of *jas* suppressed the *jas* phenotype and led to the production of reduced gametes, supporting the idea that increase of meiocyte size can bypass the requirement of the organelle band. Lastly, I discovered that *Eutrema salsugineum* that has smaller meiocytes than *Arabidopsis thaliana* was more sensitive to cold stress and produced increased numbers of diploid pollen. Combined, these results strongly support the idea that meiocyte size impacts on chromosome segregation in meiosis II and suggests that the organelle band is mainly required in species forming small meiocytes.

Altogether, this thesis provides novel insights into the mechanism leading to unreduced gamete formation and reveals a new and exciting mechanism that may have facilitated the decrease of pollen size.

Keywords: Arabidopsis, meiosis, unreduced gametes, polyploid

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Mekanismer för bildning av oreducerade gameter i *Arabidopsis thaliana*

Vetenskaplig sammanfattning

Polyploiditet är ett utbrett fenomen bland växter, som tros uppstå genom produktion av oreducerade (2n-) gameter vid misslyckad meios. Trots betydelsen av oreducerade gameter för polyploiditet, är kunskapen om de grundläggande mekanismerna fortfarande bristfälliga. Tidigare forskning i vår grupp indikerar att JASON (JAS), reglerar uppdelning av kromosomer genom att påverka organellernas position, vilka utgör en fysisk barriär mellan kärnspolarna.

Målet med min avhandling är att förstå hur JAS påverkar organellernas position under meiosen hos *Arabidopsis thaliana.* Jag fann att under meiosen var JAS sammankopplad med markörer för Golgi-apparaten och plasma membranet i ett organellaggregat under metafas II, vilket indikerar att en del av dess vesiklar utgör komponenter i organellaggregatet. Upprätthållandet av detta organellaggregat är beroende av JAS-proteinets funktion.

JAS kan koda för två typer av proteiner, en lång version som inkluderar en N-terminal sekvens med en signal för transport till mitokondrien (*JAS.1*) och en kort version utan denna sekvens (*JAS.2*). För att undersöka vilken version som är aktiv vid meios, testade jag bådas förmåga att komplementera *jas*-mutanten. Jag fann att endast *JAS.2* kunde komplementera *jas*-mutantens fenotyp. I överensstämmelse med genetiska data, visade lokalisering av JAS.2-GFP under kontroll av en konstitutiv promotor samma uttryck som JAS-GFP under kontroll av dess naturliga promotor som också innehåller N-terminalsekvensen, vilket tyder på att det troligtvis är JAS.2-proteinet som translateras vid meiosen.

För att vidare karaktärisera mekanismen för JAS funktion utförde vi en suppressor-screen med målet att hitta mutanter som bildar reducerade gameter i närvaro av *jas*-mutationen. I denna screen identifierades *telamon* (*tel*) som en stark undertryckare av *jas* som kan producera många haploida pollen i j*as*-bakrund. De meiotiska cellerna var förstorade i *jas:tel*-mutanten, men organellaggregatet var inte återställt. Tetraploidisering av *jas* undertryckte dess fenotyp och ledde till produktion av reducerade gameter, vilket stödjer idén att en ökning av storleken på meiotiska celler kan kringgå behovet av ett organellaggregat. Slutligen upptäckte jag att *Eutrema salsugineum* som har mindre meiotiska celler än *Arabidopsis thaliana*, är mer känslig för köldstress och producerar ett ökat antal diploida pollen. Dessa resultat stödjer idén att storleken på de meiotiska cellerna påverkar separationen av kromosomer i meios II och tyder på att organellaggregatet huvudsakligen behövs i arter med små meiotiska celler.

Sammanfattningsvis ger denna avhandling ny insikt om oreducerad gametbildning och uppenbarar en ny och spännande mekanism som kan ha främjat minskad pollenstorlek.

Nyckelord: Arabidopsis, meios, odödade gameter, polyploid

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Dedication

To my beloved family, my sons Ruixuan, Yixuan and my husband Hua.

We should consider every day lost on which we have not danced at least once.

Friedrich Wilhelm Nietzsche

每一个不曾迎风起舞的日子，都是对生命的辜负.

尼采

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Brownfield L. *¤, Yi J.¤, Jiang H., Minina E.A., Twell D., Köhler C. (2015). Organelles maintain spindle position in plant meiosis. *Nat Commun*. doi: 10.1038/ncomms7492.

II Cabout S., Leask M.P., Varghese S., Yi J., Peters B., Liu C.L., Köhler C., Brownfield L. (2017) The Meiotic Regulator JASON Utilizes Alternative Translation Initiation Sites to Produce Differentially Localized Forms. *J. Exp. Bot*. 68:4205-4217.

III Yi J., Kradolfer D., Köhler C.*. Meiocyte size is a determining factor for unreduced gamete formation in *Arabidopsis thaliana*. (submitted/manuscript)

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* Corresponding author. ¤ Equal contribution

The contribution of Jun Yi to the papers included in this thesis was as follows:

I Performed and analysed the data

II Performed and analysed the data

III Designed, performed, analysed the data, and wrote the paper

List of figures

Abbreviations

arabidopsis formin14	*afh14*
cell division cycle 45	*Cdc45*
cell plate	*CP*
first division restitution	FDR
gibberellic acid	GA
go-ichi-ni-san	GINS
JASON	*JAS*
minichromosome maintenance	MCM
omission of second division1	osd1
phragmoplast	PHP
PROLIFERA	*PRL*
radial microtubule arrays	RMAs
second division restitution	SDR
tardy asynchronous meiosis	*tam*
telamon	*tel*
the early endosome	EE
the Endoplasmic reticulum	ER
the Golgi apparatus	Golgi
the trans-Golgi network	TGN
transposable elements	TE
ubiquitin	UBQ
unreduced	2n

1 Introduction

1.1 Meiosis and gametophyte development

1.1.1 General introduction of plant meiosis

The plant life cycle is characterized by an alteration between two multicellular generations: an asexual sporophytic generation and a sexual gametophytic generation (Qin *et al.*, 2014). Meiosis is a specialized reductive cell division that initiates the formation of the sexual gametophytic generation. Meiosis involves one round of DNA replication followed by two rounds of chromosome divisions, termed meiosis I and meiosis II. This results in the formation of cells carrying half the number of the parental chromosomes (Ma, 2006; Zamariola *et al.*, 2014).

Meiosis I and meiosis II are further divided into four substages according to different cytological characters of the chromosomes: prophase, metaphase, anaphase, and telophase. Meiosis is a critical process and a sequence of coordinated steps must occur in these two phases to ensure successful chromosome segregation. Homologous chromosome pairing, synapsis, and recombination takes place in prophase I, which is a complex and long process and divided again into substages termed leptotene, zygotene, pachytene, diplotene, and diakinesis (Dawe, 1998; Zickler and Kleckner, 1998; Armstrong and Jones, 2003). Chromosomes begin to condense in the leptotene stage (Figure 1 A). When homologous chromosomes start recognizing each other and become

partially synapsed, they enter into the zygotene substage (Figure 1 B). In pachytene, homologs are fully synapsed and connected by a protein-RNA synaptonemal complex (Roeder, 1990) (Figure 1 C). During the pachytene stage, chromosomal crossover occurs, which not only establishes chromosome connections that are required for the following divisions, but also facilitates the exchange of genetic information and thus increases the genetic diversity in a population (Ma, 2006). These points of crossover are called chiasmata that establish connections between homologs and forming bivalent. This kind of structure not only ensures suitable positioning of spindle and chromosomes but also facilitates the segregation of homologs in balance in anaphase I(Zamariola *et al.*, 2014). Most plant species form only one to four crossovers per bivalent, independent of chromosome size (Crismani and Mercier, 2012). At least one crossover should be presented in bivalents to provide the connection between homologs, retain proper chromosome orientation and poleward movement for correct chromosome segregation in anaphase I (Dawe, 1998; Sablowski and Carnier Dornelas, 2014). After pachytene, the synaptonemal complex begins to be degraded. Homologs partially separate at the diplotene stage except at the chiasmata (Figure 1 D). After diplotene, chromosomes further condense and form bivalent pairs at the diakinesis stage (Figure 1 E). After prophase I, the bivalents begin to move to the center of the cell by being attached and dragged by meiotic spindle microtubules. This requires that the sister kinetochores from each homolog must attach to microtubules emanating from the same spindle pole (Zamariola *et al.*, 2014). At metaphase I, homologous chromosomes align at the equation plane (Figure 1 F). Following the degradation of cohesion along chromosomal arms, homologs separate and move to opposite poles of the spindle at anaphase I (Figure 1 G). Telophase I is characterized by chromosome decondensation (Figure 1 H). After the first round of nuclear division, meiosis I, homologous chromosomes are segregated and ploidy is reduced by half. Hence, meiosis I is also called a reductional division (Ross *et al.*, 1996; Armstrong and Jones, 2003; Ma, 2006).

In meiosis II, the two groups of chromosomes re-condense at prophase II (Ross *et al.*, 1996). At metaphase II, the chromosomes align prior to the second division (Fig. 1J). The sister kinetochores become bi-oriented and attach with microtubules in two directions. With the cohesion released at the sister centromeres at anaphase II, chromosomes segregate to four directions and form four clusters of new chromosomes (Figure 1 K). Chromosomes decondense at telophase II and four haploid nuclei are formed (Figure 1 L). After cytokinesis, four spores in a tetrad are formed (Figure 1 M). Meiosis II results in the segregation of sister chromosomes, which is similar to mitosis and also termed equational division (Ross *et al.*, 1996; Armstrong and Jones, 2003; Ma, 2006).

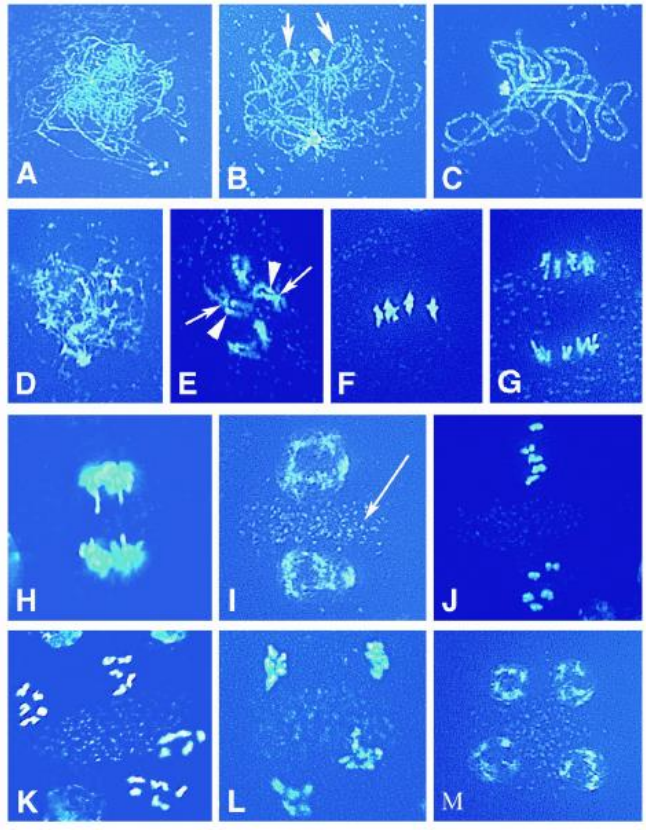

Figure 1. Arabidopsis male meiosis (Ma, 2006). A, leptotene. B, zygotene; arrows indicate regions of homologs pairing. C, pachytene. D, diplotene. E, diakinesis; arrows point to chiasmata and arrowheads indicate the centromere. F, metaphase I. G, anaphase I. H, telophase I. I, prophase II; the arrow points to

the organelle band. J, metaphase II. K, anaphase II. L, telophase II, M. Four newly formed nuclei.

1.1.2 Mechanisms ensuring faithful chromosome segregation during meiosis

The orientation of the spindle and the organization of microtubules are essential for faithful chromosome segregation. Microtubules are highly dynamic polar polymers formed of noncovalently bound α and β tubulin heterodimers that rapidly polymerize and depolymerize (Nogales, 2000). In plants, microtubules assemble in a cell-cycle particular way. Different forms of arrays are observed: radial arrays when around nuclear, cortical arrays at interphase, then forming preprophase bands, spindles, phragmoplasts (Wasteneys, 2002; De Storme and Geelen, 2013). The minus-end of microtubules usually anchors at the spindle pole and the plus-end attaches to the chromosomes (Wittmann *et al.*, 2001). The correct segregation of the chromosomes depends on the bipolar structure of the spindle and microtubule-based chromosome movement. In the majority of dicotyledonous plants that undergo simultaneous male meiotic cytokinesis, the orientation of the spindle position is essential for faithful chromosome segregation in meiosis II (Brownfield and Kohler, 2011). In such kind of cytokinesis, no cell plate is formed after the first meiotic division, which results in two sets of chromosomes which have already separated still in one common cytoplasm, contrasting the mode of successive cytokinesis where a cell plate is already formed after the first division. To ensure faithful chromosome segregation of the two chromosome sets, the two spindles have to be perpendicularly oriented. Any alteration of the spindle orientation, such as parallel, tripolar or fused spindles, causes chromosomes to reunite after division, leading to the formation of unreduced gametes (Conicella *et al.*, 2003; De Storme and Geelen, 2013). This forms the major path to polyploidy plants and will be elaborated in more detail in chapter 2.

1.1.3 Difference between male and female meiosis and postmeiotic events

Male and female meiosis takes place in anthers and ovules, respectively. The different structure of male and female organs causes a substantially higher number of cells undergoing meiosis in male than female organs. In *Arabidopsis*, male meiosis usually occurs at anther stage 6 and lasts to stage 9 of flower development (Smyth *et al.*, 1990). Each flower contains six anthers and each anther includes four lobes. There are around thirty meiocytes in one lobe; hence around seven hundred meiocytes undergo male meiosis in each flower. In contrast, there are only around fifty ovules in each of flower. Since only one cell in each ovule undergoes meiosis, there are only around fifty cells per flower undergoing female meiosis (Ma, 2006). This, together with the fact that several cell layers surround female meiocytes, makes research on female meiosis more challenging than on male meiosis. Male and female meiosis in plants, like in other organisms, share the same stages. However, in most angiosperms the product of female meiosis is a linear array of four megaspores, contrasting the tetrahedral arrangement of four haploid microspores as product of male meiosis (Brownfield and Kohler, 2011). Also the products of male and female meiosis have different fates; while all spores survive after male meiosis, in most angiosperms only one megaspore survives after female meiosis (Mccormick, 1993; Yadegari and Drews, 2004). Following degeneration of the other three megaspores, the only functional megaspore undergoes three rounds of mitosis and develops into a seven-celled mature female gametophyte, containing two gametes, the egg cell and the central cell (Yang *et al.*, 2010). In contrast, all four male meiotic products enter into a mitotic division generating a vegetative and a generative cell. In ~70% of plant families, the mature pollen grain consists of these two cells and the second mitotic division of the generative cell occurs during pollen tube germination, giving rise to two sperm cells. In other plant families, including the Brassicaceae, the mature pollen grain contains already two sperms cells. The germinating vegetative cell forms the pollen tube that delivers the sperm cells to the female gametophyte (Mccormick, 1993). After

pollination, male and female gametes fuse in a process termed double fertilization, then the formation of endosperm and embryo start in the later progress.

1.2 Polyploidization

1.2.1 Polyploidization and unreduced gamete formation

Polyploidy, the presence of three or more complete sets of genomes within a cell, is a widespread phenomenon in many eukaryotic taxa, such as yeast, insects, fish, amphibians, and reptiles (Ramsey and Schemske, 1998). In plants, it is estimated that probably all angiosperm species have undergone one or more ancient genome-wide duplication events (Cui *et al.*, 2006). Thus, polyploidy is highly prevalent in angiosperms. Much research in the past has focused on novel characteristics of polyploids (Adams and Wendel, 2005a, b; Leitch and Leitch, 2008). Genotypic plasticity of polyploids has been suggested to promote adaptation and speciation (Alix *et al.*, 2017). Importantly, polyploids are of immense commercial value. Many important crop species including coffee, potato, tobacco, wheat, banana, and many fruits are polyploids (Bretagnolle and Thompson, 1995; Udall and Wendel, 2006).

Polyploids can arise as a consequence of hybridization of different species followed by somatic doubling in meristematic tissue, giving rise to allopolyploids. Autopolyploids, which have multiple sets of chromosomes derived from the same species, most frequently arise as a consequence of meiotic defects, giving rise to unreduced gametes that contain the complete somatic chromosome number (Brownfield and Kohler, 2011). Disruption of meiosis usually results in imbalanced gametes, causing premature spore abortion and sterility. Nevertheless, under some conditions meiotic failure leads to the production of viable unreduced (2n) gametes (Bretagnolle and Thompson, 1995; Ramanna and Jacobsen, 2003). After fertilization, the union of these viable unreduced gametes with reduced gametes will lead to the formation of triploids,

which can form swarms of euploid and aneuploid gametes that after fusion can give rise to stable tetraploids (Henry *et al.*, 2005). This mechanism has been termed triploid bridge and is considered the main route to the formation of autopolyploids (Husband, 2004; Mason and Pires, 2015).

Until now, the mechanisms leading to the formation of unreduced gametes under natural conditions and their impact on the formation of polyploids are not well understood. Since polyploidy is considered a major speciation mechanism (Landis *et al.*, 2018), understanding the mechanisms leading to unreduced gametes is of vital importance.

1.2.2 Types of mutants that generate viable unreduced gametes in plants

Unreduced gametes can be the consequence of male or female meiotic defects. Three different types of meiotic mutants can produce viable unreduced gametes. To the first class of mutants belong the *Arabidopsis thaliana parallel spindle1* (*atps1*), *jason* (*jas*) and *arabidopsis formin14* (*afh14*) (d'Erfurth *et al.*, 2008; Erilova *et al.*, 2009; Li *et al.*, 2010; De Storme and Geelen, 2011; Brownfield *et al.*, 2015). Meiosis I is normal in these mutants. However, while in wild type metaphase II chromosomes are oriented in a perpendicular way, in these mutants the spindle polarity and orientation is disordered. The orientation of the metaphase II plates in these mutants is frequently parallel. Consequently, chromosomes that have already been separated in meiosis I come close again at anaphase II, generating two diploid cells that contain non-sister chromosomes. This type of meiotic restitution is termed first division restitution (FDR). To the second class of mutants belong the *tardy asynchronous meiosis (tam)* and *omission of second division1 (osd1)* mutants (Magnard *et al.*, 2001; Wang *et al.*, 2004; d'Erfurth *et al.*, 2009; d'Erfurth *et al.*, 2010). Meiosis I is also normal in these two mutants, but meiosis II does not occur, leading to dyads of diploid cells containing sister chromosomes. This type of meiotic defect is referred to as second division restitution (SDR). To the third class of mutants belongs the *tes*

mutant, in which meiosis occurs normally, but cytokinesis is disturbed (Hulskamp *et al.*, 1997; Spielman *et al.*, 1997; Yang *et al.*, 2003). The *tes* mutant contains multiple nuclei in one cell, some of which can fuse during development.

1.2.3 Environmental stress contributes to unreduced gamete formation by affecting meiotic cell division

Besides genetic factors that may contribute to the formation of unreduced gametes in natural populations, the probably more likely path to 2n gametes is meiotic failure as a consequence of environmental stress (De Storme and Geelen, 2014). Meiosis is highly sensitive to adverse environmental conditions, especially male gamete development. In particular heat and cold stress, but also treatment with the plant hormone gibberellic acid (GA) have been reported to cause meiotic defects leading to the formation of unreduced gametes (Brownfield and Kohler, 2011; De Storme *et al.*, 2012; Liu *et al.*, 2017; Wang *et al.*, 2017).

Specifically, in *Rosa hybrida*, short periods of heat stress cause alterations of spindle orientation at metaphase II (Pecrix *et al.*, 2011). Instead of the normal perpendicular orientation the spindles, parallel and tripolar spindles are formed, causing chromosome segregation failure at meiosis I and formation of unreduced diploid gametes (Pecrix *et al.*, 2011). Similarly, also cold stress leads to unreduced gamete formation in many species (Ramsey and Schemske, 1998). In *Arabidopsis*, a short period of cold stress results in the formation of dyads and triads by a combination of FDR and SDR mechanisms (de Storme et al., 2012). Cytological examination revealed that the deposition and maintenance of the organelle band and internuclear radial microtubule arrays (RMAs) at meiosis II is disrupted upon cold stress (de Storme et al., 2012), leading to defects in post-meiotic cell plate formation. This results in abnormal meiotic cytokinesis and unreduced gamete formation (De Storme *et al.*, 2012). Taken together, developing meiocytes are highly sensitive to environmental stress.

1.2.4 Possible function of the organelle band in male meiosis

Organelle aggregation has been observed to occur during plant male meiosis in different plant species (Bednara *et al.*, 1986; Tchorzewska *et al.*, 1996; Furness *et al.*, 2002; Wang *et al.*, 2010). A distinct organelle organization in the cytoplasm has been observed during metaphase I. Usually, organelles move together into a cluster around the spindle and chromosomes at this stage (Wolniak, 1976). At the end of meiosis I, organelles aggregate at the equator of meiocytes and form an organelle band between two groups of chromosomes (Ma, 2006). This organelle band can be observed at metaphase II. Following the separation of the sister chromatids at telophase II, organelles aggregate between all four haploid nuclei (Brown and Lemmon, 1988; De Storme *et al.*, 2012). Even though organelle aggregation has been frequently observed, little is known about its formation and function. It has been proposed that the organelle band in meiosis II allows equal organelle segregation into the four haploid daughter cells at end of anaphase II, or alternatively, to be essential for keeping the two spindles physically separated in the second meiotic division (Koscinska-Pajak and Bednara, 2003). It was furthermore proposed that the deposition and maintenance of the organelle band at metaphase II is critical for post-meiotic cell plate formation and cell wall establishment, which is essential for meiotic cytokinesis (Otegui and Staehelin, 2004; De Storme *et al.*, 2012). Since proper spindle orientation and cytokinesis are related to normal chromosome segregation, organelle organization in meiosis is likely an important factor impacting on unreduced gamete formation.

1.3 Plant cytokinesis and endomembrane trafficking

1.3.1 Plant somatic cytokinesis

Cytokinesis is the final step of cell division, which takes place after chromosome segregation, leading to the separation of the two daughter nuclei by a new cell wall. Although cell division is common to all eukaryotes, the way it is achieved

differs. In animals, cytokinesis proceeds from the periphery to the center, with the help of a contractile ring (Glotzer, 2001). In contrast, cytokinesis of plant somatic cells is orchestrated by a specialized structure, the phragmoplast that is a cytoskeletal array containing microtubules and microfilaments (Lipka *et al.*, 2015). A large number of membrane vesicles are transported along the phragmoplast by the endomembrane system (Figure 2). When these vesicles arrive at the center of the dividing cell, they fuse with each other to form a new membrane compartment, the cell plate. Afterwards, the phragmoplast grows towards the periphery of the cell, recruiting later-arriving vesicles to the margin of growing cell plate. Eventually, the growing cell plate fuses with the pre-existing plasma membrane to separate the forming daughter cells (Jurgens *et al.*, 2015).

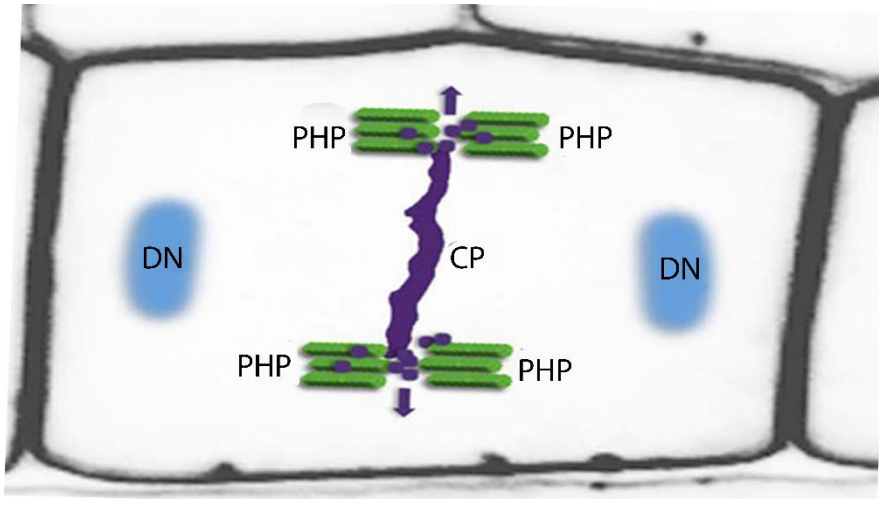

Figure 2. Dynamics of membrane fusion during plant cytokinesis (Modified from Jurgens et al., 2015). Membrane vesicles are delivered along phragmoplasts (PHPs) to the plane of cell division, and then the vesicles fuse with each other to form the cell plate (CP). This process starts at the center of the plane of cell division and expands to the CP. DN, daughter nuclei.

1.3.2 Vesicle trafficking in plant somatic cytokinesis

Electron microscopic studies revealed the dynamic change of vesicle accumulation during the cell plate formation (Otegui *et al.*, 2001; Segui-Simarro *et al.*, 2004). For a long time it was commonly accepted that vesicles required for the formation of the cell plate are mainly delivered by the secretory pathway, which includes the Endoplasmic reticulum (ER), the Golgi apparatus (Golgi), and the trans-Golgi network (TGN) (Figure 3). The ER is a place that synthesizes cell wall structural proteins, enzymes, and lipids; moreover, the ER is also the port of entry into the secretory pathway (Brandizzi and Barlowe, 2013; Stefano *et al.*, 2014; Kim and Brandizzi, 2016). After passing the quality control, newly synthesized proteins are transported to the Golgi, which is defined as a hub for protein secretion (Dupree and Sherrier, 1998; Faso *et al.*, 2009). More importantly, the Golgi is also a cellular factory for synthesizing cell wall materials and protein modifications such as glycosylation (Oikawa *et al.*, 2013). The TGN is a membrane compartment on the trans-side of the Golgi apparatus, which is responsible for the sorting and packaging of newly synthesized material from the Golgi and the transport to the plane of cell division (Roth *et al.*, 1985; Kang *et al.*, 2011; Rosquete *et al.*, 2018).

It was then realized that material from the plasma membrane and the cell wall is also delivered by endocytosis through the TGN and early endosome (EE), to the plane of cell division (Figure 3) (Dhonukshe *et al.*, 2006). The *in vivo* endocytic marker FM4-64 and multiple plasma membrane or cell wall resident proteins are localized at the forming cell plate (Dhonukshe *et al.*, 2006). However, specific disruption of endocytosis by the inhibitor Wortmannin did not impair cell plate formation, which differs from the effect of Concanamycin A and Brefeldin A that inhibit traffic at the TGN and suppress cell plate formation (Reichardt *et al.*, 2007). Hence, material delivered by both, endocytosis and the secretory pathway, are required for cell plate formation, but newly synthesized material is more crucial for cell plate formation.

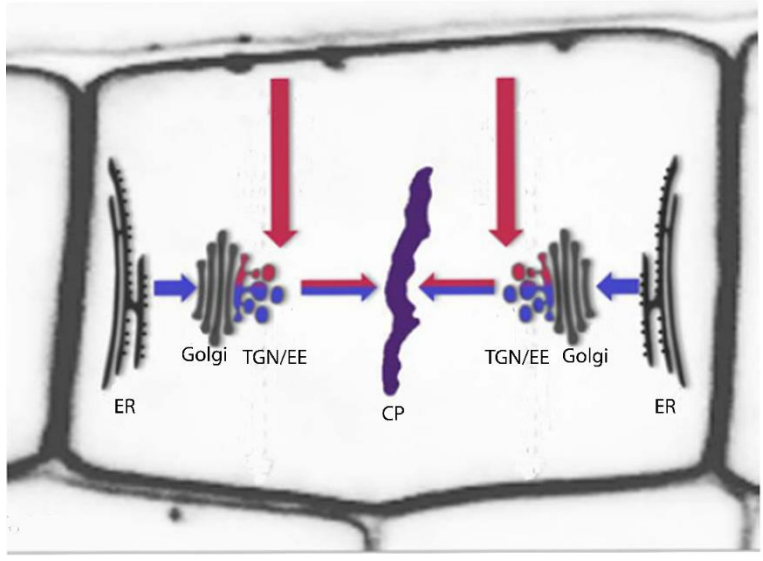

Figure 3. Endomembrane trafficking during plant cytokinesis (Modified from Jurgens et al., 2015). Both newly synthesized (blue arrows) and endocytosed (red arrows) proteins are delivered via the TGN/EE to the plane of cell division.

1.3.3 Cytokinesis in plant male meiosis

Cytokinesis in plant male meiosis can occur either successively or simultaneously. Successive cytokinesis usually occurs in monocots, where meiotic cell division is directly followed by cytokinesis. Hence, the process of male meiosis in most monocots can be considered as two rounds of mitotic cell divisions (Shamina *et al.*, 2007; De Storme and Geelen, 2013). Like in somatic cytokinesis, the phragmoplast is formed after meiosis I, and the microtubules guide vesicle deposition to generate the cell plate (Shamina *et al.*, 2007). In the end, a transient dyad is formed after first cell division and a tetrad is generated after the second cell division.

Simultaneous cytokinesis occurs in most dicots and is characterized by the suppression of cytokinesis after the first meiotic division and simultaneous completion of cytokinesis after the second meiotic division is finalized (De Storme and Geelen, 2013). The formation of the cell plate is executed by radial

microtubule arrays (RMAs), which contain microtubules and actin filaments that emerge from outside of nuclei surface at telophase II. Therefore, the position of the newly generated daughter nuclei determined the location of the cell plate at the end of meiosis II. Subsequently, the new cell walls are formed between daughter nuclei. A MAPK signalling pathway mediates RMA and cell plate formation in Arabidopsis, which includes AtNACK1/2, ANP1/2/3, MPK4, and MKK6 (Petersen *et al.*, 2000; Krysan *et al.*, 2002; Strompen *et al.*, 2002; Takahashi *et al.*, 2010; Sasabe *et al.*, 2015). MAP65s, a group of microtubule-associated proteins that is crucial for cell plate formation in somatic cytokinesis, is proposed to be targeted by this MAPK pathway in male meiotic RMA formation (Mao *et al.*, 2005; Sasabe *et al.*, 2006; Sasabe *et al.*, 2011). However, the exact targets of this MAPK pathway in male meiosis remain to be identified. Moreover, a caspase family protease, SEPARASE is also involved in male meiotic RMA formation in Arabidopsis, which is thought to occur through the control of cyclin levels (Liu and Makaroff, 2006; Yang *et al.*, 2009), but a clear connection between SEPARASE and male meiotic RMA formation is still unclear.

1.4 Cell size control

1.4.1 Interplay between cell growth and cell cycle

Cell size varies greatly within and between species (Sugimoto-Shirasu and Roberts, 2003). Nevertheless, cell size is strictly controlled and efficient cell function strongly relies on the proper cell size in both unicellular and multicellular organisms (Kwon *et al.*, 2001; Payne *et al.*, 2013). One determining factor for cell size are the movement rates of nutrients that depend on the surface-to-volume ratio, which generates a selection pressure for smaller cell size (Wu *et al.*, 2010). Cell size is controlled by genetic factors through the coupling of cell growth and cell division. This is nicely illustrated by the different growth speed of yeast depending on nutrient conditions; yeasts slow

down their growth speed in poor nutrient conditions by adjusting their cell-cycle, allowing cell division to occur at around similar sizes under high and low nutrient conditions (Jorgensen *et al.*, 2002; Buchler and Louis, 2008; Di Talia *et al.*, 2009).

Cell cycle involves mitotic cycles and endocycles (Sablowski and Carnier Dornelas, 2014). Mitotic cycles are usually divided into G1, S, G2, and M phase. In G1 phase, the cell is preparing for DNA synthesis and accomplishes most of its growth. DNA replication happens in S phase. The second G2 growth phase follows S phase, then cells enter M phase, where mitotic chromosome division occurs. Besides the mitotic cycle, the endocycle is a deviating form of the cell cycle that is important for plant growth. The endocycle is characterized by DNA replication without chromosome division and results in increased ploidy (Lee *et al.*, 2009). Cell growth and cell cycle are coupled by two feedback mechanisms (Sablowski and Carnier Dornelas, 2014). The cell-size checkpoint operates before DNA replication and coordinates cell growth and division (Jorgensen and Tyers, 2004). The other feedback connects maximum cell size and DNA content, indicating that the upper limit for cell growth is probably controlled by DNA content. The "karyoplasmic ratio" hypothesis states that the amount of cytoplasm is sustained by the amount of DNA, thus the upper limit of cytoplasmic growth is settled by the ploidy level (Sugimoto-Shirasu and Roberts, 2003; Jorgensen and Tyers, 2004). It is nevertheless not clear how these two feedbacks are regulated.

Together, maintaining a characteristic cell size could be reached either by regulating the growth rate or by the cell size sensors coordinating cell division (Amodeo and Skotheim, 2016).

1.4.2 The MCM complex and DNA replication

DNA replication starts with the unwinding of the double stranded DNA at an origin of replication. Then DNA synthesis is carried out by a multi-protein

assembly complex called the replisome (Bell, 2014). The replisome is usually assembled at the origin of DNA replication. DNA replication only starts at the origins when the double helix unwinds to two single strands. Thus, precise DNA replication in eukaryotes depends on the strict regulation of DNA unwinding by the replicative helicase (Deegan and Diffley, 2016). The central component of the replicative helicase is a ring-shaped DNA helicase termed the MCM (minichromosome maintenance) complex. It is composed of six related subunits, Mcm2 to Mcm7 (Davey *et al.*, 2003; Bochman *et al.*, 2008; Remus *et al.*, 2009; Costa *et al.*, 2011; Li *et al.*, 2015).

The loading of the MCM complex at the origin of DNA replication begins in G1 phase, encircling double strand DNA (Gillespie *et al.*, 2001; Evrin *et al.*, 2009; Gambus *et al.*, 2011). After entering into S phase, the replicative helicase activates. Two proteins, go-ichi-ni-san (GINS) and cell division cycle 45 (Cdc45) are recruited to the MCM complex. The MCM double hexamer is remodeled and develops into two active replicative helicases, termed as CMG (Cdc45–MCM– GINS) helicases (Tanaka and Araki, 2013). The CMG complex is constituted of GINS, Cdc45, and a single MCM hexamer. After DNA unwinding and loading of DNA polymerases, DNA replication commences in a bidirectional way. When two directional replisomes encounter each other and the replication forks converge, the MCM subunit MCM7 (termed PROLIFERA in Arabidopsis), is modified by a chain of ubiquitin proteins (Bell, 2014). MCM7 is ubiquitylated by the ubiquitin ligase SCF^{Dia2} in yeast and then targeted by Cdc48, leading to CMG disassembly (Maric *et al.*, 2014; Moreno *et al.*, 2014). The SCF complex contains Skp1, Cullin, and the DIA2 F-box protein. DNA replication terminates after the CMG complex and the replisome are disassembled.

1.4.3 Cell size and genome size

Cell size varies greatly among tissues within a single organism, but is remarkably constant within tissue types. What decides the particular cell size in a tissue? Two hypotheses were proposed addressing this question. The first one proposes that there is a fitness consequence for a special size in a given cell type (Gregory, 2005). Based on this hypothesis, a particular cell size is determined by natural selection optimizing cell size in relation to metabolic rate (Hughes and Hughes, 1995; Waltari and Edwards, 2002), or developmental rate (Gregory, 2002). Another hypothesis focuses on the correlation between genome size and cell size. Based on this hypothesis, genomic expansion by transposable element (TE) accumulation in the genome causes expansion in cell size (Roth *et al.*, 1994). Combining both hypotheses, it has been proposed that natural selection shapes a particular cell size followed by changes in genome size. The alternative hypothesis is that changes in cell size follow changes in genome size (Mueller, 2015).

In the animal kingdom, a positive correlation between genome size and cell size has been frequently reported (Horner and Macgregor, 1983; Hardie and Hebert, 2003; Organ *et al.*, 2007). In the plant kingdom, many studies investigated correlations between genome size and cell size across varying ploidy series. These studied revealed that cells in polyploid plants are substantially larger than cells in their diploid progenitors (Mowforth and Grime, 1989; Melaragno *et al.*, 1993; Kudo and Kimura, 2002). Also a strong positive correlation was reported between genome size, guard cell length, epidermal cell area across 101 angiosperms species (Beaulieu *et al.*, 2008). While it remains difficult to test whether genome size follows cell size or conversely, cell size follows genome size, the fact that polyploid plants have larger cells than diploid plants would strongly favor the second hypothesis.

1.4.4 Pollen size

Pollen size varies over three order of magnitude across plant species (Muller, 1979). Variation in pollen size is impacted by different factors. One factor is strong selection pressure correlated with pollen dispersal strategies. Wind-pollinated species prefer small, light pollen, while pollen of insect-pollinated species can be quite large (Schwendemann *et al.*, 2007). Another factor is the length of the style, where it is assumed that species with longer styles produce larger pollen that is able to produce sufficiently long pollen tubes to fertilize the ovules (Roulston *et al.*, 2000; Jurgens *et al.*, 2012; Knight *et al.*, 2010)

It has been proposed that there is a trade-off between the number and size of pollen produced (Knight *et al.*, 2010), leading to the evolutionary trend to decrease pollen size. Small pollen produced in large quantities maximize the pollination success by wind and many insect pollinators (Schwendemann *et al.*, 2007), which may be the driving force causing pollen size reduction.

As discussed above, there is a correlation between genome size and cell size, raising the question whether this is reflected in a correlation between genome size and pollen size. Previous studies based on few species revealed that pollen size increases with ploidy (Bennett, 1972; Orjeda *et al.*, 1990; Altmann *et al.*, 1994). Nevertheless, a study investigating the relationship between genome size and pollen size of 464 species made a more differentiated conclusion (Knight *et al.*, 2010). At the microevolutionary level, particularly when divergence involves variation in ploidy level, the data support that increasing genome size correlates with increasing pollen size. However, analyzing phylogenetically distant species did not show a simple positive correlation between genome size and pollen size for small pollen size. The authors proposed that increased genome size by polyploidization causes an initial increase of pollen size, but strong selection pressure for small pollen causes eventually again a reduction of pollen size.

2 Aims of the study

The aims of the study are to:

1. Characterize the origin and function of the organelle band in meiosis;
2. Identify the version of JAS that functions in meiosis;
3. Identify the mechanisms that maintain chromosome segregation in meiosis.

3 Results and discussion

3.1 JAS co-localizes with markers of the endomembrane system

The *jason* (*jas*) mutant was identified in a genetic screen for mutants that affect *PHE1* expression in seed development. The *jas* mutant forms about 60% diploid pollen due to a defect in male meiosis (Erilova *et al.*, 2009). In wild-type meiosis, two chromosome groups are divided by an organelle band in metaphase II. In the *jas* mutant, the organelle band is usually lost in metaphase II, which results in parallel or fused chromosome groups at this stage. At anaphase II, parallel or fused chromosome groups are separated in two or three directions, which finally results in dyads or triads after meiosis (De Storme and Geelen, 2011).

To identify the localization of JAS and the composition of the organelles in the meiotic organelle band, a construct containing the *JAS* native promoter and coding sequence fused to GFP was generated. This construct could complement the *jas* phenotype, leading to reduced pollen formation. JAS–GFP was localized in many subcellular compartments, including the organelles and membranes in meristematic root cells. To clarify in which organelles JAS was localized, we introduced organelle markers into the JAS-GFP expressing background. The results revealed that JAS was colocalized with the Golgi apparatus, trans-Golgi network, and tonoplast. There was also a weak JAS-GFP signal colocalized with the plasma membrane, indicating that JAS is also presented in plasma membrane

vesicles. However, JAS-GFP was not colocalized with mitochondria, the endoplasmatic reticulum (ER), and peroxisome markers. Overall, these data revealed that JAS is associated with the endomembrane system, trafficking from the Golgi apparatus through the trans-Golgi network to the tonoplast and plasma membranes in meristematic root cells.

JAS plays a vital role in maintaining the organelle band in meiosis; therefore, we investigated JAS localization during meiosis. The results revealed that JAS was localized in clustered organelles at metaphase I and in the organelle band at metaphase II, consistent with the role of JAS in maintaining these structures. I observed the localization of different endomembrane organelles in meiosis by using markers for the Golgi apparatus, the tonoplast, and the plasma membrane, and further compared their localization with JAS–GFP. Markers for the Golgi apparatus and the plasma membrane were located in the organelle band, but the tonoplast marker was largely undetectable during meiosis. JAS–GFP co-localized with a subset of the Golgi and largely co-localized with the plasma membrane marker at metaphase II. Overall, I conclude that JAS is located in the endomembrane system in meiosis, consistent with the localization of JAS in root cells.

The localization of these endomembrane vesicles indicates that a subset of the Golgi apparatus and endomembrane vesicles are components of the organelle band. In mitotic cytokinesis, endomembrane vesicles are transported to the phragmoplast and further develop to the new membrane of the cell plate (Dhonukshe *et al.*, 2006; Van Damme *et al.*, 2008). In plants undergoing simultaneous male meiotic cytokinesis, where cytokinesis only takes place after the completion of the second meiotic division, phragmoplast-like structures have been observed at the end of meiosis I in some species (Brown and Lemmon, 1988; Dinis and Mesquita, 1993; Tchorzewska and Bednara, 2011). We propose that, in male meiocytes, the JAS-containing vesicles could be transported to the centre of the cell at the end of meiosis I. The cytokinesis process is initiated but halted, which fails to result in cell plate formation. But the organelle band still

plays a very important role, forming a physical barrier between the two groups of chromosomes in metaphase II to support the separation of the spindles. Maintaining the organelle band relies on the function of the JAS protein.

3.2 Alternative translation initiation leads to the two versions of JAS

JAS encodes a functionally unknown protein. There are two potential start codons in the JAS cDNA, but it was not clear which ATG is used as a start codon in wild-type Arabidopsis. The longer version of JAS contains a putative mitochondrial targeting peptide at the N-terminus, while the shorter version lacks the mitochondrial targeting peptide. To investigate which version of JAS is functional, I generated two constructs; a long version of JAS including the N-terminal targeting sequence (*JAS.1*) and a short version lacking this sequence (*JAS.2*). A similar pattern to the genomic JAS-GFP was observed in *UBQ:JAS.1-GFP*, with JAS-GFP being localized in organelles that overlap with the Golgi and tonoplast marker, but not in the plasma membrane. In contrast, JAS.2–GFP was present in the tonoplast and the plasma membrane, but not colocalizing with the Golgi marker. The localization pattern of genomic JAS-GFP driven by the *JAS* native promoter likely reflects a combination of JAS.1-GFP and JAS.2-GFP, indicating endogenous JAS.1 localizes to the Golgi and only JAS.2 localizes to the plasma membrane, but both JAS.1 and JAS.2 localize to the tonoplast.

We next asked if the two different versions had different functional roles. As the *jas* mutant does not have a root phenotype, I focused on meiotic cells where the *jas* mutation leads to the formation of unreduced gametes. To test which version functions in maintaining the organelle band during male meiosis, I tested which version can complement the *jas* mutant. I found that only *JAS.2* can complement the *jas* mutant phenotype, revealing that the plasma membrane-localized, short version is sufficient for JAS function in meiotic cells. Consistent with the genetic data, JAS.2-GFP localization in meiosis II was the same as that

of pNative:JAS-GFP that was restricted to the organelle band. In contrast, JAS.1–GFP protein was also present in other areas of the cell as well as the organelle band, suggesting JAS.2 is the predominant form in meiotic cells, but there is little, if any, of JAS.1 produced during meiosis.

3.3 Meiocyte size is a determining factor for unreduced gamete formation in *Arabidopsis thaliana*

To further characterize the mechanisms maintaining chromosome segregation in meiosis II, we performed a suppressor screen with the aim to find mutants that form reduced gametes in the presence of the *jas* mutation. In this screen *telamon* (*tel*) was isolated as a strong suppressor of *jas* that can produce many haploid pollen in the *jas* background.

In the *jas* mutant concomitantly with the disappearance of the organelle band in meiosis II, the chromosome groups are often parallel or fused at metaphase II and the distance between the two groups of chromosomes is often much shorter than in wild-type plants (De Storme and Geelen, 2011). Although the production of diploid pollen was significantly reduced in *jas tel*, the organelle band was not restored in meiosis II, suggesting an organelle band-independent mechanism maintaining chromosome segregation. But the distance of the chromosome groups in the *jas tel* double mutant was significantly increased compared to *jas*, probably explaining the high percentage of haploid pollen formation. Measuring the size of meiotic cells in *jas tel* revealed that the meiotic cells are enlarged, supporting chromosome segregation in meiosis II independently of the organelle band. Polyploid plants have larger cells than diploid plants; therefore, I used tetraploid *jas* as a tool to test whether increased meiocyte size can bypass the requirement of JAS function. Tetraploid *jas* was generated by colchicine treatment. Strikingly, the percentage of reduced pollen formation was significantly increased in tetraploid *jas*. The organelle band was still missing in tetraploid *jas*, but the distance of the chromosome groups was increased,

coinciding with the increased size of meiotic cells. This data strongly supports the idea that cell size is an important factor in maintaining chromosome segregation in meiosis. Recent studies demonstrated that cold stress induces diploid pollen production in Arabidopsis (Nico De Storme et *al*., 2012). Based on my finding that cell size is important in maintaining chromosome segregation, I hypothesized that species with smaller meiocytes may produce more diploid pollen under cold stress conditions than species with larger ones. *Eutrema salsugineum* produces smaller meiocytes than *Arabidopsis thaliana*, forming an ideal model to test whether male meiosis in *Eutrema* is more sensitive to low-temperature stress than in *Arabidopsis*. Enlarged pollen was detected from 5 to 10 days after cold treatment. Indeed, *Eutrema* produced significantly more large pollen grains than *Arabidopsis*. The shape of sperm in enlarged *Eutrema* pollen was elongated and flattened, which is strikingly different from the round shape of sperm in normal pollen. Sperm elongation and flattening occurs in *Arabidopsis* during pollen tube growth (Ge *et al.*, 2011), when sperms are completing S phase (Friedman, 1999). This indicates that the elongated and flattened sperm in enlarged *Eutrema* pollen is a consequence of the increased DNA content. Together, our result strongly supports the idea that meiotic cell size is important in maintaining chromosome segregation during meiosis.

Using a Next Generation Mapping approach (Michael L Metzker, 2010), we have identified the candidate gene of *TEL*. The mutation in the candidate gene co-segregated with the *jas tel* phenotype. The mutation was found to be dominant; therefore, we tested whether a construct with the mutated *TEL* candidate gene could mimic the *jas tel* phenotype. Several transgenic lines were identified that mimicked the *jas tel* phenotype, revealing that the candidate gene was *TEL*. Further analysis showed that TEL^{DtoN} could enlarge meiotic cell size in the *jas-3* background in both metaphase I and metaphase II stage, indicating that TEL^{DtoN} starts its function from the early stage of meiosis. However, *tel* had no effect in male meiosis in a wild-type background and *tel* did not enlarge root cells, revealing that *tel* only causes an effect during meiosis in the *jas*

background. We failed to detect TEL-GFP and TEL^{DtoN}-GFP in male meiocytes, however, ectopic expression in root cells revealed that wild-type TEL-GFP was localized in the cytoplasm, while TEL^{DtoN}-GFP mainly accumulated in nuclei in root cells. Therefore, the point mutation changes the subcellular localization of TEL. Whether this causes a difference in TEL function remains to be shown.

TEL encodes a putative F-box protein, which is one of the three components of the SCF complex; the other two components are ASK1 and CULLIN. The complex mediates ubiquitination of proteins targeted for degradation by the proteasome (Hershko and Ciechanover**,** 1998). ASK1 is crucial for male meiosis and proposed to be required for degrading a protein regulating homolog association before anaphase I (Yang *et al.*, 1999). In a yeast two-hybrid screen using wild-type TEL and TEL^{DtoN} as baits, we identified several interacting proteins. One of them is PROLIFERA (PRL), a DNA helicase essential for DNA replication that is part of the minichromosome maintenance (MCM) complex (Springer *et al.*, 1995). We further found that TEL^{DtoN} was able to interact with ASK1. Importantly, the wild-type version of TEL did not interact with ASK1, indicating that TEL^{DtoN} gained a new function. We speculate that interaction of TEL^{DtoN} with ASK1 may cause enhanced degradation of PRL, resulting in impaired MCM complex function and thus increased time of premeiotic DNA replication. Cell growth can continue when the cell cycle is blocked (Sablowski and Carnier Dornelas, 2014; Amodeo and Skotheim, 2016); therefore, impaired MCM function during premeiotic replication may potentially increase meiocyte size. Together, the above results provide novel insights into the role of meiocyte size for successful chromosome segregation in male meiosis.

4 Conclusions

By investigating the localization of JAS and the composition of the organelle band during male meiosis in *Arabidopsis*, I found that JAS is associated with a subset of the Golgi apparatus and components of the plasma membrane at metaphase II. I thus conclude that a subset of the Golgi apparatus and endomembrane vesicles are components of the organelle band, and maintenance of the organelle band depends on the function of JAS. I furthermore was able to show that from the two versions of JAS proteins encoded by *JAS*, only the short version (JAS.2) lacking the mitochondrial targeting peptide in the N-terminus was functional during meiosis, consistent with its localization in the organelle band in meiosis II. Finally, I was able to show that increased meiocyte size can bypass the requirement of the organelle band during meiosis, suggesting a requirement of the organelle band with decreasing meiocyte size. This idea was corroborated by my findings revealing that *Eutrema salsugineum*, which has smaller meiocytes than *Arabidopsis thaliana*, is more sensitive to cold stress and produced increased numbers of diploid pollen. In conclusion, meiocyte size is crucial for maintaining chromosome segregation in meiosis II and the organelle band is mainly required in species forming small pollen.

5 Future perspectives

Meiosis is regulated by a complicated network of molecular factors. Failure at different levels may cause the formation of unreduced gametes, resulting in sexual polyploidization. While JAS was found to be required to maintain the organelle band in male meiosis, how JAS achieves this function is still not clear, neither the relevant proteins that function in the same pathway. Elucidating the pathway through which JAS functions could reveal important new insights of potential relevance for plant breeding. Generating polyploids is an important breeding tool that is mainly achieved using the highly toxic drug colchicine. Interfering with organelle band formation during meiosis could be a valuable and possibly non-toxic alternative. Therefore, knowing the proteins that interact with JAS could open new avenues to polyploidy breeding.

This work unravelled that meiotic cell size is important for maintaining chromosome segregation during meiosis, implicating that species with smaller meiocyte size are more sensitive to low-temperature stress. This could explain why there are more polyploid species in harsh environments and at higher altitudes. Systematically testing whether indeed meiocyte size correlates with increased incidence of polyploidy would be an exciting future project. Furthermore, the size of male meiocytes could be used as a predictor for the success of producing unreduced gametes by cold, which could have potential value for plant breeding.

The point mutation in *TEL* caused TEL to become mainly nuclear localized, for reasons that remain to be explored. Identifying the underlying mechanism will be important for understanding how the localization of F-box proteins is regulated between nucleus and cytoplasm. Through yeast two hybrid screening I identified the MCM complex member PROLIFERA as interaction partner for TEL. Moreover, one of the core components of the SCF complex, ASK1, could only interact with TEL^{DtoN} but not with the wild-type version of TEL. It will be important to confirm the interaction among TEL^{DtoN}, ASK1, and PRL by co-immunoprecipitation and pulldown assays. If the interaction can be confirmed, it will be exciting to elucidate whether the mutated TEL causes increased degradation of PROLIFERA and whether impaired MCM complex function affects the balance between cell division and cell expansion. This research could substantially broaden our understanding of cell size regulation in plants.

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Popular science summary

Many plants have more than two chromosome sets in their cells and are referred to as polyploids. Polyploidy is frequently associated with increased biomass production, increased yield, and increased resistance to biotic and abiotic stress. For that reason, most of our currently used crops are polyploids. In nature, it is generally assumed that new polyploids arise as a consequence of meiotic failure, leading to the formation of unreduced gametes. My thesis provides new insights into the underlying mechanism leading to unreduced gamete formation. I found that an organelle band aligning at the center of the meiotic cell is required to separate chromosomes groups that belong to different cells after meiosis. Without the organelle band, chromosome groups easily fuse with each other, leading to the formation of unreduced gametes. I furthermore found that meiotic cell size impacts on the formation of unreduced gametes. Large meiotic cells could successfully complete meiosis without the organelle band. Therefore, variation in size of the male meiotic cells in different plant species may underlie the different frequency of unreduced gamete formation among species. Insights generated by this thesis may also be of relevance for plant breeding. This far, polyploids are mainly generated using highly toxic drugs. My findings revealed that factors affecting the organelle band, like cold, cause the formation of unreduced gametes, which could be exploited for chemical-free polyploidy breeding.

Populärvetenskaplig sammanfattning

Många växter har mer än två uppsättningar av kromosomer i sina celler och är därmed polyploida. Polyploiditet associeras med ökad biomassa, högre avkastning och ökad resistens mot stress, som t ex torka och skadedjur. Därför är många av våra nuvarande grödor polyploida. Det är en generell uppfattning att i naturen är polyploiditet en konsekvens av att delningen av könsceller misslyckats och därmed ger upphov till att könscellerna får för många kromosomer (s k oreducerade könsceller). Min avhandling ger ny kunskap om mekanismerna bakom bildandet av oreducerade könsceller. Jag fann att ett aggregat av organeller i mitten av den delande cellen krävs för att separera kromosomer som hör till olika celler efter meiosen. Utan organellaggregatet kan kromosomer lätt smälta samman vilket leder till att oreducerade könsceller bildas. Vidare fann jag att storleken på den delande cellen påverkar bildandet av oreducerade könsceller. I stora celler kan meiosen fullbordas utan organellaggregatet. Därför kan variationen i storlek hos de hanliga delande cellerna i olika växtarter ligga till grund för varierande frekvenser av oreducerade könsceller bland arter. Insikter som genererats i denna avhandling kan komma till användning inom växtförädlingen. Hittills har polyploiditet huvudsakligen uppnåtts på kemisk väg med mycket giftiga ämnen. Mina resultat visar att faktorer som t ex kyla kan påverka bildandet av oreducerade könsceller, vilket kan komma till användning för en kemikaliefri växtförädling.

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