European Journal of Cell Biology 89 (2010) 258–261

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Analyzing female gametophyte development and function: There is more than one way to crack an egg

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ARTICLE INFO

Keywords:
Female gametophyte
Fertilization
Segregation distortion
Pollen tube attraction
Reproduction

ABSTRACT

In flowering plants, gametes are formed in specialized haploid structures, termed gametophytes. The female gametophyte is a few-celled structure that integrates such diverse functions as pollen tube attraction, sperm cell release, gamete fusion and seed initiation. These processes are realized by distinct cell types, which ensure reproductive success in a coordinated manner. In the past decade, much progress has been made concerning the molecular nature of the functions carried out by the different cell types. Here, we review recent work that has shed light on female gametophyte development and function with a particular focus on approaches that have led to the isolation of genes involved in these processes.

Introduction

The formation and specification of gametes is of paramount importance for sexually reproducing organisms. The haploid status, characteristic of gametes of diploid organisms, is accomplished by meiosis. In contrast to animal development, in most flowering plants, meiosis is followed by a small number of mitotic divisions. The resulting few-celled haploid structure is termed gametophyte. The female gametophyte of *Arabidopsis* comprises two gametic cells, the egg and central cell, and the non-gametic synergids and antipodal cells (Fig. 1A). Whereas the function of the antipodal cells, which degenerate prior to cellularization, is unclear, synergids have been demonstrated to play an essential role in pollen tube attraction and sperm cell release (Higashiyama et al., 2001; Huck et al., 2003; Rotman et al., 2003; Escobar-Restrepo et al., 2007). The two female gametes differ morphologically and with respect to their molecular profiles. Importantly, they contribute in a distinct manner to seed formation: Whereas the egg cell upon gamete fusion gives rise to the embryo, the diploid central cell fuses to a second sperm cell resulting in the formation of endosperm, which nurtures the developing embryo (Fig. 1B, C). The respective sperm cell pair is delivered by a single pollen tube (Fig. 1B). It is unclear whether the male gametes are similarly predetermined with respect to their contribution to either embryo or endosperm (Moll et al., 2008a). Up to now, no molecular differences have been attributed to either of the two *Arabidopsis* sperm cells. The male gametes are, at best, told apart by their differential association to the vegetative nucleus, as only one is cytoplasmically connected to the vegetative cell (Lalanne and Twell, 2002). However, in some plant species the male gametes display marked morphological differences (Weterings and Russell, 2004) and express a different bias towards the female gametes (Russell, 1985).

In the past, quite different approaches have been taken to identify the factors necessary for female gametophyte development and function. Here, we aim to introduce and compare the distinct strategies and highlight recent findings gained through the various approaches.

Forward genetic screens to identify genes involved in female gametophyte development and function

A prominent approach to identify mutants with gametophytic defects is screening for distorted segregation. The rationale behind this strategy is that mutations affecting the gametophytic phase are likely to compromise fertility. In plants heterozygous for a deleterious gametophytic mutation, the mutant allele is transmitted to the next generation less efficiently than the respective wild-type allele (Page and Grossniklaus, 2002). Depending on the severity of the defect, this leads to a concomitant shift of the normal Mendelian segregation (Fig. 1D). By this means T-DNA insertion mutants have been isolated by many labs (Feldmann et al., 1997; Bonhomme et al., 1998; Howden et al., 1998; Christensen et al., 2002). A particularly comprehensive study, making use of Ac/Ds transposon mutagenesis, was published by Pagnussat et al. (2005). The group screened 24,000 lines resulting in the identification of 129 female gametophytic mutants, and their respective T-DNA insertion sites. The majority

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doi:10.1016/j.ejcb.2009.11.005

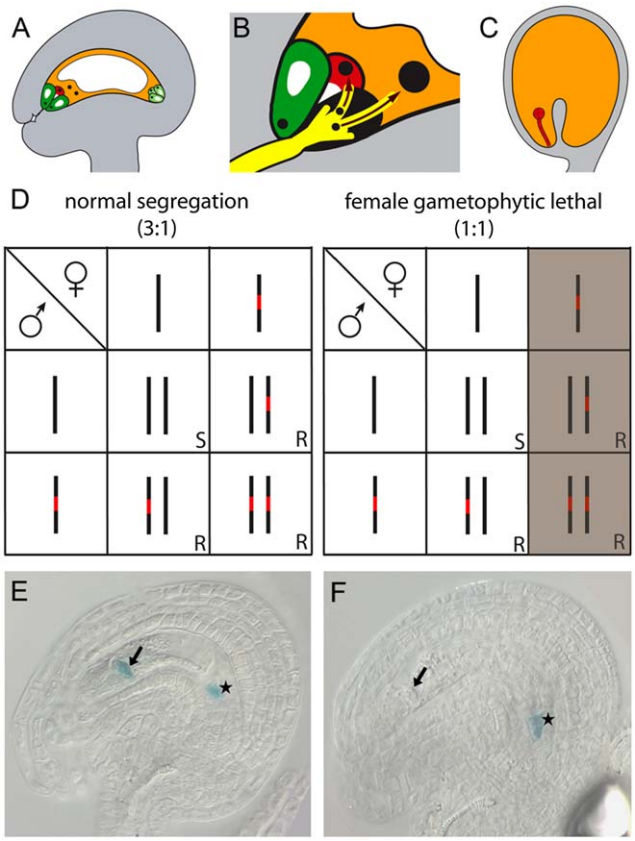

Fig. 1. Analyzing female gametophyte development and function. (A-C) Female gametophyte and seed formation. (A) The female gametophyte contains seven cells, which differentiate into four distinct cell types: two synergids (dark green), one egg cell (red), one central cell containing two polar nuclei (orange) and three antipodal cells (light green). Shortly before fertilization the two polar nuclei fuse to a secondary nucleus (B). The pollen tube (yellow) delivers two sperm cells, one of which fuses to the egg cell giving rise to the diploid embryo (red in C). The second sperm cell fuses to the central cell, thereby initiating the development of the triploid endosperm (orange in C). (D) In plants heterozygous for a deleterious gametophytic mutation, the mutant allele is transmitted to the next generation less efficiently than the respective wild-type allele. Depending on the severeness of the defect, this leads to a concomitant shift of the normal Mendelian segregation. In the case of mutants that co-segregate with readily traceable traits, which is the case for T-DNA insertions that confer antibiotic resistance (red), the ratio of resistant (R) to sensitive (S) plants can be determined. Modified after (Page and Grossniklaus, 2002). (E, F) Ovules expressing GUS reporter genes. (E) Ovule showing *GUS* expression of an egg and antipodal cell marker. (F) Ovule with antipodal marker gene expression only.

of the mutants appeared to be primarily affected in post-fertilization processes. This is surprising as only a few mutants with maternal effects on seed development have been described (Ohad et al., 1996, 1999; Chaudhury et al., 1997; Grossniklaus et al., 1998; Ngo et al., 2007; Tiwari et al., 2008), indicating that the influence of the female gametophyte on post-fertilization processes is underestimated.

Among the mutants isolated for distorted segregation are several mutants defective in polar nuclei fusion. Interestingly, some of the responsible genes code for mitochondrial proteins, suggesting that these organelles play a critical role in nuclear membrane fusion (Christensen et al., 2002; Portereiko et al., 2006b). A slightly later developmental defect is displayed by a T-DNA insertion in the transcription factor AGL80 (Portereiko et al., 2006a). Central cells of *agl80* mutant gametophytes show polar nuclei fusion but fail to maturate, which is reflected by the reduced size of both nucleolus and vacuole and a failure to initiate endosperm formation. The finding that two of three central cell markers are downregulated in the mutant indicates that AGL80 regulates key central cell functions. In contrast, *eostre* mutant gametophytes form a functional central cell but contain one supernumerary egg cell as a result of synergid missspecification (Pagnussat et al., 2007). The phenotype is caused by ectopic expression of the Bell-like homeodomain protein BLH1 and provides a unique tool to address the longstanding question of sperm cell predetermination: By fertilizing *eostre* mutants with wild-type pollen, Pagnussat et al. (2007) were able to demonstrate that both sperm cells can target egg cells to give rise to zygote formation. Although an intrinsic bias of any of the sperm cells to either egg or central cell cannot be excluded, this is the first demonstration that both *Arabidopsis* sperm cells are capable of fertilizing the egg cell.

T-DNA insertion screens for segregation distortion have demonstrated a high potential in revealing a plethora of novel female gametophytic mutants. Due to the associated T-DNA insertion the responsible gene is readily identified – at least in theory. T-DNA insertions can also turn out to be a pitfall: the integration of foreign DNA can cause chromosomal rearrangements and multi-gene deletions, which can hamper the identification and subsequent analysis of the respective gene (Page et al., 2004). One such example is *glauce*: In most *glauce* mutants the central cell cannot be fertilized and the mutants are unable to form endosperm. The Ds insertion in *glauce* causes a 215-kb genomic deletion (Ngo et al., 2007). How many of the deleted genes contribute to the phenotype is hence unclear. Still, T-DNA insertion screens had an advantage over non-insertion screens in times when the isolation of mutants by map-based approaches was laborious and time consuming. However, the advent of novel high-throughput in-depth sequencing techniques is about to neutralize this disadvantage in favor of non-insertion screens. Segregation distortion screens require extensive re-screening, as developmental defects at various gametophytic stages can cause reproductive failure. Noteworthy, the gametophyte is the first stage at which the developmental requirement of a gene is possibly revealed. Accordingly, also mutations in housekeeping genes, which do not necessarily affect gametophyte-specific functions, are likely to accumulate in this generation.

Different forward-genetic screens target seed abortion or misexpression of cellular markers (Huck et al., 2003; Groß-Hardt et al., 2007). The advantage of marker-based screens is the possibility to discriminate unspecific female gametophytic mutations. Nevertheless they are very time-consuming and accordingly one has to lower one's sights in matters of quantity and screen saturation. The screen performed by Groß-Hardt et al. (2007) targeted 5200 lines. Of these lines, three non-allelic mutants, *lachesis, clotho* and *atropos*, showed deregulated egg cell marker expression (Groß-Hardt et al., 2007; Moll et al., 2008b). An in-depth analysis revealed ectopic egg and central cell marker gene expression in synergids and antipodal cells, respectively, demonstrating that all the cells in the female gametophyte are competent to adopt gametic cell fate. Intriguingly, the respective genes all code for putative core spliceosomal components. Although the link between this class of proteins and the regulation of gametic cell fate is unclear, the result demonstrates the specificity of the approach.

Marker-based screens for mutants defective in the specification of a given cell type are complicated by the fact that the expression of most cell-specific marker genes described to date is only initiated after cellularization (Kägi and Groß-Hardt, 2007). The failure of a given mutant to express the marker does, hence, not necessarily indicate a cell specification defect, but might point towards an early female gametophytic arrest. One option for circumventing this problem is to introduce a second marker gene, which can serve as an internal stage control. Making use of a combined egg cell, antipodal cell marker gene approach (Fig. 1E,