Development 139, 498-502 (2012) doi:10.1242/dev.075234

LACHESIS-dependent egg-cell signaling regulates the development of female gametophytic cells

Ronny Völz[1], Ludwig von Lyncker[1], Nadine Baumann[1], Thomas Dresselhaus[2], Stefanie Sprunck[2] and Rita Groß-Hardt[1,*]

SUMMARY

In contrast to animals, plant germ cells are formed along with accessory cells in specialized haploid generations, termed gametophytes. The female gametophyte of flowering plants consists of four different cell types, which exert distinct functions in the reproductive process. For successful fertilization, the development of the four cell types has to be tightly coordinated; however, the underlying mechanisms are not yet understood. We have previously isolated the *lachesis* (*lis*) mutant, which forms supernumerary gametes at the expense of adjacent accessory cells. *LIS* codes for the *Arabidopsis* homolog of the pre-mRNA splicing factor PRP4 and shows a dynamic expression pattern in the maturing female gametophyte. Here, we used *LIS* as a molecular tool to study cell-cell communication in the female gametophyte. We show that reducing *LIS* transcript amounts specifically in the egg cell, affects the development of all female gametophytic cells, indicating that cell differentiation in the female gametophyte is orchestrated by the egg cell. Among the defects observed is the failure of homotypic nuclei fusion in the central cell and, as a consequence, a block in endosperm formation. *LIS*-mediated egg cell signaling, thus, provides a safeguard mechanism that prevents the formation of nurturing tissue in the absence of a functional egg cell.

KEY WORDS: Cell-cell communication, Egg cell, Female gametophyte

INTRODUCTION

Plant germ cells develop in specialized haploid structures, termed gametophytes. The female gametophyte of flowering plants comprises two gametes, the egg and the central cell. Both cells are fertilized by one of two sperm cells delivered by the pollen tube. The fertilized egg cell subsequently develops into an embryo, whereas the central cell generates the endosperm, which nurtures the developing embryo. This double-fertilization process distinguishes the reproductive mode of angiosperms from that of other plants and is considered to be one of the key traits responsible for the success of flowering plants (Friedman, 2006). Importantly, it ensures that the formation of nurturing tissue and hence the allocation of resources is only initiated upon sperm cell delivery. The formation of endosperm is of high metabolic cost to the plant and probably affects other life history constraints, suggesting that double fertilization is of high adaptive value. Apart from the two gametic cells, the female gametophyte forms two types of accessory cells. Two synergids lie at the entrance point of the pollen tube and are required for both the attraction of the pollen tube and the reception of the sperm cells (Higashiyama et al., 2001; Huck et al., 2003; Rotman et al., 2003; Amien et al., 2010). The function of the three antipodal cells at the opposite pole of the female gametophyte is less clear; however, they might have a nourishing function that is achieved by transferring nutrients from the surrounding sporophyte to the female gametophyte (Raghavan, 1997). The development of the distinct cell types has to be closely coordinated to ensure reproductive success and there is some evidence for cell-cell communication in the female gametophyte: plants with reduced mitochondrial activity in the central cell exhibit an increased antipodal lifespan, indicating that programmed cell death of antipodal cells is under regulatory control by the central cell (Kägi et al., 2010). Moreover, it has been shown that synergids, egg cells and central cells are symplastically connected in the early eight-nucleate female gametophytes of *Torenia fournieri* (Han et al., 2000). In maize, small peptides have been identified that, owing to their size, have the potential to move between cells (Dresselhaus, 2006). A further hint for communication between single female gametophytic cells comes from the analysis of the *lachesis* (*lis*) mutant (Groß-Hardt et al., 2007). *lis* mutants form supernumerary egg cells, resulting from misspecification of the synergids and central cell, which express morphological and molecular attributes of the egg cell. Furthermore, antipodal cells adopt a central cell fate in *lis* mutants, demonstrating that all cells in the female gametophyte can differentiate into gametic cells. *lis* is defective in the *Arabidopsis* homologue of the yeast pre-mRNA splicing factor PRP4 (Groß-Hardt et al., 2007), and promoter-reporter analysis has suggested that cell specification in wild-type female gametophytes correlates with a strong upregulation of *LIS* expression in gametic cells. By contrast, *LIS* expression appears to be downregulated in accessory cells of mature female gametophytes, which has led to the hypothesis that the gametic potential of accessory cells is repressed by lateral inhibition from the gametic cells (Groß-Hardt et al., 2007). Whereas *LIS* appears to be involved in the maintenance of cell fates, the plant hormone auxin has been implicated in the establishment of the distinct female gametophytic cells. Pagnussat et al. have studied the effects of altered auxin synthesis and response and analyzed the expression of the auxin response regulator DR5 (Pagnussat et al., 2009). The presented data are consistent with a model whereby a micropylar-chalazal auxin gradient determines the different cell fates in the female gametophyte.

[1]Center for Plant Molecular Biology (ZMBP), University of Tübingen, Tübingen, Germany. [2]Cell Biology and Plant Biochemistry, University of Regensburg, Regensburg, Germany.

*Author for correspondence (rita.gross-hardt@zmbp.uni-tuebingen.de)

Accepted 22 November 2011

Here, we have made use of *LIS* as a molecular tool to study cell-cell communication in the female gametophyte. We show that reducing *LIS* transcript amounts specifically in the egg cell affects synergids, central cell and antipodals, suggesting that the egg cell acts as a signaling center for the development of all female gametophytic cells. Moreover, reducing *LIS* transcript levels in the egg cell interferes with homodiploidization of the central cell and, consequently, endosperm formation. *LIS*-mediated egg cell signaling thus reveals a safeguard mechanism, which prohibits the formation of nurturing tissue in the absence of a functional egg cell.

MATERIALS AND METHODS

Plant material and growth conditions

Plants were grown on soil in growth chambers under long-day conditions (16 hours light/8 hours dark) at 18°C.

Molecular cloning

The promoters *DD2*, *DD45*, *EC1*, *MEA*, *HSFa2* and *35S* used in front of *2xGFP_LIS* and/or *lis (RNAi)* were amplified (see supplementary material Table S3) and cloned into the *pGEM-T vector* (Promega). The *DD45*, *MEA* and *EC1* promoters were cloned into the binary vector *pGIIBar-LIS::2xGFP_LIS* (Moll et al., 2008) in replacement of the *LIS* promoter. To generate *DD45::NLS_3xGFP* and *DD2::NLS_3xGFP*, the respective promoters were introduced into *pGIIbar-NLS_3xGFP* (Takada and Jürgens, 2007). For the *lis (RNAi)* construct, a 500 bp fragment was amplified from *LIS* cDNA (Groß-Hardt et al., 2007) with primers P-ASs and P-ASas containing *Bam*HI and *Pac*I restriction sites, and with primers P-Ss and P-Sas containing *Mlu*I and *Not*I restriction sites and cloned into *pGEM-T* (Promega). The two fragments were subsequently introduced into *pGIIBar-EC1* containing an additional *MCS-adaptor* between the *Pac*I and *Spe*I restriction sites (see supplementary material Table S3). Between the two *lis (RNAi)* fragments, a *FAD2* intron of vector *pEC1-if2* (S.S., unpublished) was inserted using *Bam*HI/*Mlu*I restriction sites. Subsequently, the *DD2*, *DD45*, *MEA*, *HSFa2* and *35S* promoters were introduced in front of the *lis (RNAi)* construct. For generating *pGIIbar-EC1::NLS_3xdsRED::tNOS*, the *EC1* promoter and the transcription termination sequence *tNOS* were amplified with primers RV3s/RV3as containing *Eco*RI/*Pst*I restriction sites and RV4s/RV4as containing *Hin*dIII/*Eco*RI restriction sites and cloned into *pGEM-T vector*. Subsequently, the *EC1* promoter and *tNOS* were introduced into *pGIIbar*. The *NLS_3xdsRed* was excised from *ER126* (T. Laux, unpublished) using the *Bam*HI restriction site and introduced into *pGIIbar* containing *EC1* and *tNOS*.

mRNA isolation and semi-quantitative PCR

mRNA was isolated from leaves, buds and flowers with Dynabeads mRNA Purification Kit (Invitrogen). For RT-PCR and first-strand synthesis, the Revert Aid H-Minus First Strand cDNA Synthesis Kit from Fermentas was used with Oligo(dT)-primers. For semi-quantitative PCR equal amounts of cDNA were used. The cDNA fragment of c*LIS* was amplified using P-LISs and P-LIS2as, and *cACTIN* was amplified using P-ACTs and P-ACTas (see supplementary material Table S3).

Histology and microscopy

For analysis of mature female gametophytes, the oldest closed flower bud of a given inflorescence was emasculated and harvested 2 or 3 days later. Whole-mount clearing was performed as described previously (Groß-Hardt et al., 2007). Cleared whole mounts were visualized using a Zeiss Axioscope Microscope (Zeiss, Oberkochen, Germany). *GFP*- and *dsRED*-expressing specimens were observed using a Leica DMI6000B.

RESULTS AND DISCUSSION

In the female gametophyte, LIS is non-mobile and preferentially accumulates in gametic cells

We have previously shown that the *LIS* expression pattern in maturing female gametophytes appears to be dynamic: a nuclear localized *β-glucuronidase* (*GUS*) reporter, fused to the *LIS* promoter, confers expression to all cells of young eight-nucleate gametophytes. However, shortly after cellularization, expression is hardly detectable in accessory cells and is upregulated in gametic cells (Groß-Hardt et al., 2007). To address the question of whether the transcriptional dynamics were reflected at the protein level, we tagged the *LIS* cDNA N-terminally with two copies of the green fluorescent protein (2×GFP). We have previously shown that the chimeric LIS protein complements the *lis* mutant, indicating that the GFP did not interfere with LIS protein function (Moll et al., 2008).

In mature female gametophytes of wild-type plants containing the *LIS::2xGFP_LIS* construct, the fluorescence signal was detected in all female gametophytic cells, with the strongest expression in the egg cell and the central cell, indicating that the upregulation of *LIS* promoter activity in gametic cells translates into increased protein amounts (Fig. 1A). The fact that the GFP-tagged LIS protein was detected in both accessory cell types suggests that the transcriptional downregulation of *LIS* in these cells is masked by the stability of the protein. Alternatively, we could not rule out the possibility that the LIS protein is mobile. We, therefore, expressed *2xGFP_LIS* specifically in the egg cell and in the central cell using the *DD45* and the *MEA* promoters, respectively (Groß-Hardt et al., 2007; Steffen et al., 2007). The expression profile of *DD45* was originally described for the Columbia accession and by analyzing a *DD45::NLS_3xGFP* reporter construct, we could confirm the egg cell-specific expression profile in Landsberg *erecta* plants (Fig. 1B).

Importantly, in *DD45::2xGFP_LIS* and *MEA::2xGFP_LIS* plants, we detected the fluorophore specifically in the egg cell and the central cell, respectively (Fig. 1C,D). This indicates that the GFP-tagged LIS protein is not mobile in the time window covered by the respective two promoters and suggests that the presence of 2×GFP_LIS in the accessory cells is due to protein stability.

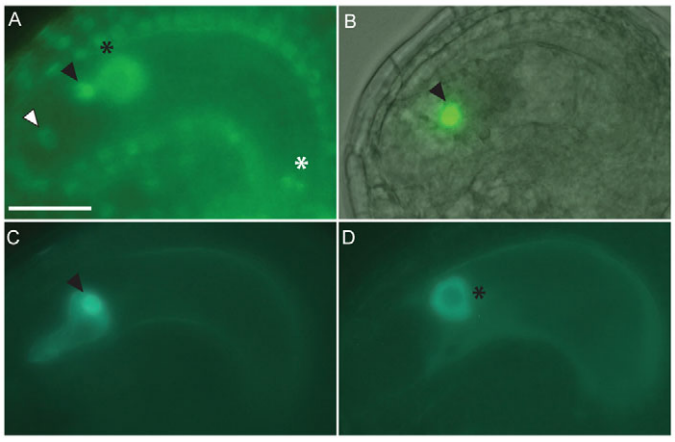

Fig. 1. The LIS protein is immobile and preferentially accumulates in gametic cells. (**A**) *LIS::2xGFP_LIS* is detected ubiquitously with strongest expression in the egg and central cell. (**B**) In Landsberg *erecta DD45::NLS_3xGFP* plants, the fluorescence signal is observed only in the egg cell. (**C**,**D**) In *DD45::2xGFP_LIS* plants (C), GFP is specifically detected in the egg cell (*n*=208) and in *MEA::2xGFP_LIS* plants (D), GFP is specifically detected in the central cell (*n*=276). White arrowhead, synergid nucleus; black arrowheads, egg cell nucleus; black asterisk, central cell nucleus; white asterisk, antipodal nucleus. Scale bar: 25 μm.

The upregulation of *LIS* in gametic cells correlates temporally with the establishment of the distinct cell types, and we next asked whether enhanced *LIS* expression in gametic cells was of functional relevance for the development of female gametophytic cells. In order to reduce *LIS* transcript amounts specifically, we made use of a RNA interference (RNAi) approach.

Constitutive *lis (RNAi)* lines exhibit reduced *LIS* mRNA levels and growth retardation

To analyze whether a *lis (RNAi)* construct could effectively reduce *LIS* transcript levels, we expressed the construct ubiquitously in sporophytic tissue using the *35S* promoter. Of 12 independent *35S::lis (RNAi)/–* lines, six showed a dwarf-like phenotype (see supplementary material Fig. S1A). Semi-quantitative PCR of three independent lines revealed that the respective plants contained substantially reduced *LIS* transcript amounts, suggesting that *35S::lis (RNAi)/–* plants are *lis* hypomorphs (see supplementary material Fig. S1B). We had previously shown that homozygous *lis* plants are lethal (Groß-Hardt et al., 2007) and the severe growth retardation in *35S::lis (RNAi)/–* plants is in line with an essential role of *LIS* in plant development.

Reduced *LIS* expression in the egg cell compromises the development of all female gametophytic cells

To modulate *LIS* transcript dynamics in maturing egg cells, we next expressed the RNAi construct from the *DD45* promoter, which is specifically active in the egg cell and early embryos (Steffen et al., 2007). Of 19 independent transformants, seven lines exhibited a reduced fertility (see Table S1A in the supplementary material). Of these seven lines, four showed morphological abnormalities in the female gametophyte strongly resembling *lis* mutants (Fig. 2A,C,E,F; Fig. 4A). We consistently observed a failure of polar nuclei fusion in the central cell. Additionally, synergid nuclei size was increased and comparable with egg cell nuclei size in three lines. In four lines, degeneration of antipodals was impaired and in some cases we observed that the antipodals had protruded towards the central cell (Fig. 4F). As in *lis* mutants, the morphological defects in *DD45::lis (RNAi)/–* correlated with reduced fertility, albeit to a lower extent (see supplementary material Table S1A). A hallmark of the *lis* mutant defect is ectopic expression of the egg cell marker ET1119 in central cell and synergids. However, in *DD45::lis (RNAi)/–* plants, we did not observe ectopic expression of this reporter (data not shown), and expression of a second egg cell marker line, *EC1::NLS_3xdsRED*, was also comparable between wild-type and *DD45::lis (RNAi)/–* plants (see supplementary material Fig. S3). This molecular discrepancy might be accounted for by temporal differences in *LIS* transcript depletion, which in *lis* initiates with the onset of megagametogenesis and in *DD45::lis (RNAi)* only after cellularization.

lis mutant female gametophytes become infrequently fertilized and the resulting seeds contain undeveloped endosperm, which is a likely consequence of central cell mis-specification (Groß-Hardt et al., 2007) (Fig. 2B,D). Notably, this defect was also observed in *DD45::lis (RNAi)/–* plants, indicating that the central cell is functionally impaired (Fig. 2G). At first glance, these results suggest that defects in the egg cell impair the development of other female gametophytic cells. However, it has been established that RNAi can spread between cells (Baulcombe, 2004) and we could therefore not exclude that the broad spatial range of symptoms observed in *DD45::lis (RNAi)* was due to systemic downregulation

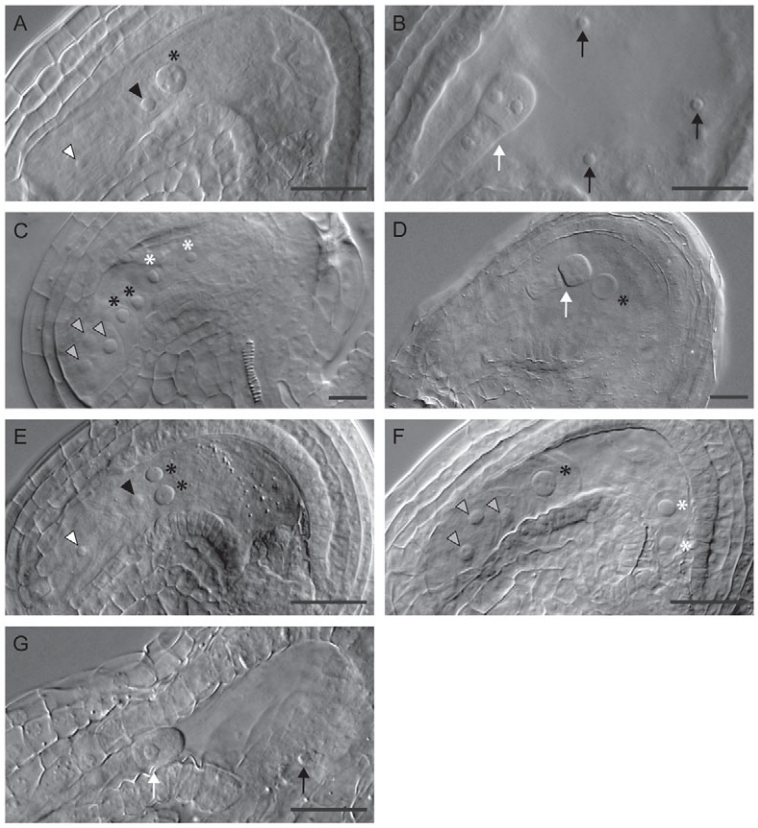

Fig. 2. Effect of *lis (RNAi)* on gametophyte development. (**A**) Wild-type female gametophyte showing one of two synergid nuclei and one egg cell nucleus. The secondary central cell nucleus results from fusion of two polar nuclei. Antipodals have undergone programmed cell death and are no longer visible. (**B**) Wild-type ovule after fertilization containing embryo and endosperm. (**C**) *lis* female gametophyte with enlarged and mis-polarized synergid nuclei. The egg and the synergids are consequently indistinguishable (grey arrowheads). Polar nuclei are unfused and antipodal nuclei are protruded. (**D**) *lis* ovule after fertilization containing a young embryo and undeveloped endosperm. (**E**) Ovule of T2-4 *DD45::lis (RNAi)* transgenic line exhibiting unfused polar nuclei. (**F**) Female T2-7 gametophyte exhibiting enlarged and mis-polarized synergid nuclei and protruded antipodal cells. (**G**) T2-4 ovule after fertilization containing a young embryo and undeveloped endosperm. White arrowheads, synergid nucleus; black arrowheads, egg cell nucleus; grey arrowhead, egg cell-like nucleus; black asterisks, central cell nucleus; white asterisks, antipodal nucleus; white arrows, embryo; black arrows, endosperm. Scale bars: 25 μm.

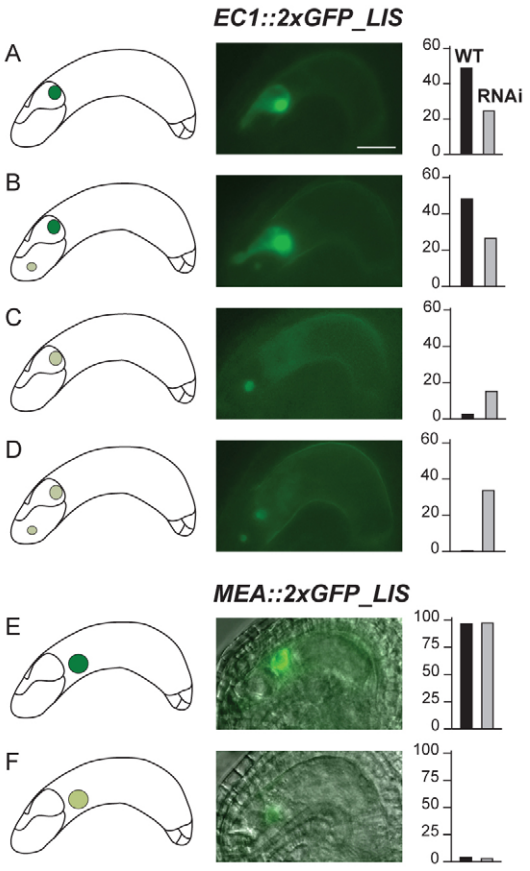

Fig. 3. *DD45::lis (RNAi)* results in egg cell-specific reduction of a 2xGFP_LIS reporter. (A-F) Frequencies of different fluorescence patterns (in %) observed in *DD45::lis (RNAi)/-* (T2-4) plants containing *EC1::2xGFP_LIS/–* (A-D) and *MEA::2xGFP_LIS/–* (E,F). (**A**,**B**) Strong egg cell expression with (B) and without (A) weak synergid expression. (**C**,**D**) Weak egg cell expression with (D) and without (C) weak synergid expression. (**E**,**F**) Strong (E) and weak (F) expression in the central cell. Only GFP-positive ovules were scored (for *EC1::2xGFP_LIS/–*: 37% in wild type, *n*=506; 37.4% in *DD45::lis (RNAi)/–*, *n*=524; for *MEA::2xGFP_LIS/–*: 47.6% in wild type, *n*=277; 46.7% in *DD45::lis (RNAi)/–*, *n*=287). Black bar, wild type; grey bar, *DD45::lis (RNAi)/–*.

of *LIS* in the entire female gametophyte. To test whether *DD45::lis (RNAi)* specifically reduced *LIS* transcript amounts in the egg cell, we introduced a *GFP*-tagged *LIS* cDNA under the control of the *EC1* promoter (Ingouff et al., 2009) into the *DD45::lis (RNAi)/–* T2-4 line. *EC1* confers expression specifically to the egg cell in Columbia accessions (Ingouff et al., 2009). However, in Landsberg *erecta* plants, we occasionally detected an additional moderate expression in synergids and a very weak signal in the central cell. In wild-type plants containing *EC1::2xGFP_LIS/–*, 96.8% of all ovules showing fluorescence exhibited a strong GFP signal in the egg cell, whereas 3.2% of the gametophytes showed a weak egg cell signal (Fig. 3A-D). In 48.6% of the GFP positive ovules, we detected an additional signal in the synergids. By contrast, in *DD45::lis (RNAi)/–* (line T2-4), *EC1::2xGFP_LIS/–* plants, strong egg cell expression was only observed in 51% of GFP-positive ovules, whereas 49% exhibited only a weak egg cell GFP signal. Importantly, the GFP signal in the synergids was not reduced (Fig. 3A-D). Similar results were gained with a second *DD45::lis (RNAi)/–* line (T2-7) (see supplementary material Fig. S2). Together, these results indicate that *DD45::lis (RNAi)* reduced *LIS* transcript amounts significantly in the egg cell but not in synergids. To assess *LIS* transcript amounts in the central cell of *DD45::lis (RNAi)/–* plants, we introduced a central cell-specific *MEA::2xGFP_LIS* construct. The GFP signal in the central cell of *MEA::2xGFP_LIS/–* plants was indistinguishable from that observed in *MEA::2xGFP_LIS/–, DD45::lis (RNAi)/–* line T2-4 (Fig. 3E,F), suggesting that *DD45::lis (RNAi)* did not significantly affect *LIS* mRNA levels in the central cell.

We could not, however, exclude the possibility that *LIS* transcript amounts were reduced in the synergids and central cell below the detection level. To assess the effects of reduced *LIS* transcript in the cells adjacent to the egg cell, we therefore targeted the *RNAi* construct to the synergids, central cell and antipodal cells, using the cell-specific promoters *DD2* (Steffen et al., 2007), *MEA* (Groß-Hardt et al., 2007) and *HSFa2* (Kägi et al., 2010), respectively. Fortunately, not only the promoter of *MEA* but also the one of *DD2* is additionally active after fertilization in the endosperm allowing direct assessment of the functionality of both constructs (Fig. 4 B,C) (Köhler et al., 2003; Steffen et al., 2007). Of 15 independent *MEA::lis (RNAi)/–* transformants, six showed defects after fertilization (see supplementary material Table S1B). These plants exhibited various degrees of reduced endosperm formation and the seeds eventually aborted. Two lines arrested at the megaspore stage resulting in sterile ovules. As we never detected *MEA* expression at this stage (data not shown), the defect is likely to be due to nonspecific defects caused by the T-DNA insertion. Importantly, female gametophyte development was indistinguishable from wild type in the remaining lines. Experiments using the *DD2* promoter yielded similar results. Of 18 transgenic lines analyzed, 15 exhibited an increased number of aborted seeds (see supplementary material Table S2A), which could be traced back to severe defects in endosperm development. Two of the *DD2::lis (RNAi)/–* lines revealed defects in the maturing female gametophyte. However, by introducing *EC1::2xGFP_LIS* as a reporter for *LIS* transcript amounts, we could show that in line O *LIS* mRNA levels appeared to be not reduced, whereas in line C *LIS* mRNA levels were additionally reduced in the egg cell (see supplementary material Fig. S4). These results suggest that the defects are not caused by a synergid-specific reduction of *LIS* transcripts.

Finally, we expressed *lis (RNAi)* under control of the antipodal promoter *HSFa2* (Fig. 4D, supplementary material Table S2B). None of the 20 independent transformants analyzed exhibited a defect in the mature female gametophyte. Together, our data indicate that a moderate reduction of *LIS* transcript amounts in the synergids, central cell and antipodal cells is tolerated, whereas reduction of *LIS* transcript in the egg cell affects all female gametophytic cells. This indicates that cell differentiation in the female gametophyte is orchestrated by the egg cell through a *LIS*-dependent lateral inhibition signal (Fig. 4E).

Together, we have shown that *DD45::lis (RNAi)* targets *LIS* specifically in the egg cell. Surprisingly, downregulating *LIS* in the egg cell only affects the development of the entire female gametophyte with terminal consequences: the resulting female gametophyte does not only show severe morphological abnormalities but additionally reduced fertility. These results imply that egg cell integrity is required for completion of female gametophyte development. Among the defects observed in *DD45::lis (RNAi)* gametophytes is the failure of polar nuclei fusion, indicating that this process, which is a prerequisite for

A

genotype	unfused pn (%)	syn nuclei = ec nuclei (%)	ap present (%)	n
wild type	4.9	0.0	4.2	321
lis/LIS	51.2	56.9	42.4	514
DD45::lis (RNAi)/- T2-4	45.9	0.0	19.2	356
DD45::lis (RNAi)/- T2-7	25.5	33.0	35.5	341
DD45::lis (RNAi)/- T2-9	27.9	4.1	7.3	368
DD45::lis (RNAi)/- T2-17	33.2	4.5	14.5	378

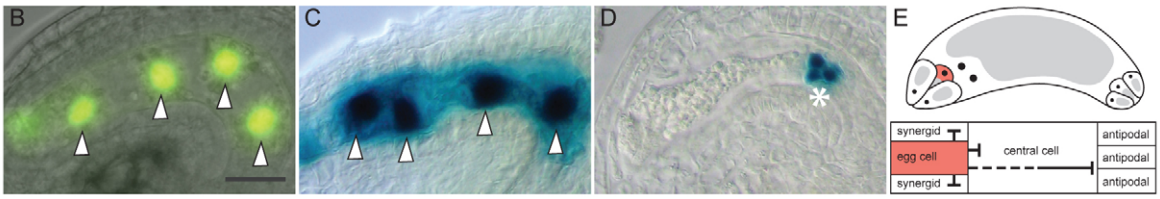

Fig. 4. ***DD45::lis (RNAi)* affects the development of all female gametophytic cells.** (**A**) Morphological characterization of four *DD45::lis (RNAi)/–* lines. (**B**,**C**) Expression of *DD2::NLS_3xGFP* (B) and of *MEA::NLS_GUS* (C) in the four-nucleate endosperm. (**D**) Expression of *HSFa2::NLS_GUS* in antipodals. (**E**) Model of LIS function. pn, polar nuclei; syn, synergids; ec, egg cell; ap antipodals; white arrowheads, endosperm nucleus; white asterisk, antipodal cells. Scale bar: 25 μm

endosperm formation, is possible only in the presence of a functional egg cell. The *LIS* dependent cell-cell communication thus suggests a safeguard mechanism, whereby the formation of endosperm, which is of high metabolic cost, is only enabled in the presence of a fertilizable egg cell.

Acknowledgements
We thank Ramona Joachim for technical support, Gerd Jürgens and Thomas Laux for providing the *NLS_3xGFP* and *NLS_3xdsRed* reporters, respectively, and Felicity De Courcy and members of the Groß-Hardt lab for critical reading of the manuscript.

Funding
This work was supported by the Rosenkranz holding and by a grant from the Deutsche Forschungsgemeinschaft to R.G. [GR 1833/5-1].

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.075234/-/DC1

References

Amien, S., Kliwer, I., Marton, M. L., Debener, T., Geiger, D., Becker, D. and Dresselhaus, T. (2010). Defensin-like ZmES4 mediates pollen tube burst in maize via opening of the potassium channel KZM1. *PLoS Biol.* **8**, e1000388.
Baulcombe, D. (2004). RNA silencing in plants. *Nature* **431**, 356-363.
Dresselhaus, T. (2006). Cell-cell communication during double fertilization. *Curr. Opin. Plant Biol.* **9**, 41-47.
Friedman, W. E. (2006). Embryological evidence for developmental lability during early angiosperm evolution. *Nature* **441**, 337-340.
Groß-Hardt, R., Kägi, C., Baumann, N., Moore, J. M., Baskar, R., Gagliano, W. B., Jürgens, G. and Grossniklaus, U. (2007). LACHESIS restricts gametic cell fate in the female gametophyte of Arabidopsis. *PLoS Biol.* **5**, e47.
Han, Y. Z., Huang, B. Q., Zee, S. Y. and Yuan, M. (2000). Symplastic communication between the central cell and the egg apparatus cells in the embryo sac of Torenia fournieri Lind. before and during fertilization. *Planta* **211**, 158-162.
Higashiyama, T., Yabe, S., Sasaki, N., Nishimura, Y., Miyagishima, S., Kuroiwa, H. and Kuroiwa, T. (2001). Pollen tube attraction by the synergid cell. *Science* **293**, 1480-1483.
Huck, N., Moore, J. M., Federer, M. and Grossniklaus, U. (2003). The Arabidopsis mutant feronia disrupts the female gametophytic control of pollen tube reception. *Development* **130**, 2149-2159.
Ingouff, M., Sakata, T., Li, J., Sprunck, S., Dresselhaus, T. and Berger, F. (2009). The two male gametes share equal ability to fertilize the egg cell in Arabidopsis thaliana. *Curr. Biol.* **19**, R19-R20.
Kägi, C., Baumann, N., Nielsen, N., Stierhof, Y. D. and Groß-Hardt, R. (2010). The gametic central cell of Arabidopsis determines the lifespan of adjacent accessory cells. *Proc. Natl. Acad. Sci. USA* **107**, 22350-22355.
Köhler, C., Hennig, L., Spillane, C., Pien, S., Gruissem, W. and Grossniklaus, U. (2003). The Polycomb-group protein MEDEA regulates seed development by controlling expression of the MADS-box gene PHERES1. *Genes Dev.* **17**, 1540-1553.
Moll, C., von Lyncker, L., Zimmermann, S., Kägi, C., Baumann, N., Twell, D., Grossniklaus, U. and Groß-Hardt, R. (2008). CLO/GFA1 and ATO are novel regulators of gametic cell fate in plants. *Plant J.* **56**, 913-921.
Pagnussat, G. C., Alandete-Saez, M., Bowman, J. L. and Sundaresan, V. (2009). Auxin-dependent patterning and gamete specification in the Arabidopsis female gametophyte. *Science* **324**, 1684-1689.
Raghavan, V. (1997). *Molecular Embryology of Flowering Plants*. Cambridge, UK: Cambridge University Press.
Rotman, N., Rozier, F., Boavida, L., Dumas, C., Berger, F. and Faure, J. E. (2003). Female control of male gamete delivery during fertilization in Arabidopsis thaliana. *Curr. Biol.* **13**, 432-436.
Steffen, J. G., Kang, I. H., Macfarlane, J. and Drews, G. N. (2007). Identification of genes expressed in the Arabidopsis female gametophyte. *Plant J.* **51**, 281-292.
Takada, S. and Jürgens, G. (2007). Transcriptional regulation of epidermal cell fate in the Arabidopsis embryo. *Development* **134**, 1141-1150.