Nucleoporin MOS7/Nup88 is required for mitosis in gametogenesis and seed development in *Arabidopsis*

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Angiosperm reproduction is characterized by alternate diploid sporophytic and haploid gametophytic generations. Gametogenesis shares similarities with that of animals except for the formation of the gametophyte, whereby haploid cells undergo several rounds of postmeiotic mitosis to form gametes and the accessory cells required for successful reproduction. The mechanisms regulating gametophyte development in angiosperms are incompletely understood. Here, we show that the nucleoporin Nup88-homolog MOS7 (Modifier of Snc1,7) plays a crucial role in mitosis during both male and female gametophyte formation in *Arabidopsis thaliana*. Using a mutagenesis screen, we identify the *mos7-5* mutant allele, which causes ovule and pollen abortion in *MOS7/mos7-5* heterozygous plants, and preglobular stage embryonic lethality in homozygous *mos7-5* seeds. During interphase, we show that MOS7 is localized to the nuclear membrane but, like many nucleoporins, is associated with the spindle apparatus during mitosis. We detect interactions between MOS7 and several nucleoporins known to control spindle dynamics, and find that in pollen from *MOS7/mos7-5* heterozygotes, abortion is accompanied by a failure of spindle formation, cell fate specification, and phragmoplast activity. Most intriguingly, we show that following gamete formation by *MOS7/mos7-5* heterozygous spores, inheritance of either the *MOS7* or the *mos7-5* allele by a given gamete does not correlate with its respective survival or abortion. Instead, we suggest a model whereby MOS7, which is highly expressed in the Pollen- and Megaspore Mother Cells, enacts a dosage-limiting effect on the gametes to enable their progression through subsequent mitoses.

microtubule dynamics | gametogenesis | nuclear pore complex | mitosis | plant reproduction

Amost striking difference between the developmental approaches of plants and animals is the intervention of mitotic divisions between meiosis and gamete formation in plants (1). Following meiosis, successive mitotic divisions of haploid cells produce both mature gametes and complex gametophytic structures encasing them, facilitating fertilization. The regulation of gametophyte formation is not completely understood, but is characterized by elegant cell, nuclear and organelle migration, led by microtubule activity (2, 3).

Arabidopsis thaliana female gametophyte formation begins with meiosis of the diploid megaspore mother cell (MMC), forming four haploid megaspores. Female Gametogenesis stages (FG) follow, with megaspores migrating to the micropylar end of the gametophyte (FG1) (4). Three megaspores degenerate, one undergoes mitosis and nuclei migrate to opposite poles, likely facilitated by development of a large central vacuole (FG2). Two additional mitotic divisions generate the eight-nuclear FG5 female gametophyte. Nuclei migrate according to their cell-fate and simultaneous cytokinesis (cellularization) takes place, followed by polar nuclei fusion to form the homo-diploid central cell (FG6). At the micropylar pole lie the synergid cells and egg, and three antipodal cells are located at the opposite pole, which degenerate, marking formation of the mature female gametophyte (FG7) (5).

Male gametogenesis also involves precise nuclear migration and both symmetric and asymmetric cell divisions. Meiosis of the diploid pollen mother cell (PMC) produces a tetrad of haploid microspores (6). Unlike female megaspores, however, and reminiscent of mammalian spermatogenesis, all four microspores survive to undergo asymmetric mitotic division, Pollen Mitosis I (PMI), each producing a generative cell engulfed in the cytoplasm of a vegetative cell. The generative cell undergoes a second mitosis (PMII) to form two identical sperm cells. The vegetative nucleus gives rise to the pollen tube (7).

Around half of the genes so far identified as functioning in gametogenesis regulate both female and male gametophyte formation, and are involved in common cellular processes that occur in both such as mitosis, vacuole formation, cellularization, nuclear migration and cell expansion (8). To identify genes involved in gametogenesis, we performed a mutagenesis screen for lines showing seed and ovule lethality. We identified *mos7-5*, a *MOS7* (*Modifier Of Snc1,7*) mutant. MOS7 is homologous to human and *Drosophila melanogaster* nucleoporin protein Nup88. Nucleoporins comprise nuclear pore complexes (NPCs) which traffic proteins and RNA between the nucleus and cytoplasm (9). Previously identified *mos7* mutant alleles were *mos7-1*, a hypomorphic loss-of-function allele that revealed the importance of MOS7-mediated nucleocytoplasmic passage of defense proteins for plant innate immunity (10); *mos7-2* and *mos 7–4*, both embryonic lethal when homozygous (10). Thus, MOS7, as well as an immune function,

Significance

During plant reproduction, meiosis generates haploid spores that undergo mitoses, forming gametophytes, in male and female parts of the flower. Haploid cells within gametophytes differentiate into sperm and eggs, which form the next generation. We discovered that the *MOS7* (Modifier of Snc1,7) gene plays a critical role in microtubule organization and dynamics during mitosis in *Arabidopsis* sexual reproduction. MOS7 absence results in impaired gametogenesis, failure of ovule and pollen development, and seed abortion. Although mitoses during gamete formation are unique to plants, the regulation of mitosis itself is highly conserved between plants and vertebrates. Notably, we find that MOS7 protein primarily produced prior to meiosis is inherited by gametophytes, and, only if there is sufficient MOS7 protein, will mitosis correctly occur during gametogenesis.

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likely has a developmental function that is currently unknown, although alterations in human *Nup88* expression result in multipolar spindles and promote carcinogenesis (11), so MOS7 may have a role in plant cell division.

Results

A T-DNA Mutant Screen for Defects in Reproduction Identifies a Nucleoporin Gene. We mutagenized *Arabidopsis thaliana* using a T-DNA activation vector that activates flanking sequences and inactivates inserted genes (12). Mutagenized seed pools were screened for 50% abortion by opening siliques and counting normal and aborted seeds. We identified such a mutant and found that seeds were either aborted due to ovule arrest (Fig. 1*A*) or arrest at the globular stage of embryo development (Fig. 1*C*). Mutants also displayed a large amount of aborted pollen (Fig. 1*B*). Mutant lines were maintained as heterozygotes because their defects in reproduction made it impossible to generate homozygous mutant progeny.

PCR-based fine-mapping of the T-DNA located its insertion in exon 3 of At5g05680, known as *MOS7* (*Modifier Of Snc1,7*) (Fig. 1*D*), which encodes a homolog of human nucleoporin88 (Nup88), and we denoted the mutant allele *mos7-5*. Using the mutant as a pollen donor, we backcrossed the *mos7-5* mutation to wild-type Col-0 five times to remove unlinked mutations, germinating each generation on plain agar plates and genotyping using PCR to amplify the T-DNA/exon 3 junction in *MOS7*. For each backcross, we observed absolute cosegregation of the reproductive phenotypes with the T-DNA in *MOS7*. That is, none of the wild-type progeny showed any reproductive defects, whereas all heterozygous progeny displayed seed abortion, ovule abortion, and the production of defective pollen (Fig. 1 *A–C*, and *SI Appendix*, Table S1). Therefore, either the T-DNA inserted in *MOS7*, or another very closely linked mutation, is the cause of the phenotypes we observe in *mos7-5*.

Next, we obtained three other mutant lines from the ABRC at Ohio State University: *mos7-2* (*Salk_129301*, T-DNA in intron 3), *mos7-3* (*Salk_085349*, T-DNA in exon 6) and *mos7-4* (*CS822857*, T-DNA in exon 3) (Fig. 1*D*). As with *mos7-5*, no homozygous plants were obtained from any of *mos7-2*, *mos7-3*, or *mos7-4* mutant lines, and heterozygotes for each mutant allele exhibited the same level of seed abortion as *mos7-5* (*SI Appendix*, Table S1). All except *mos7-3* exhibited the same severity of ovule and pollen abortion (*SI Appendix*, Table S1). In contrast, the *mos7-1* allele analyzed by Cheng et al. (10) is due to a 12-bp (four amino acid) in-frame deletion at the MOS7 N terminus, that still makes some functional MOS7, and displays no reproductive phenotypes. We analyzed expression levels of *MOS7* in the floral buds of *mos7-2*, *mos7-3*, *mos7-4*, and *mos7-5* heterozygotes, compared with wild-type, and found levels to be reduced to half in each mutant (Fig. 1*E*), contrary to the four genes immediately flanking *MOS7*, whose expression did not change in *mos7-5* (*SI Appendix*, Fig. S1). These data strongly suggest that loss-of-function mutations in *MOS7* cause a distinctive reproductive syndrome, epitomized by 50% ovule abortion, pollen abortion and embryo arrest.

To prove that the *mos7-5* mutation was responsible for the observed phenotypes, we generated a transgene consisting of 2.0 kb of sequence upstream of *MOS7*, followed by the *MOS7* genomic sequence and GFP (*Pro2.0kb:MOS7:GFP*), which did not contain any intact *Arabidopsis* genes other than *MOS7*. When we introduced the transgene into the *mos7-5* heterozygous genetic background, we observed rescue of the seed, ovule and pollen abortion. In fact, the transgene enabled the appearance of homozygous *mos7-5/mos7-5* plants in the progeny of our rescue lines (*SI Appendix*, Tables S1 and S2). Thus, it is the mutation in *MOS7* that causes the 50% ovule abortion, pollen abortion and embryo arrest we observed.

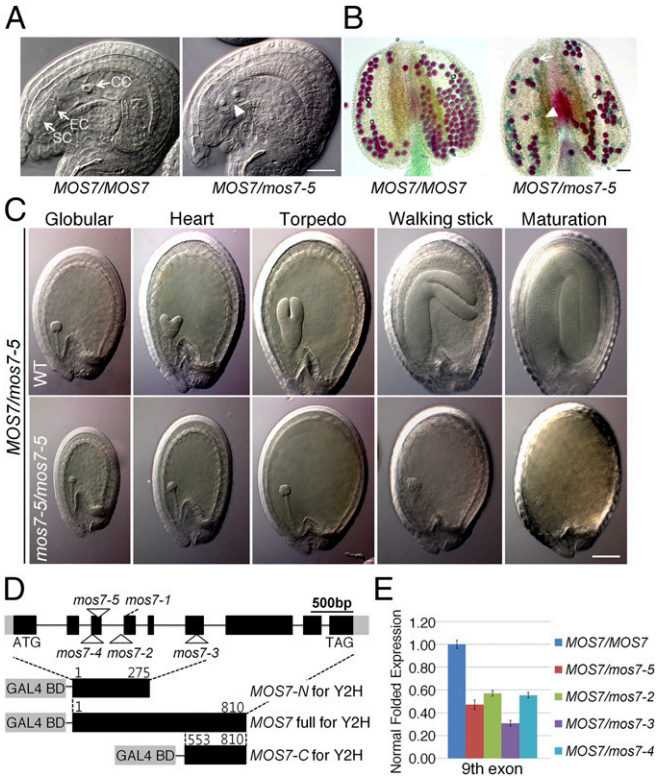

Fig. 1. Characterization of *mos7-5* mutant gametophytic and seed development. (*A*) Prefertilization MOS7/MOS7 ovule and arrested ovule from *MOS7/mos7-5* plants, containing two-nucleated female gametophyte (arrowhead). (*B*) Alexander staining of wild-type *MOS7/MOS7* and *MOS7/mos7-5* stamen. Viable pollen grains stain red (arrow) and nonviable shrunken grains green (arrowhead). (*C*) Developing seeds from *MOS7/mos7-5* heterozygous plants. Normally developing (*Upper*) and defective seeds (*Lower*) at each stage taken from same siliques. (*D*) *MOS7* gene structure, showing the four mutant alleles (TDNA = triangle). Domains used for Y2H also shown. Black box, translated exon; gray box, untranslated exon; line, intron. (*E*) Quantitative RT-PCR for MOS7 exon 9, showing floral bud expression decrease in each of the mutants. Error bars indicate SEM of three biological replicates. CC, central cell; EC, egg cell; SC, synergid cell. (Scale bars: 25 μm in *A* and *B* and 100 μm in *C*.)

Mutations in *MOS7* Cause Mitotic Defects During Female Gametogenesis and a Failure of Cells to Acquire Proper Identity. In *mos7-2*, *mos7-4*, and *mos7-5* heterozygotes, ∼50% of the ovules were arrested (*SI Appendix*, Table S1). To delineate the cause of ovule arrest, we analyzed developing ovules using confocal microscopy. We found no discernible differences within *MOS7/mos7-5* ovule populations during meiosis and up to FG1 (6) (Fig. 2*B*). However, following FG1, an unusually large and strongly autofluorescent mass was seen in half of the ovules from *mos7-5* and *mos7-2* plants (Fig. 2*C*). Subsequent mitotic divisions were reduced in number, resulting in either a single nucleus or two nuclei inside the female gametophyte (Fig. 2 *D–F*). In the gametophytes with two nuclei, a small vacuole appeared in some cases, but did not develop further (Fig. 2 *E* and *F*, *Middle*). As such, either minimal or no nuclear migration occurred, and no large central vacuoles were detected (Fig. 2 *E* and *F*). Thus, at the morphological level, megasporogenesis in *mos7* mutants seems to proceed normally, with the 50% ovule arrest apparently due to a failure of mitosis and cellular migration in female gametogenesis.

To reveal the cellular identity of the large autofluorescent mass and the nuclei that were formed, we introduced cell-specific markers into *mos7-5* heterozygous plants using genetic crosses with the following transgenic lines: *DD1:GFP* (antipodal cell expression), *DD2:GFP* (synergid cell expression), *DD45:GFP* (egg cell expression), and *DD7: GFP* (central cell expression) (13). Nonarrested ovules showed GFP expression for all markers, however none of the arrested ovules displayed GFP expression (*SI Appendix*, Fig. S2). In contrast, when we introduced *FM2:GUS*,

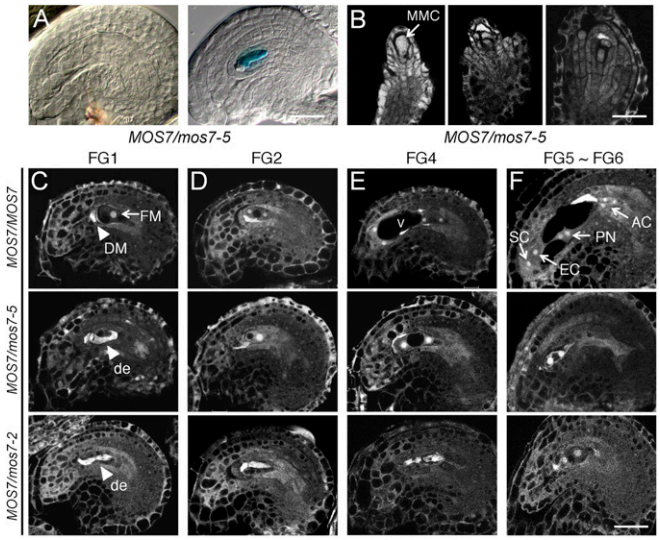

Fig. 2. Defects in female gametogenesis of the *MOS7/mos7* mutants. (*A*) DIC image of *FM2:GUS*, a functional megaspore (FM) marker, absent from mature wild-type ovule at FG7, but expressed in arrested ovule of the same pistil in a *FM2:GUS/FM2:GUS MOS7/mos7-5* plant. (*B*) Apparently normal meiotic divisions of diploid MMC (arrow) to FG1 stage ovule of a *MOS7/mos7-5* plant. (*C–F*) Wild-type (*Top*), *MOS7/mos7-5* (*Middle*), and *MOS7/mos7-2* (*Bottom*) ovules at same growth point. (*C*) Meiotic products of an FG1 WT ovule, FM (arrow) and DM (arrowhead). FM undergoes three mitoses to form an octonucleate female gametophyte (*F*; FG5 stage). *MOS7/mos7-5* and *MOS7/mos7-2* ovules had abnormal FG1 female gametophytes and subsequent mitoses failed. Degenerated nuclei (arrowhead) were visualized by their strong autofluorescence. AC, antipodal cell; de, degenerated embryo sac; DM, degenerated megaspore; EC, egg cell; FM, functional megaspore; MMC, megaspore mother cell; PN, polar nucleus; SC, synergid cell; V, vacuole. (*B–F*) Confocal images, in which the cytoplasm is gray, vacuoles are black, and nucleoli are white. (Scale bars: 25 μm.)

a functional megaspore marker (14), GUS was strongly expressed in arrested ovules (Fig. 2*A*). Therefore, the cells in the arrested female gametophyte have a functional megaspore identity, even after completing the first mitosis in megagametogensis. Thus, although megasporogenesis appears to proceed normally, megagametogenesis does not occur in *mos7* mutants.

Mutations in *MOS7* Cause Mitotic Defects in Male Gametogenesis. Pollen development in *MOS7/mos7-5* heterozygous mutant lines was also abnormal, with an abundance of aborted pollen within *MOS7/mos7-5* anthers (Fig. 1*B*). To visualize mitotic defects during microgametogenesis in *mos7-5*, the *ProHTR12:HTR12:GFP* transgene, a centromere marker (15), was crossed into a *MOS7/mos7-5* heterozygote. Consistent with Chen et al. (16), in microspores from wild-type plants, HTR12:GFP was observed at all five chromosomal centromeres in the haploid microspore (Fig. 3*A*). This pattern was also seen in the pollen of *MOS7/mos7-5* plants (Fig. 3*E*). Following wild-type pollen mitosis I (PMI) (Fig. 3*B*), in the early bicellular stage, HTR12:GFP was observed at all five centromeres in both the vegetative and generative cells. However, in ~50% of the pollen from *MOS7/mos7-5* plants, division does not appear to have occurred, with only five centromeres stained (white arrowheads, Fig. 3*F*). In addition, localization of HTR12:GFP to the centromeres is not punctate as in the wild type, and diffuse GFP fluorescence is observed throughout the nucleus (Fig. 3*F*). Approximately 45% of the pollen from *MOS7/mos7-5* plants was found to be at this stage and did not develop further (Fig. 3*G*). In late bicellular stage wild-type pollen, coincident with decondensation of chromatin within the vegetative nucleus, HTR12:GFP disappears from the vegetative cell, but is still observed at all five centromeres of the generative cell nucleus (Fig. 3*C*). In the pollen from *MOS7/mos7-5*, there were rare occasions (5%) of apparent mitosis, and two distinct nuclei could be observed (Fig. 3*I*). However, division was delayed and the two nuclei displayed abnormal morphology. Although some centromeres could be identified (white arrows, Fig. 3*I*), HTR12:GFP fluorescence was unusually diffuse throughout both nuclei (Fig. 3*I*). During wild-type pollen mitosis II (PMII), the generative cell divides to produce two sperm cells, and HTR12:GFP staining can be observed at the five centromeres in each sperm cell, but remains absent in the vegetative nucleus (Fig. 3*D*). The aberrantly developing pollen, comprising half of the pollen in *MOS7/mos7-5* anthers, had all aborted by this stage (Fig. 3 *H* and *J* and *K*), and the external periphery of pollen grains had shrunk (Fig. 3 *H* and *J*). Thus, we show that *mos7* mutants exhibit extensive defects in both female and male gametogenesis due to a failure of mitosis and of the appropriate specification of nuclei required to form functional gametophytes.

Inheritance of the *mos7-5* Mutant Allele Does Not Cosegregate with Defects in Either Female or Male Gametophytes. Heterozygous *mos7-5* pistils before fertilization contained approximately equal

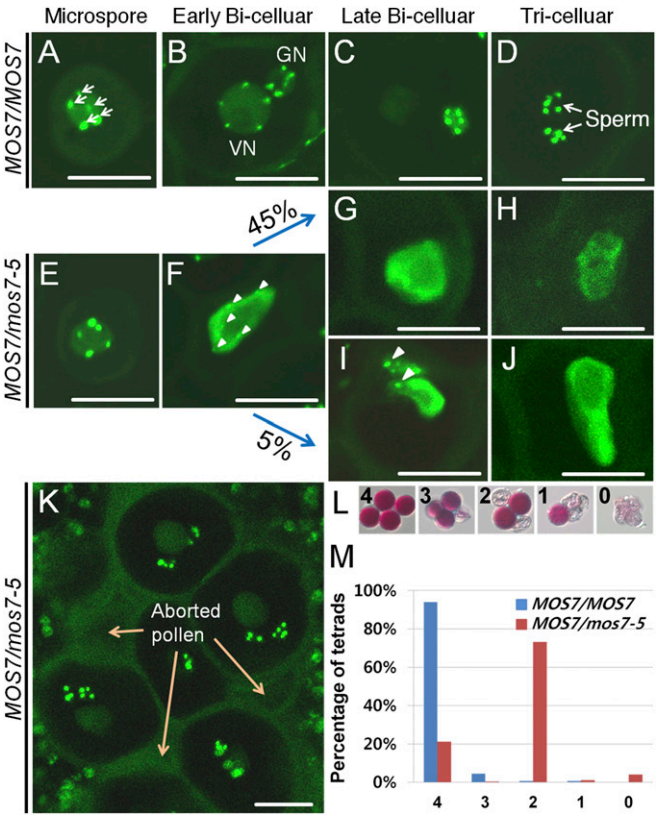

Fig. 3. Male gametogenesis defects in *MOS7/mos7* mutants. (*A–K*) HTR12:GFP protein observed as centromeric bright dots. Confocal images of developing pollen grains from wild-type (*A–D*) and *MOS7/mos7-5* mutants (*E–J*). (*A* and *E*) Microspore with five centromeres (arrow). (*B*) Early bicellular stage after PMI. (*C*) Late bicellular stage, GFP expression only in generative cell. (*D*) Tricellular stage after PMII, GFP expression in two sperms (arrows). (*F*) Defect in karyokinesis with diffuse GFP. Five arrowheads indicate strong GFP fluorescence at centromeres. (*G*) Nondivided, abnormal nuclear structure with diffused GFP. (*H*) Shrunken pollen grain. (*I*) 5% of defective pollen undergo delayed cell division. Arrowheads indicate centromeres. (*J*) Shrunken pollen grain. (*K*)Viable and aborted pollen present together in *MOS7/mos7-5* anthers, after PMII. (*L* and *M*) Transmission analysis of the *mos7-5* mutant allele using *qrt/qrt MOS7/mos7-5* mutants. (*L*) DIC images of Alexander staining of the *MOS7/mos7-5* pollen in a *qrt/qrt* background. 4, normal tetrad; 3, 3:1 viable:aborted tetrad; 2, 2:2 viable:aborted tetrad; 1, 1:3 viable:aborted tetrad; 0, all aborted tetrad. (*M*) % tetrads containing 4, 3, 2, 1 or 0 normal pollen grains in wild type versus *MOS7/mos7-5* mutant. GN, generative cell; VN, vegetative cell. (Scale bars: 10 μm.)

numbers of normal full-size ovules versus small aborted ovules (420:400, 1:1, $\chi^2 = 0.49$, $P > 0.48$; Fig. 1*A* and *SI Appendix*, Table S1). We therefore hypothesized that the aborted ovules contained a female gametophyte with a mutant *mos7-5* allele, which would result in 50% ovule abortion, thus, viable progeny from a *mos7-5* heterozygous plant pollinated with wild-type pollen would be homozygous for the wild-type allele, *MOS7*. Surprisingly, however, we obtained approximately equal numbers of homozygous wild-type *MOS7* and heterozygous *mos7-5* F1 progeny (134:110, 1:1, $\chi^2 = 2.36$, $P > 0.12$; *SI Appendix*, Table S3). Therefore, in the above genetic cross, wild-type and *mos7-5* female gametophytes functioned equally well in transmitting their respective alleles. Presumably, the wild-type and mutant alleles are represented equally in the female gametophytes of the aborted ovules, as well.

Approximately half of all pollen grains produced by heterozygous *mos7-5* plants abort their development and are nonviable (Figs. 1*B* and 3*K*). When wild-type plants were pollinated with pollen from *mos7-5* heterozygous plants, of the F1 progeny, 44% were *mos7-5* heterozygotes (*SI Appendix*, Table S3). Thus, pollen from a *mos7-5* heterozygous plant transmitted the wild-type *MOS7* allele only slightly more efficiently than the mutant *mos7-5* allele (424:328, 1:1, $\chi^2 = 12.3$, $P > 0.0005$). So, in heterozygous *mos7-5* plants, the *mos7-5* genotype of the male gamete does not predict whether or not it will be viable either. To explore this idea further, we analyzed the products of individual premeiosis pollen precursors, the PMC. *QUARTET* (*qrt*) mutants fail to undergo microspore separation, releasing viable pollen tetrads (>95% shown by Alexander Staining, Fig. 3 *L* and *M*), allowing the fate of each of the four progeny from individual PMCs to be assessed (17). We observed a distribution of tetrads ($n = 2{,}093$) with all possible viable to nonviable ratios (Fig. 3 *L* and *M*). Whereas a 1:1 viable:nonviable ratio was the most common (73%), we also detected a significant number of 4:0 (21%) and 0:4 (4%) tetrads, these latter two groups demonstrating complete lack of cosegregation of the *mos7-5* mutation with the abortion phenotype. Taken together, these results suggest that MOS7 has a critical function before meiosis and gametogenesis in the MMC and PMC. Consistent with this, although *MOS7* is expressed throughout the plant (*SI Appendix*, Fig. S3 *A–S*), we observed particularly high expression of *MOS7:GFP* in the MMC and PMC (*SI Appendix*, Fig. S3 *H*, *I*, and *M*).

Mutations in *MOS7* Cause Seed Abortion. Heterozygous *mos7-5* plants have siliques with approximately equal numbers of normal full-size ovules and small aborted ovules (Fig. 1 and *SI Appendix*, Table S1). The full-size ovules contain viable female gametophytes, which when fertilized, produce seeds. When a *mos7-5* heterozygote is pollinated with wild-type pollen, all of the F1 seeds are viable (*SI Appendix*, Table S1). In contrast, when pollen from a *mos7-5* heterozygote is used, ~25% of the fertilized F1 seed abort their development at the preglobular stage of embryogenesis (Fig. 1*C* and *SI Appendix*, Table S1). We carried out quantitative PCR and established that the aborting seeds were likely homozygous for the *mos7-5* allele, and that the viable seeds segregated 1.65 (612):1(370) *mos7-5* heterozygote: wild-type *MOS7* homozygote (*SI Appendix*, Fig. S4 and Table S4). This result is consistent with *mos7-5* heterozygous plants producing 50% viable female and male gametophytes, approximately half with a wild-type *MOS7* allele and half with a mutant *mos7-5* allele, as shown above (*SI Appendix*, Tables S1 and S3). Thus, MOS7 plays an essential role after fertilization during embryo development, during which time the mutant *mos7-5* allele behaves in a manner recessive to the wild-type allele.

MOS7 Colocalizes with Mitotic Microtubules During Cell Division. To gain a better understanding of MOS7 function, we searched for MOS7-interacting proteins using a yeast -2- hybrid (Y2H) library screen. Nucleoporins form large multiprotein complexes with one another, and consistent with this, we found that MOS7 interacts with nucleoporins RNA export 1 (Rae1) and Nup98a, its binding partner (*SI Appendix*, Fig. S5*A*) (18). Interestingly, in a preliminary analysis we observed arrest after the first mitosis in *rae1* mutant female gametophytes (*SI Appendix*, Fig. S6). Both Rae1 and Nup98 colocalize with spindle microtubules, specifically alpha-tubulin, and are required for normal spindle assembly (19, 20). We then investigated whether MOS7 also colocalizes with the spindle in wild-type plants. We visualized the localization of both MOS7:RFP (red) and the microtubule marker, alpha-tubulin 6 TUA6:GFP (green), in dividing root tip cells of plants expressing both transgenes. As cells progressed into mitosis, we observed MOS7 colocalization with mitotic microtubules and the cell plate-forming zone until the end of cytokinesis (Fig. 4 *A* and *B* and *SI Appendix*, Fig. S7 *A–C*). After mitosis, MOS7 was found to be enriched at the newly-formed nuclear membrane (Fig. 4*C*).

Our Y2H screen also identified dynein light chain type 1 family protein (At4g15930) and a kinectin-related protein (At2g17990) as MOS7-interacting proteins (*SI Appendix*, Fig. S5*A*). We investigated the in vivo interaction of these proteins to MOS7 in *Arabidopsis* protoplasts using a Bimolecular Fluorescence Complementation (BiFC) assay, detecting a strong reconstituted YFP signal in the cytoplasm (*SI Appendix*, Fig. S5*B*). We were unable to detect a positive BiFC interaction between MOS7 and alpha-tubulin (*SI Appendix*, Fig. S5*D*). However, we detected a strong reconstituted YFP fluorescence between alpha-tubulin and dynein light chain and kinectin-related protein (*SI Appendix*, Fig. S5*C*). We therefore suggest that MOS7 localizes at the mitotic microtubules via either this interaction or that with Rae1 and Nup98a during cell division.

The *mos7-5* Allele Causes Defects in Microtubule Dynamics During Cell Division. To investigate whether *MOS7* mutations cause defects in spindle assembly or action during mitosis, we introduced the *ProUBQ14:GFP:TUA6* marker which labels the alpha-tubulin of microtubules, and monitored their dynamics during male gametogenesis (21).

In wild-type male gametogenesis, before the asymmetric division of Pollen Mitosis 1 (PMI), the microspore becomes polarized through nuclear migration to the radial cell wall, where it is surrounded by a directional array of cortical microtubules (Fig. 4*D*, white arrows). Interzonal microtubules then rearrange into a bipolar phragmoplast array between the two newly-forming nuclei (Fig. 4*E*, white star), the plus-end of the phragmoplast microtubules seen as a dark line (Fig. 4*F*, white bracket). During phragmoplast microtubule polymerization (Fig. 4*G*), they envelop a small amount of cytoplasm containing the generative cell nucleus (GN), and the microspore undergoes PMI (GN, Fig. 4*H*). The generative cell then undergoes a second mitosis, PMII, to form two sperm cells within the mature pollen grain (Fig. 4*I*).

In contrast, in *mos7-5* heterozygous mutants, half of the microspores contained disorganized cortical microtubules that did not surround the nucleus, and the nucleus itself did not migrate to the radial wall (Fig. 4*J*). As the normally developing microspores underwent division, aborting pollen could be identified by abnormal bundles of microtubules (white arrow, Fig. 4*K*) and an almost complete lack of normal spindle assembly (white arrowhead, Fig. 4 *L* and *M*). As such, the nucleus did not segregate its chromatin (white star, Fig. 4 *L* and *M*). The microtubule bundles were lost, microtubules seeming to rearrange to a state similar to wild-type interphase, although only a single nucleus is seen (Fig. 4*N*). Finally, aborting pollen grains degenerate and shrink (Fig. 4*O*).

During PMI, the asymmetric division is achieved through specific microtubule dynamics characterized by phragmoplast-mediated cell plate formation. The cell plate forms at the midline of the phragmoplast, visualized here using aniline blue staining, between a small, lens-shaped generative cell and a large vegetative cell (Fig. 4*P*) (22). Pollen from *mos7-5* heterozygous plants displayed highly disorganized phragmoplasts (Fig. 4*Q*). In summary, the failure of microtubules to promote polarity of the microspore, to form a functional spindle, or a normal

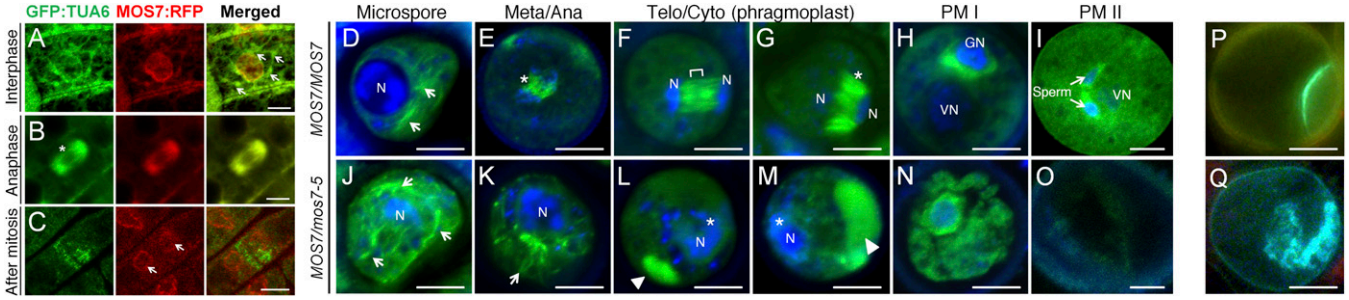

Fig. 4. MOS7-microtubule colocalization and defective dynamics during male gametogenesis in *MOS7/mos7-5* mutants. (*A*–*C*) MOS7 colocalizes with microtubules during mitosis. All mitotic root cells coexpress GFP:TUA6 (*Left*) and MOS7:RFP (*Center*) at mitotic spindles and phragmoplasts as seen in merged view (*Right*). Mitotic stages listed on the left. (*A*) Interphase, with cortical microtubule arrays (arrows), nuclear-localized MOS7 (*B*) Spindle-midzone (asterisk) at Anaphase, MOS7 and TUA6 colocalized. (*C*) MOS7:RFP localization at newly formed nuclear membrane (arrows), no longer colocalized with TUA6. (*D*–*Q*) CLSM micrograph composits of *ProUBQ14:GFP:TUA6* expression merged DAPI (Green; GFP:TUA6, Blue; DAPI). (*D*–*I*) GFP:TUA6 in WT pollen grains during gametogenesis. (*D*) Microspore with cortical microtubules (arrows). (*E*) Spindle at meta-anaphase (asterisk). (*F* and *G*) Telophase, phragmoplast midline (bracket), its polymerization (asterisk). (*H*) Bicellular pollen. (*I*) Mature pollen with sperm cells and vegetative nucleus (VN). (*J*–*O*) GFP:TUA6 in pollen from *MOS7/mos7-5* mutants at the same growth period as wild type (*D*–*I*), respectively. (*J*) Microspore nucleus surrounded by disoriented cortical microtubules (arrows). (*K*) Bipolar microtubule configuration defects (arrow). (*L* and *M*) Spindle assembly failure (arrowhead) and cytokinesis (asterisk). (*N*) Irregular cytoplasmic microtubule accumulation (*O*) Shrunken pollen grain. (*P* and *Q*) Aniline blue-stained bicellular pollen (*P*) WT Cell plate appearance between two cells after phragmoplast formation. (*Q*) Irregular cell plate formation in defective pollen. GN, generative cell; N, nucleus; VN, vegetative cell. (Scale bars: 5 μm.)

phragmoplast, leads to the complete failure of PMI and II. Thus, MOS7 plays a pivotal role in microtubule dynamics and is required to set up nuclear polarity, for normal spindle assembly, and for the cell plate formation in cytokinesis during sexual reproduction in *Arabidopsis*.

Discussion

In this paper, we describe the discovery of the *mos7-5* mutant allele, and analyze its reproductive defects. We find that mutations in the *MOS7* nucleoporin gene result in aberrant microtubule dynamics during the mitoses that follow meiosis in both male and female gametogenesis, resulting in pollen and ovule abortion. In addition, MOS7 is required during seed development and homozygous *mos7-5* mutants abort at the globular stage of embryogenesis.

MOS7 Homologs and Interacting Partners from Disassembled NPCs Are Important During Mitosis. Nucleoporin proteins are the main components of NPCs. Although NPCs are stable throughout interphase, they are dynamic during cell division, disassembling into subcomplexes and reassembling at the newly-formed nuclear envelope at the end of the cell cycle (23). Evidence from several organisms demonstrates that, aside from their roles in macromolecular transport, nucleoporins and their subcomplexes colocalize with mitotic apparatus and play a variety of important roles in mitosis (24). In both male and female *MOS7/mos7-5* gametophytes, the mitotic divisions that lead to mature gametophyte formation fail to progress normally, arresting either before or directly after the first division. Previous data demonstrate that Rae1, with binding partner Nup98, bind to, and regulate, the activity of the Anaphase Promoting Complex in mice (25). Haploinsufficiency of both proteins results in premature sister chromatin separation and aneuploidy (25). Rae1 binds to spindle microtubules, and is required for spindle formation in vertebrates and plants (19, 26). Here, we demonstrate that MOS7 interacts with Rae1 and Nup98, and that *rae1* mutants appear to exhibit female gametophytic abortion similar to *mos7-5*. Nup88 is known to form a complex with Nup214 that localizes to mitotic spindles (27). Nup88/Nup214 complexes are required for kinetochore-spindle interaction in a dosage sensitive manner, so that their loss resulted in multipolar spindle formation and aneuploidy in humans (11). Interestingly, *Arabidopsis* LNO1, required for mRNA export, is a homolog of human Nup214, and *LNO1* mutations cause seed abortion (28). In *lno1* homozygotes, there were no gametogenesis defects, but mitotic abnormalities were detected from the first zygotic division onwards. Thus, LNO1 may be required for proper mitosis in embryogenesis, similar to the requirement for MOS7 in mitosis in gametogenesis.

In *Arabidopsis*, we show that MOS7 localizes to the spindle during mitosis, suggesting that its role in the regulation of microtubule dynamics may be conserved (18). Finally, during male gametogenesis in aborting pollen from *MOS7/mos7-5* plants, we observed disorganized microtubule structures, a failure of spindle formation, and a lack of chromatin segregation or cytokinesis.

We also found a direct interaction between MOS7, dynein light chain type 1 family protein and kinectin-related protein, and in turn, a direct interaction of the latter proteins with microtubules. Their functions in plants has not yet been shown, and no homologs of the animal dynein heavy chain have been found in the *Arabidopsis* genome, so the dynein light chain cannot assemble a conventional dynein motor (29). We also demonstrate that the phragmoplast array, responsible for cytokinesis in plant cells, is aberrant in male gametogenesis. It has been shown that in dividing microspores, pollen mutant for Kinesin-12A and B fail to organize phragmoplast microtubules, resulting in a lack of cytokinesis and no cell plate formation, preventing formation of the generative cell and, subsequently, the sperm (22). These phenotypes are highly reminiscent of those we observe in *mos7-5*, and as such, it is inviting to suggest that MOS7 may interact with phragmoplast kinesins through its interaction with kinectin-related protein, promoting phragmoplast formation in the male gametophyte.

MOS7 Expression in the PMC and MMC Is Critical for Progression Through Mitosis. Meiosis is a conserved process required for eukaryotic sexual reproduction, however, subsequent gametophytic mitoses are specific to plants. *MOS7* mutations do not seem to be detrimental to meiosis, instead causing arrest during gametophytic mitoses. Most interestingly, our results show that during gametophytic mitoses, the genotype with regards to *mos7-5*, or the independently derived *mos7-2* and *mos7-4* mutant alleles, is not important. Instead, it is the genotype of the PMCs and MMCs that dictate the behavior of their progeny through mitosis. The clearest explanation for this phenomenon is that of haploinsufficiency of the MOS7 protein during gametogenic mitoses. A half-dose of the protein is inherited stochastically from heterozygous PMCs and MMCs by each of the meiotic progeny. During pollen mitosis 1 in the male gametophyte, and FG1 in the female gametophyte, the amount of MOS7 protein in half of the progeny reaches a threshold. After this point, the protein is limiting, resulting in aberrant division and subsequent arrest, whereas those gametes inheriting sufficient MOS7 develop normally. A dose-dependent

requirement for nucleoporins in cell division has been observed previously, whereby the level of Rae1/Nup98 complexes in double heterozygous mutants is shown to correlate with the severity of aneuploidy in mouse embryonic fibroblasts (25).

The heightened sensitivity of the gametophytes to dosage of proteins such as MOS7 may be linked to the fact that different regulatory pathways control symmetric and asymmetric divisions, and cell and nuclear divisions of male and female gametogenesis are often asymmetric. In *Arabidopsis* shoot and root, for example, a higher dose of the cell-cycle dependent protein kinase CDKA:1 is required for asymmetric divisions (30). Intriguingly, mutations in *CDKA:1* also cause arrest of male gametophytic development at PMII. CDKA:1 acts in the microspore, and cytoplasmic inheritance of it is required for progression through mitosis, so the effect of the null allele is delayed (31, 32). In this way, the effect of the *mos7-5* allele is reminiscent of a loss of *CDKA:1*.

In the seed, the transmission of both maternal and paternal mutant alleles of *MOS7* can be seen most clearly, whereby homozygous *mos7-5*/*mos7-5* seeds are produced but arrest at the preglobular stage of embryo development, indicating that the seed is able to progress through the earliest stages of development without MOS7. This may be for similar reasons to the gametophytic abortion that we observe. However, there are 3–4 mitoses in between zygote specification and the preglobular stage embryo, so it seems unlikely that inherited MOS7 would not be limiting until then. Instead, we suggest that zygotic abortion is due to the requirement of MOS7 in its NPC capacity, whereby around the globular stage of embryogenesis an as-yet unidentified protein becomes either limiting or excessive, in the embryo or endosperm, due to aberrant NPC activity in the absence of MOS7. Indeed, it has been shown that MOS7 is required for the nuclear retention of the defense R protein SNC1 in *Arabidopsis* (10). Furthermore, the preglobular stage of embryogenesis seems to act as a kind of "checkpoint," notably in cases of endosperm dysfunction. Mutants with early defects in endosperm development exhibit normal embryo development until the globular stage, aborting after this point (33).

In this work, we show that *MOS7* expression in the PMC and MMC is absolutely required for the respective development of the male and female gametophytes. Through an as-yet unidentified process, MOS7 is required for proper spindle assembly and phragmoplast formation during cell divisions in male gametogenesis, possibly through its interaction with nucleoporins Rae1 and Nup98 and/or dynein light chain type 1 family protein and kinectin-related proteins. Thus, we delineate a pathway shared between male and female gametogenesis in *Arabidopsis* whereby nucleoporin MOS7 is required for the postmeiotic mitoses characteristic of angiosperm reproduction.

Materials and Methods

Full details of methods are presented in *SI Appendix, SI Materials and Methods*.

Quantitative Real-Time RT-PCR. qRT-PCR product was amplified using the iQ SYBR Green Supermix (Bio-Rad) on a CFX96 machine (Bio-Rad), and the data were analyzed using CFX Manager software (Bio-Rad). The primer sequences for quantitative RT-PCR are listed in the *SI Appendix*, Table S5.

Yeast Two-Hybrid Assay. The pGBKT7 bait vector and pGADT7 prey vector in the Matchmaker Two-Hybrid system (Clontech) were used. A yeast two-hybrid screening with MOS7 was performed by Panbionet Corp. (www.panbionet.com). See *SI Appendix*, Table S6 for a list of plasmid constructed and primer sequences used.

BiFC Analysis. *pSAT4-nEYFP-C1* (E3801) and *pSAT4-cEYFP-C1-B* (E3802) were used to generate constructs.

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