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ORIGINAL PAPER

The *Arabidopsis* kinesin gene *AtKin-1* plays a role in the nuclear division process during megagametogenesis

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Abstract
Key message ***Atkin-1*****, the only Kinesin-1 member of *Arabidopsis thaliana*, plays a role during female gametogenesis through regulation of nuclear division cycles.**
Abstract Kinesins are microtubule-dependent motor proteins found in eukaryotic organisms. They constitute a superfamily that can be further classified into at least 14 families. In the Kinesin-1 family, members from animal and fungi play roles in long-distance transport of organelles and vesicles. Although Kinesin-1-like sequences have been identified in higher plants, little is known about their function in plant cells, other than in a recently identified Kinesin-1-like protein in a rice pollen semi-sterile mutant. In this study, the gene encoding the only Kinesin-1 member in *Arabidopsis*, *AtKin-1* was found to be specifically expressed in ovules and anthers. *AtKin-1* loss-of-function mutants showed substantially aborted ovules in siliques, and this finding was supported by complementation testing. Reciprocal crossing between mutant and wild-type plants indicated that a defect in *AtKin-1* results in partially aborted megagametophytes, with no observable effects on pollen fertility. Further observation of ovule development in the mutant pistils indicated that the enlargement of the megaspore was blocked and nuclear division arrested at the one-nucleate stage during embryo sac formation. Our data suggest that *AtKin-1* plays a role in the nuclear division cycles during megagametogenesis.

Keywords Kinesin · *Arabidopsis* · Ovule · Megagametogenesis

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Introduction

Kinesins constitute a superfamily of microtubule-associated motor proteins. They are able to move along microtubules using energy released from adenosine triphosphate (ATP) hydrolysis (Reddy 2001). Kinesins were initially identified in animals (Vale et al. 1985a, b), and numerous kinesin isoforms have since been identified in plants and lower eukaryotic organisms. Kinesins share a conserved motor domain of approximately 350 amino acid residues, which has ATPase and microtubule-binding activities (Vale and Fletterick 1997). A short neck region adjacent to the motor domain works in concert with the motor domain to produce motility (Miki et al. 2005). Some members of the superfamily have a less conserved coiled-coil region important for dimerization, and a non-conserved tail region that is thought to bind cargoes directly or via light chains (Richardson et al. 2006). The motor domain can be located in the N-terminus, C-terminus, or even internally in some kinesins (Reddy 2005; Reddy and Day 2001; Vale and Fletterick 1997). Phylogenetic analysis based on the amino acid sequence of motor domains could classify kinesins into at least 14 families (Lawrence et al. 2004). The diversity of the kinesin structure contributes to its various functions, and they participate in many cellular processes, including the

transport of vesicles (Goldstein and Philp 1999), organelles (Leopold et al. 1992), chromosomes (Barton and Goldstein 1996), RNA location (Carson et al. 1997), and regulation of microtubule dynamics (Sawin et al. 1992).

Since the immunochemical identification of plant kinesin in tobacco pollen (Tiezzi et al. 1992), numerous plant genes encoding kinesins have been isolated. These are involved in various microtubule-related biological processes, including cell division (Yang et al. 2003; Tanaka et al. 2004; Strompen et al. 2002; Spielman et al. 1997; Nishihama et al. 2002; Liu et al. 1996; Lee and Liu 2000; Lee et al. 2001; Hulskamp et al. 1997), morphogenesis (Reddy and Day 2000), cell wall deposition (Oda and Fukuda 2013; Zhong et al. 2002), regulation of organelle movement (Cai and Cresti 2012; Suetsugu et al. 2010a, b; Romagnoli et al. 2007, 2003; Reddy 2001; Cai et al. 2000), synthesis of phytohormones (Li et al. 2011), and regulation of respiration (Yang et al. 2011). Increasing data have suggested that plant kinesins have evolved to take on specialized functions dependent on their unique microtubule arrays during the plant cell cycle (Zhu and Dixit 2012; Richardson et al. 2006; Reddy and Day 2001). There are 61 predicted loci that encode kinesins in *Arabidopsis*, 58 of which can be classified into 10 families, with the remaining three ungrouped (Richardson et al. 2006; Reddy and Day 2001). The functions of over 60 % of *Arabidopsis* kinesins are still unknown (Zhu and Dixit 2012). Members of the Kinesin-1 family were found to be involved in long-distance cargo transport mediated by a light chain-binding domain in animals (Vale 2003; Hirokawa and Takemura 2005; Arimoto et al. 2011). There is only one member of the Kinesin-1 family present in *Arabidopsis*; this is also the only member present in rice (Richardson et al. 2006; Reddy 2001; Reddy and Day 2001). Nucleotide acid sequence deduced amino acid sequences have revealed that the plant members of Kinesin-1 family do not have a light chain-binding domain (Reddy and Day 2001). Recently, a rice *pollen semi-sterility1* (*PSS1*) mutant, displaying 50 % pollen viability and defective anther dehiscence, has been found to be caused by a defect of a Kinesin-1-like protein (Zhou et al. 2011).

This study characterizes the expression pattern of the Kinesin-1 member in *Arabidopsis* and investigates the phenotype of T-DNA insertion mutagenesis lines. The results showed that the gene plays a role in female gametogenesis at the nuclear division stage.

Materials and methods

Plant materials and growth conditions

The *Arabidopsis thaliana* wild and mutant plants used in this study were of the Columbia ecotype. All plants were grown in an air-conditioned room under a 16 h light/8 h dark photoperiod at 22 °C. Seeds from the T-DNA insertion SALK lines were obtained from the *Arabidopsis* Biological Resource Center at the Ohio State University.

Reverse-transcription-polymerase chain reaction (RT-PCR) detection of *AtKin-1* transcripts

Total RNAs were extracted from different *Arabidopsis* organs using TRIzol reagent (Gibco, Grand Island, NY, USA). Roots were collected from 10-day-old seedlings grown on MS solid medium. Stems, leaves, inflorescences, and siliques were obtained from flowering plants grown on soil. First-strand cDNAs were synthesized with M-MLV reverse transcriptase (Sangon, Shanghai, China) and oligo$(dT)_{18}$. The cDNA of *AtKin-1* was amplified using first-strand cDNA as a template with the primer combination KHCF (5′-GCGGTACCATGTCTAACGTAACCGTC TGTG-3′) and KHCR (5′-GCGGTACCGGACGTAAAGA ACGATGCATA-3′). As an internal control, primers for the *CBP20* gene (Kmieciak et al. 2002), CBP20F (5′-GGTT CGTGATGAATACCGTACAG-3′) and CBP20R (5′-AAG ATTACATTGACCATTCAGCAAC-3′), or *actin2*, ACT2F (5′-ATTCAGATGCCCAGAAGTCTTGTT-3′) and ACT2R (5′-GAAACATTTTCTGTGAACGATTCCT-3′) were also included. PCR products were fractionated by 0.7 % agarose gel electrophoresis.

Construction of the *AtKin-1* promoter-*GUS* reporter and GUS assay of transgenic plants

A 2119-bp promoter fragment containing the ATG start codon was amplified by PCR using a 5′ primer PKCF (5′-GCAAGCTTACACTCCTTTAGCTCCCTTTGGC-3′) containing a *Hind*III site, and a 3′ primer PKCR (5′-GCGGATCCCATCTCCTCTCCTTATCGGAACG-3′) containing a *Bam*HI site. PCR products of the desired size were cloned into a pUCm-T vector (Sangon, Shanghai, China) and confirmed by sequencing. The fragment was released using *Hind*III and *Bam*HI, and used to replace the *35S* promoter upstream of the *GUS* (*β-Glucuronidase*) gene in the vector pBI121 (Clontech, CA, USA). Transgenic plants were obtained through *Agrobacterium tumefaciens*-mediated floral dip infiltration (Clough and Bent 1998). Kanamycin plates containing 50 mg/L kanamycin supplemented to MS medium with 0.3 % sucrose were used for transgenic screening of lines. Transgenic plants were selected and stained in GUS staining solution (Jefferson et al. 1987). For inflorescences, stained inflorescences were decolorized with 95 % ethanol, and observed and photographed using an Olympus SZX stereomicroscope (Olympus, Tokyo, Japan) equipped with a Nikon Coolpix 4500 camera (Nikon, Wuxi, China). For ovules,

stained pistils were first cleared in 20 % lactic acid and 20 % glycerol, and then observed under a Leica DMIRE2 inverted microscope (Leica Microsystems GmbH, Wetzlar, Germany), and documented with a Nikon Coolpix 4500 digital camera.

PCR-based screening of T-DNA insertions

The T-DNA insertions were confirmed using a PCR-based method. Confirmation of *kin-1a* and *kin-1c* used the gene-specific primer KHC12 (5′-CACATGGAGCAAGAAACC GTT-3′) and the T-DNA border-specific primer Lba1 (5′-TGGTTCACGTAGTGGGCCATCG-3′) to test for the T-DNA insertion, and the gene-specific primers KHC12 and KHC13 (5′-CTTGTCAGGCGTTACAGAGGC-3′) for testing homozygosity. Confirmation of *kin-1b* used the gene-specific primer KHC10 (5′-TCTATGCGCGCTCTT CATCT-3′) and primer Lba1 for verifying the T-DNA insertion, and the gene-specific primers KHC9 (5′-GCTGT TTGGGATGATAAAGATGG-3′) and KHC10 for testing homozygosity.

Phenotypic analysis

Siliques from wild-type or mutant plants were dissected, and the development of the ovule observed. Confocal observation of ovule development was performed according to the method described by (Christensen et al. 1997) using a LEICA-SP2 laser scanning microscope with a 488-nm argon laser and a LP530 filter (Leica Microsystems GmbH, Wetzlar, Germany).

Complementation testing of *kin-1a* mutant

For functional complementation testing of the *kin-1a* mutant, a 6299-bp genomic fragment, starting 2116 bp upstream of the ATG start codon and ending 1446 bp downstream of the stop codon, was amplified with LA Taq polymerase (Takara, Dalian, China) using the primers PKCF (5′-GCAAGCTTACACTCCTTTAGCTCCCTTTG GC-3′) and KHC7 (5′-GCGAATTC ATAAAGTGTGTTG GAAATAATTAACA-3′). The desired PCR products were cloned into the pUCm-T vector (Sangon, Shanghai, China) for sequence verification. Next, the fragment was released, and inserted into pCAMBIA1300 (CAMBIA, Canberra, Australia) at the *Pst*I site to generate the pCAMBIA-AtKin-1 construct. The construct was then introduced to the homozygous *kin-1a* mutant, as described above for the promoter-*GUS* fusion. Transformants were obtained by selection on MS plates supplemented with 20 mg/L hygromycin. Siliques from homozygous T_3 plants were detected for the restoration of the normal ovule development.

Results

Analysis of AtKin-1 sequences

Previous predictions of kinesin members at the genome level revealed that there is only one possible KHC-type kinesin member, MAA21.110, in *Arabidopsis* (Reddy and Day 2001), this is encoded by the locus At3g63480. Comprehensive analysis using the deduced amino acid sequence from the available mRNA sequence grouped this kinesin into the Kinesin-1 family of the kinesin superfamily (Richardson et al. 2006), and it is here named as AtKin-1.

Amino acid sequence analysis indicated that AtKin-1 was an N-terminal motor kinesin with 465 amino acid residues, and an estimated molecular weight of 51.3 kD. Phylogenetic analysis (Tamura et al. 2007) of motor domains revealed that AtKin-1 and two kinesin members of *Vitis vinifera* and *Oryza sativa* ssp. Japonica clustered into a clade within the Kinesin-1 family (Fig. 1a), implying that plant-type Kinesin-1 members are closely related.

Lupas algorithm prediction (Lupas et al. 1991) revealed that AtKin-1 had a coiled–coiled region, spanning amino acids 404–441, adjacent to the motor domain (Fig. 1b) as previously reported by (Richardson et al. 2006). Besides the conserved motor domain, there were no other functional domains detected. AtKin-1 and its homologs from grape and rice have a very short tail region compared with other kinesin-1 members (Fig. 1b, c).

AtKin-1 is expressed specifically in anthers and ovules

To determine the expression pattern of *AtKin-1*, total RNAs were isolated from different organs, and RT-PCR performed using gene-specific primers at the 5′ and 3′ ends of the *AtKin-1* coding region. A single band with an expected size of ~1.4 kb was detected in total RNAs from inflorescences, but no transcripts were found in roots, stems, leaves, or siliques (Fig. 2a), indicating that *AtKin-1* is specifically expressed in inflorescences.

To further profile the temporal and spatial expression pattern of *AtKin-1* in inflorescences, the CAMV 35S promoter in the pBI121 vector was replaced with promoter regions from *Atkin-1*, thus fusing the *AtKin-1* promoter to the 5′ end of a *GUS* reporter gene. The resulting construct was introduced into wild-type *Arabidopsis*, and GUS activity in the inflorescences observed. GUS activity was seen in the pistils before pollination, and in young anthers (Fig. 2b–d). Cleared ovules showed ubiquitously GUS activity throughout tissues in the ovules, with a stronger signal observed in embryo sac regions (Fig. 2e).

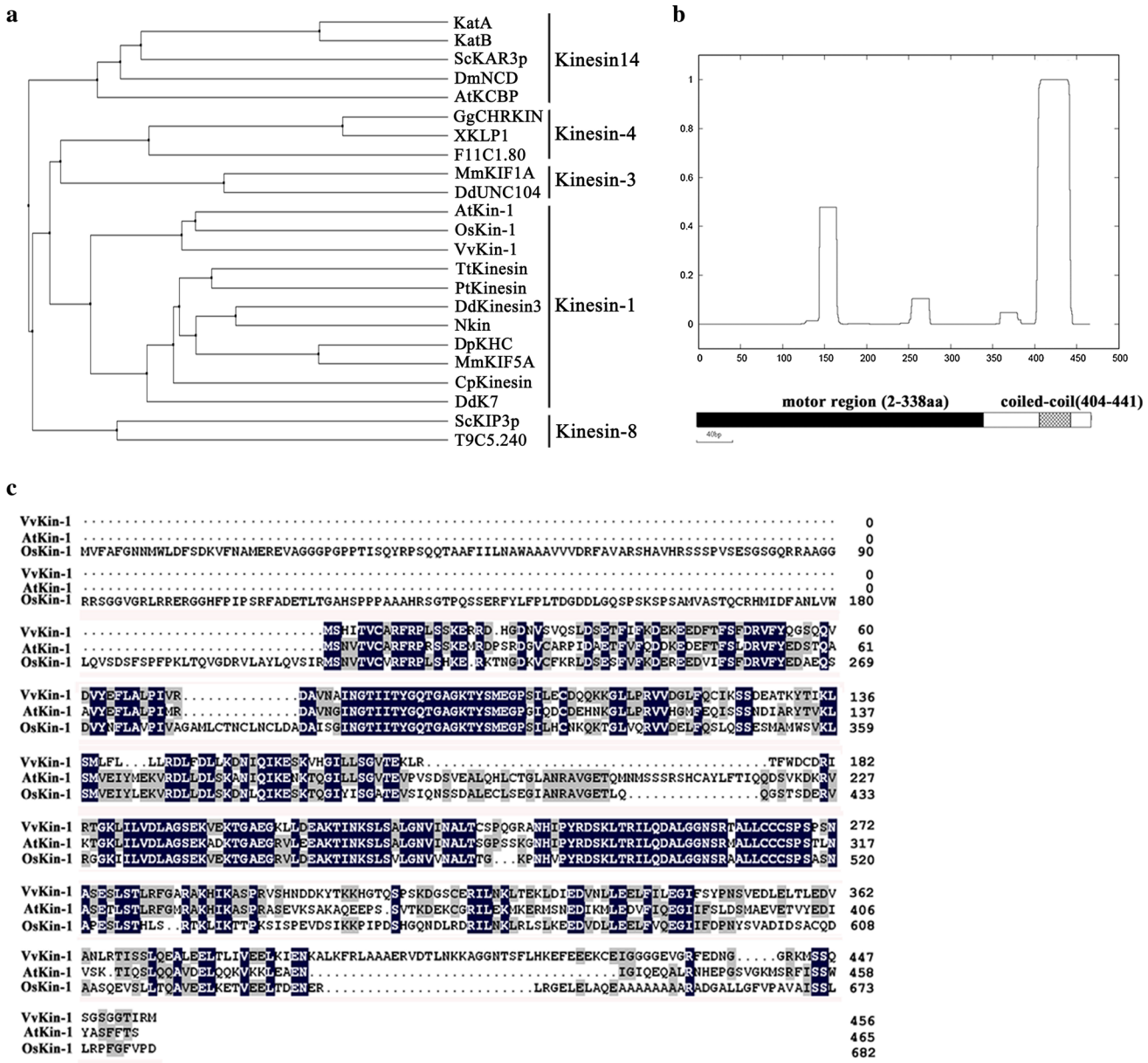

Fig. 1 Analysis of AtKin-1. **a** Phylogenetic analysis between AtKin-1 and other kinesins. KatA (D11371), KatB (D21137), AtKCBP (L40358), T9C5.240 (AL132964), and F11C1.80 (AB061676) are from *Arabidosis thaliana*; ScKAR3p (M31719) and ScKIP3p (Z72739) from *Saccharomyces cerevisiae*; DmNCD (X52814) from *Drosphila melanogaster*; GgCHRKIN (U18309) from *Gallus gallus*; XKLP1 (X82012) from *Xenopus laevis*; MmKIF1A(D29951) and MmKIF5A (AF067179) from *Mus musculus*; DdUNC104(AF245277), DdKinesin3 (XP640847), and DdK7(U41289) from *Dictyostelium discoideum*; OsKinesin-1 (BAD33096) from *Oryza sativa* spp japonica; VvKinesin (CAN82039) from *Vitis vinifera*; TtKinesin (XP001027745) from *Tetrahymena thermophila*; PtKinesin (XP001444178) from *Paramecium tetraurelia*; Nkin (L47106) from *Neurospora crassa*; DpKHC (J05258) from *Dorytheuthis pealeii*; and CpKinesin (XP001388331) from *Cryptosporidium parvum*. **b** Protein structure prediction. The relative positions of the motor domain and coiled-coil region are indicated. **c** Alignment of AtKin-1 with Kinesin-1 members from *Oryza sativa* spp. japonica (OsKin-1) and *Vitis vinifera* (VvKin-1)

Mutation of the *AtKin-1* gene causes partial abortion of seeds

To test whether AtKin-1 plays a role in anther and ovule development, T-DNA insertion mutagenesis lines (Fig. 3a) were grown, and their phenotypes observed. The phenotypic observation of *kin-1a* (Salk_120399) revealed that both heterozygous and homozygous plants produced significantly fewer seeds in their siliques than wild type. Siliques of the homozygous and the heterozygous *kin-1a* mutants had ~58.62 and ~78.84 % normally developing seeds, respectively, while the wild type had nearly a full

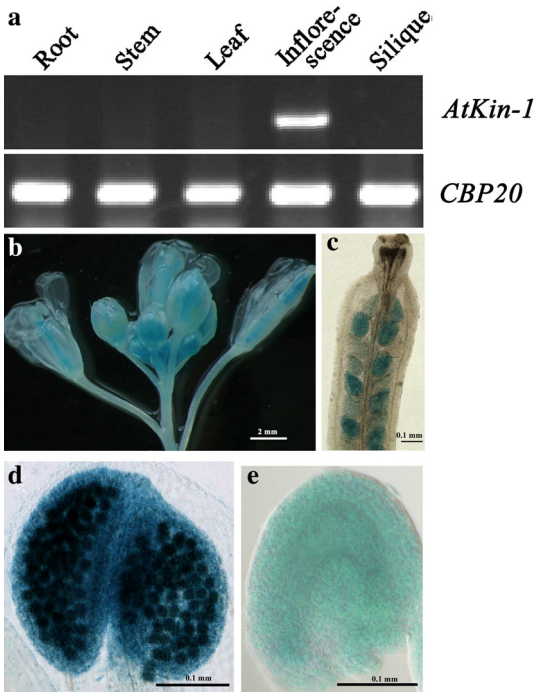

Fig. 2 Expression analysis of *AtKin-1* in different organs and tissues. **a** RT-PCR analysis of *AtKin-1* in different organs. The *CBP20* gene was used as an internal control. **b–e** *GUS* expression driven by the *AtKin-1* promoter in transgenic *Arabidopsis*, showing GUS reporter activity in inflorescences (**b**), siliques (**c**), anthers (**d**), and ovules (**e**)

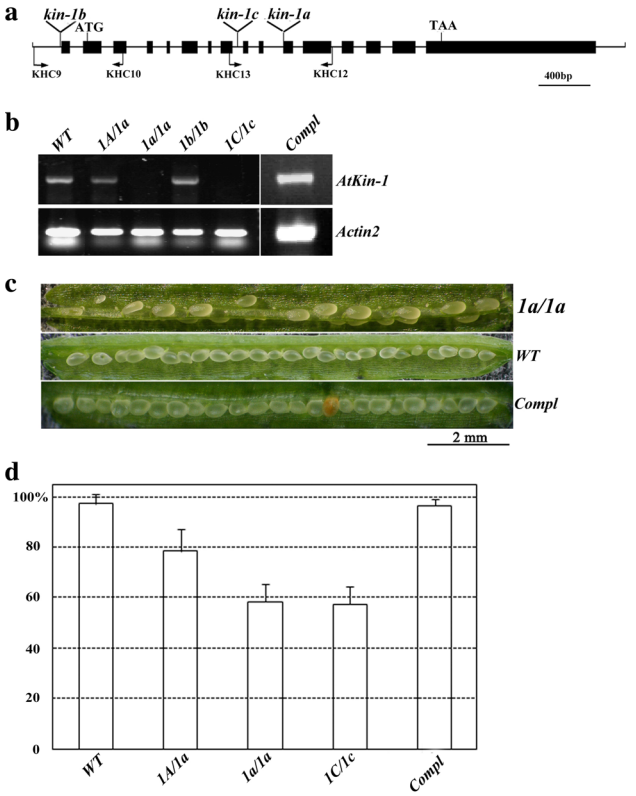

Fig. 3 Phenotype of T-DNA insertional mutants and expression detection of the *Atkin-1* gene. **a** Diagrammatical representation of *Atkin1* gene structure. Introns are shown as *lines*, and exons as *solid boxes*. Positions of the T-DNA insertions are shown at the top of diagram. Gene-specific primers for verifying T-DNA insertions are shown as arrows at the bottom of the diagram. **b** RT-PCR detection of *AtKin-1* transcripts in mutants and wild-type plants. *Atkin-1* transcript was detected in wild-type plants (*WT*), heterozygous *kin-1a* mutant (*1A/1a*), homozygous *kin-1b* mutant (*1b/1b*) and genetic complementation lines (*Compl*). Homozygous *kin-1a* mutant (*1a/1a*) and heterozygous *kin-1c* mutant (*1C/1c*) showed no *Atkin-1* transcript. *Actin2* gene was used as the internal control. **c** The homozygous *kin-1a* mutant (*1a/1a*) produced significantly fewer normally developing seeds, while wild-type plants (*WT*) and genetic complementation lines (*Compl*) of the homozygous *kin-1a* mutant showed nearly a full seed set. **d** Quantification of seed production in siliques of wild-type plants (*WT*), heterozygous (*1A/1a*) and homozygous (*1a/1a*) *kin-1a* mutants, heterozygous *kin-1c* mutant (*1C/1c*), and genetic complementation lines (*Compl*)

seed set (~97.7 %) (Fig. 3c, d). For *kin-1b* (Salk_135192) and *kin-1c* (Salk-024926) mutants, only homozygous and heterozygous lines were recovered, respectively. Screening of the *kin-1c*-derived population identified no homozygous plants. Phenotyping of heterozygous *atkin-1c* indicated ~57 % normally developing seeds, which is comparable to homozygous *kin-1a* (Fig. 3c, d). However, homozygous *kin-1b* showed nearly a full seed set (Fig. 3d). To test whether T-DNA insertions caused alternation of *Atkin1* expression, the transcripts of the three mutation lines were detected by RT-PCR. In homozygous *kin-1b* mutant plants, RT-PCR detection revealed that the T-DNA insertion, located near the transcriptional start site, did not affect *AtKin-1* expression (Fig. 3b). No transcripts were detected in heterozygous *kin-1c* plants (Fig. 3b), suggesting that the wild-type *AtKin-1* allele was silenced in the heterozygous *kin-1c* plants, the reason for this remains unknown. Moreover, it is likely that *kin-1c* mutant had other mutations tightly linking with *kin-1c* allele, which results in lethal homozygous sporophyte or totally aborted male gametophyte. No transcripts were amplified in the homozygous *kin-1a* plants, while correctly sized bands were detected in the wild type and the heterozygous type (Fig. 3b); this was consistent with the phenotyping results. Therefore, *kin-1a* mutant was used for further genetic testing and phenotypic observation.

A genetic complementation test was performed to confirm whether the seed abortion was caused by the *AtKin-1* gene mutation. The descendants of homozygous *kin-1a* plants transformed with a wild-type *AtKin-1* genomic fragment showed a similar seed set to wild-type plants (Fig. 3b–d). By combining the phenotyping data and *AtKin-1* expression detection results, it can be concluded that the partial seed abortion phenotype was due to the functional defect of *AtKin-1* gene.

To verify if the seed abortion caused by the mutation of *AtKin-1* could be due to a defect of the male or female gametophyte, reciprocal crosses were conducted. When

Table 1 Transmission efficiency of the *kin-1a* allele in reciprocal crosses between mutant and wild-type plants

Female parent	Male parent	Genotype of progeny		Transmission efficiency of *kin-1a* gamete (%)	*P* value*
		1A/1A	*1A/1a*		
1A/1a	*1A/1A*	59	36	37.89 (♀)	<0.025
1A/1A	*1A/1a*	45	48	51.61 (♂)	0.836

* *P* values calculated using the χ^2 test based on the expected value of a 1:1 segregation ratio

KIN-1A/kin-1a was used to pollinate the wild type, the transmission efficiency of the *kin-1a* allele (51.61 %) was at an expected ratio of 1:1 (Table 1). However, when the heterozygous mutant was pollinated with pollen from wild-type plants, transmission efficiency of the *kin-1a* allele (37.89 %) through the female gametophyte was significantly reduced (Table 1). These results revealed that mutation of *AtKin-1* caused a defect in female gametophyte development.

Gametophytic division is blocked in *kin-1a* mutant ovules

To further investigate the mutant phenotype, ovule development in *kin-1a* mutant plants was observed using a confocal laser scanning microscope (CLSM). Our observations of ovule development in the pistils at different developmental stages in the mutant plants indicated that there were no distinguishable differences found between ovules at the early stage before megaspore formation. The first aberration became apparent during the procession of megagametogenesis, which showed arrested nuclear division (Fig. 4b, c, e, h, k) or functional megaspore degeneration (Fig. 4f, i) in a substantial number of ovules. Normally, the three micropylar megaspores underwent degeneration following meiosis, with the remaining chalazal most one becoming enlarged, with its nucleus becoming prominent and moving to the center (Fig. 4a). When normally developing ovules showed two- or four-nucleated embryo sacs (Fig. 4d, g), some megaspores did not show enlargement and remained at the one-nucleated stage (Fig. 4b, c, e, h), or degenerated (Fig. 4f, i). At pistils with normally matured ovules (Fig. 4j), a few ovules displayed arrested division cycle megaspores (Fig. 4k) or degenerated embryo sacs (Fig. 4l).

Discussion

In wild-type *Arabidopsis* plants ovule development initiates at a hypodermal archesporial cell of the nucellus, and this further differentiates into a megasporocyte (Christensen et al. 1997; Mansfield et al. 1991). The megasporocyte produces four haploid megaspores through meiosis, of these the chalazal most one survives, and then undergoes three cycles of nuclear division to form an eight-nucleate embryo sac (Christensen et al. 1997; Mansfield et al. 1991). Subsequent nuclear migration, nuclear fusion, and cellularization occur to form a seven-celled female gametophyte, which is composed of three antipodal cells, two synergids, one egg, and a diploid central cell (Christensen et al. 1997; Mansfield et al. 1991). Death of the antipodal cells ultimately leads to the formation of mature four-celled female gametophyte (Christensen et al. 1997; Mansfield et al. 1991). Female gametogenesis is subject to regulation by a complicated network, of which the full details remain unclear (Shi and Yang 2011; Sundaresan and Alandete-Saez 2010; Yang et al. 2010). With regard to nuclear division, several mutants showing impaired mitotic division circles have been isolated in *Arabidopsis* and maize. A few of the associated gene mutations have been characterized (Drews et al. 1998; Ebel et al. 2004; Grossniklaus and Schneitz 1998; Kwee and Sundaresan 2003; Li et al. 2009; Liu and Qu 2008; Moore et al. 1997; Pagnussat et al. 2005; Shi et al. 2005; Shi and Yang 2011; Springer et al. 1995, 2000; Yang and Sundaresan 2000; Yang et al. 2010), suggesting that genes involved in the cell-cycle process play a role during female gametogenesis in higher plants. Here, we found that the gene encoding the Kin-1 family member of kinesin superfamily, *AtKin-1*, is specifically expressed in pollen grains and ovules (Fig. 2b–e). Loss-of-function mutants revealed that a defect in the *AtKin-1* gene results in a significantly increased number of aborted ovules (Fig. 3c), and this was confirmed by genetic complementation testing (Fig. 3d). Reciprocal crossing between mutant and wild-type plants indicated that mutation of *AtKin-1* causes defective female gametophyte development (Table 1). CLSM observation further demonstrated that the progression of female gametogenesis was blocked at the one nuclear stage (Fig. 4). In the cell cycle, the E2F pathway that controls the transition from G1 to S phase is conserved in both mammals and plants (De Veylder et al. 2003; Dewitte and Murray 2003). E2F transcription factors induce the transcription of genes required for cell-cycle progression and DNA replication (Helin 1998; Lavia and Jansen-Durr 1999). Previous microarray analysis revealed that ectopically expressing genes encoding E2Fa and DPa transcription factors could activate E2F target genes in *Arabidopsis* seedlings, including *AtKin-1* (Vandepoele et al. 2005). Characterization of E2F target genes established that a cis-element, TTTCCCGCC, is conserved in their promoter region (Mariconti et al. 2002; Stevens et al. 2002; Vandepoele et al. 2005). Although previous research did not identify a

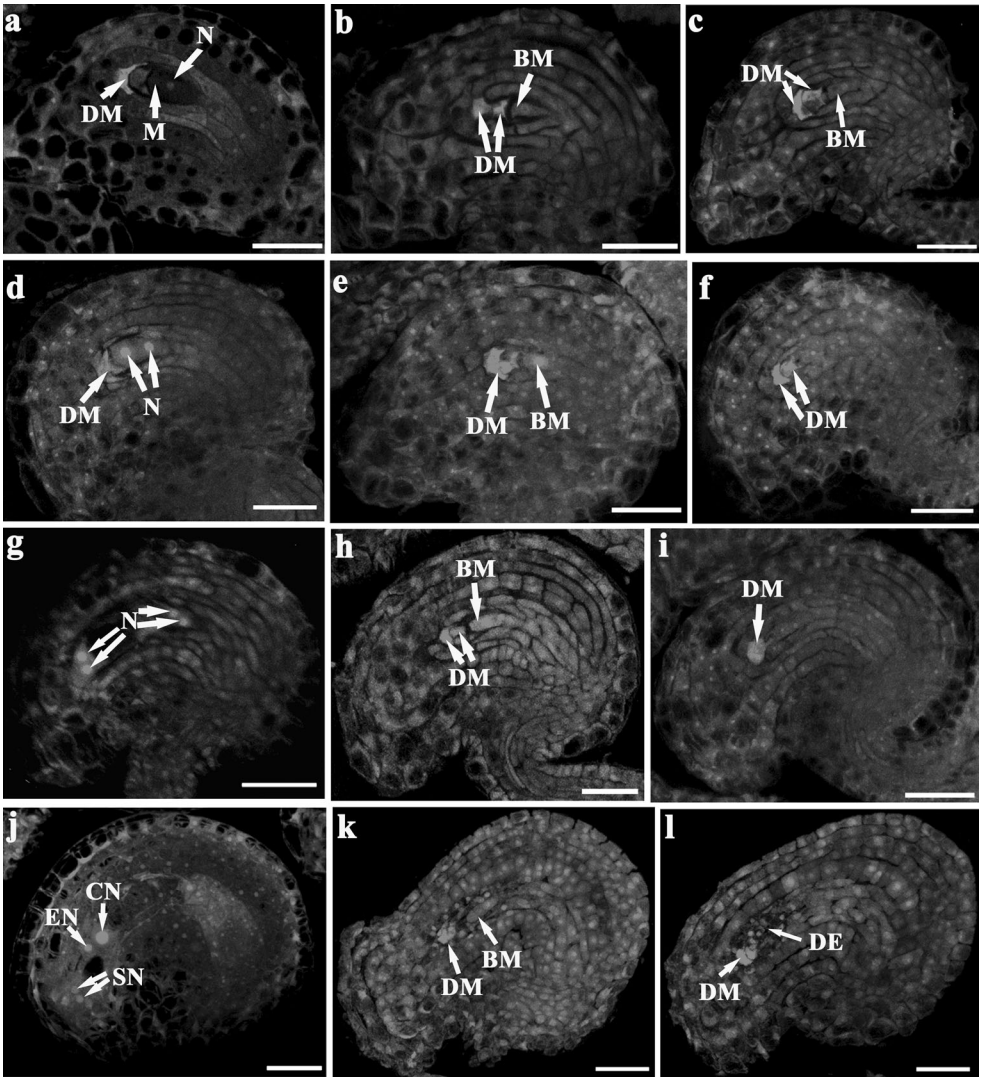

Fig. 4 *atkin-1* mutant ovule development as revealed by CLSM. **a–f** Ovules from a pistil of a *kin-1a* mutant showing one- (**a**), two-nucleated megaspores (**d**), nuclear division-blocked (**b**, **c**, **e**) and degenerated (**f**) functional megaspores. **g–i** Ovules from a pistil of a *kin-1a* mutant showing four-nucleated megaspore (**g**), nuclear division-blocked (**h**) and degenerated (**i**) functional megaspores. **j–l** Ovules from a pistil of a *kin-1a* mutant showing matured embryo sac (**j**), nuclear division-blocked megaspore (**k**) and degenerated embryo sac (**l**). *BM* blocked megaspore, *CN* central cell nucleus, *DE* degenerated embryo sac, *DM* degenerated megaspore, *EN* egg nucleus, *M* megaspore, *N* nucleus, *SN* synergid nucleus, *bars* 10 μm

conserved motif upstream of the *AtKin-1* coding region (Vandepoele et al. 2005), our observations revealed a conserved putative E2Fa binding sequence in the untranslated region (Fig. 5). Interestingly, *AtKin-1* is directly connected with a gene encoding a DP-E2F-like protein in the *Arabidopsis* coexpression network (http://www.arabidopsis.org). Therefore, *AtKin-1* plays a role in the regulation of nuclear division cycles during megagametogenesis.

Recent identification of a rice pollen semi-sterility mutant, pss1, revealed that a member of the Kinesin-1 family plays a role in male meiotic chromosomal dynamics and in anther dehiscence in rice (Zhou et al. 2011).

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-94 TATGGACCATTAGATCTTTTAACAACCCACACGCGTATCCTCGTG
-49 ACCTCTTCTCCTTCTCCTTCTTCTTCTTCCTTCTCTTTTCTCAAA
 -4 AATCAGATTTCGAGTTTCTTCTTTCCCGCCCGTAAGAGAAACCCT
+42 ACTCTGACTCTCCACTCCATTTGAGAGGTAAGTTCCGATTCCTTT
+87 TCTCTCAGCTTCAAATTTCCTGTGCTACTATTTCCCCAATTTCAT
+132 TCATCTTCTGCTGACTCATTTTGGGTGTTTCACTGTAGACTGAGC
+177 TCGTTCCGATAAGGAGAGGAGATG
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Fig. 5 The upstream sequence of *AtKin-1* contains a conserved E2F binding cis-element (*boxed*). Exons are shown as *bold letters*, with the transcription start site indicated by a solid triangle; the translation start codon is *underlined*

Although *PSS1* is expressed in various organs, expression peaks during male meiosis (Zhou et al. 2011). Our observation of the *Arabidopsis* ortholog revealed that *AtKin-1* was expressed specifically in pollen grains and in pre-pollinated ovules (Fig. 2b–e). Phenotyping of the SALK T-DNA lines revealed defective ovules (Fig. 3c, d), but no effects on pollen grain development were detected (Table 1). One possibility is that there are kinesins of other families such as ATK1 and AtK5 (Chen et al. 2002; Marcus et al. 2003; Quan et al. 2008) or other motor protein family members playing equivalent roles during *Arabidopsis* pollen formation. In addition, the discrepancy of the knock-out phenotypes and gene expression patterns between rice and *Arabidopsis* implies that the Kinesin-1 members in higher plants might be somehow functionally diverged. Compared with the Kinesin-1 members in *Arabidopsis* and grape, the rice ortholog possesses an extra region of 209 amino acid residues at the N-terminus (Fig. 1c). Whether this extra fragment of PSS1 contributes to its novel function in rice remains unknown.

The Kinesin-1 members of the kinesin superfamily were formerly called KHCs or conventional kinesins (Diefenbach et al. 1998; Lawrence et al. 2004; Miki et al. 2005; Reddy and Day 2001). They are known to play roles in organelle/vesicle transport and in nuclear movement in eukaryotes, and a non-conserved tail region is thought to bind the organelles and vesicles, either directly or mediated by light chains (Goldstein and Philp 1999; Leopold et al. 1992; Richardson et al. 2006). For conventional kinesins, identification of the site for binding of the light chain suggested that four highly conserved heptad repeats can form a predicted coiled-coil interacting with the N-terminus of the light chain, which also contains the heptad repeats (Diefenbach et al. 1998; Lawrence et al. 2004; Miki et al. 2005; Reddy and Day 2001). However, data from fungi have suggested that no light chains interact with conventional kinesins (Reddy and Day 2001; Steinberg and Schliwa 1995; Steinberg et al. 1998). Of the 61 kinesins in *Arabidopsis*, AtKin-1 is the only member belonging to the Kinesin-1 family (Reddy and Day 2001; Richardson et al. 2006). Like *Arabidopsis*, it appears that only one Kinesin-1 gene is present in *O. sativa* (Richardson et al. 2006). A search against GenBank identified only one Kinesin-1 member in the complete sequence of *V. vinifera* (Jaillon et al. 2007), although the expression pattern and the function of the gene remain unknown. Our phylogenetic analysis of motor domains (Fig. 1a) revealed that there are higher-plant-type members in the Kinesin-1 family, as previously speculated by (Reddy and Day 2001). Although the higher-plant-type Kinesin-1 tail contains a region of coiled-coil domain, it has a very short tail with little similarity to animal or fungi members. The data from rice and from our study suggest that the higher-plant-type Kinesin-1 members play a role in gametogenesis. Cellular localization observations and the identification of organelles or proteins interacting with Atkin-1 are expected to elucidate the mechanism underlying the regulation of this process.

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Conflict of interest The authors declare that they have no conflict of interest.

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