

Antipodal cells persist through fertilization in the female gametophyte of *Arabidopsis*

Xiaoya Song · Li Yuan · Venkatesan Sundaresan

Received: 5 July 2014 / Accepted: 29 October 2014 / Published online: 12 November 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract

Key message Extended antipodal life-span.

Abstract The female gametophyte of most flowering plants forms four cell types after cellularization, namely synergid cell, egg cell, central cell and antipodal cell. Of these, only the antipodal cells have no established functions, and it has been proposed that in many plants including *Arabidopsis*, the antipodal cells undergo programmed cell death during embryo sac maturation and prior to fertilization. Here, we examined the expression of female gametophyte-specific fluorescent reporters in mature embryo sacs of *Arabidopsis*, and in developing seeds shortly after fertilization. We observed expression of the fluorescence from the reporter genes in the three antipodal cells in the mature stage embryo sac, and continuing through the early syncytial endosperm stages. These observations suggest that rather than undergoing programmed cell death and degenerating at the mature stage of female gametophyte as previously supposed, the antipodal cells in *Arabidopsis* persist beyond fertilization, even when the other cell types are no longer present. The results support the concept that the *Arabidopsis* female gametophyte at maturity should be

considered to be composed of seven cells and four cell types, rather than the previously prevailing view of four cells and three cell types.

Keywords Mature female gametophyte · Early developing seed · Antipodal cell · Stage of degeneration

Introduction

The *Polygonum*-type embryo sac, which exists in the majority of the angiosperm species, forms seven cells with four cell types including two synergid cells, one egg cell, one central cell and three antipodal cells, in order along the micropyle–chalaza axis (Fig. 1a) (Maheshwari 1950; reviewed in Sundaresan and Alandete-Saez 2010). Most of the cells perform essential functions for double fertilization. Typically, the pollen tube is guided by the synergid cells to grow into the embryo sac and release two sperm cells (Higashiyama et al. 1998; Leshem et al. 2013). The two female gametes, the egg cell and central cell, then fuse with the two sperm cells, resulting in the embryo and endosperm, respectively (reviewed in Drews and Koltunow 2011; Ma and Sundaresan 2010). However, the function of the antipodal cells has not been established in any plant species. In many cereals, including maize and rice, the antipodal cells continue to proliferate following fertilization to form a cluster of cells (Randolph 1936; Diboll and Larson 1966). Ultrastructural studies in maize have found the invaginated cell walls of antipodal cells toward the surrounding nucellar cells leading to the hypothesis that in maize and other cereals, they may function as transfer cells to transport the nutrient from the surrounding sporophytic cells into the embryo sac (Diboll and Larson 1966; Dong and Yang 1989; Maeda and Miyake 1997).

Communicated by Weicai Yang.

Electronic supplementary material The online version of this article (doi:10.1007/s00497-014-0251-1) contains supplementary material, which is available to authorized users.

X. Song · L. Yuan · V. Sundaresan (✉)
Department of Plant Biology, University of California-Davis,
One Shields Ave., Davis, CA 95616, USA
e-mail: sundar@ucdavis.edu

V. Sundaresan
Department of Plant Sciences, University of California-Davis,
Davis, CA, USA

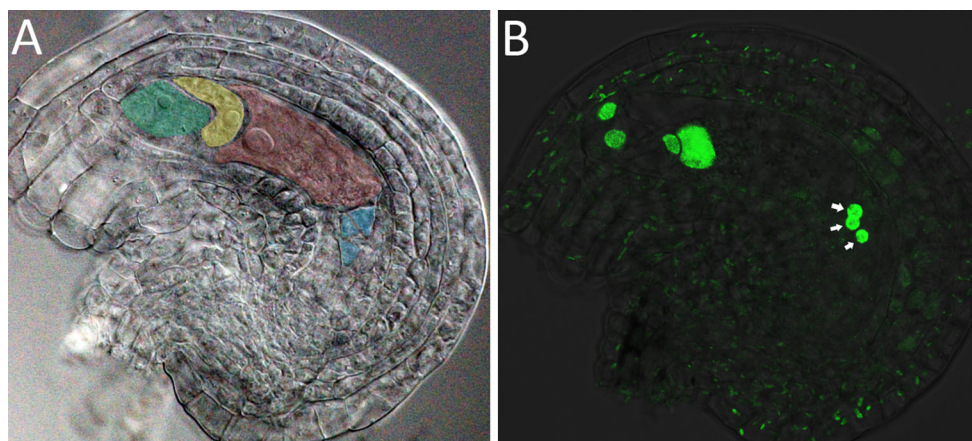


Fig. 1 The female gametophyte contains seven cells at the mature stage 1 day after emasculation. **a** Cleared embryo sac corresponding to ovule stage III–VI (Schneitz et al. 1995) showing seven cells. Note that fusion of the polar nuclei has occurred. False coloring has been used to highlight each cell type. *Green*, synergid cell. *Yellow*, egg

cell. *Red*, central cell. *Blue*, antipodal cells. **b** CDC123::H2B:YFP reporter labels the seven nuclei corresponding to the seven cells in a mature female gametophyte. Note that signals from the three antipodal cells are very strong (*white arrows*)

Unlike maize and rice, the antipodal cells in *Arabidopsis* do not undergo proliferation in the mature embryo sac, and instead undergo diminution in size with maturation (Christensen et al. 1997) and have been reported to undergo degeneration prior to fertilization (Heydlauff and Gross-Hardt 2014; Murgia et al. 1993; Schneitz et al. 1995; Sprunck and Gross-Hardt 2011; Yadegari and Drews 2004). Degeneration of antipodal cells has also been reported in other plants with *Polygonum*-type embryo sacs (Maheshwari 1950). In most of these studies, the degeneration of antipodal cells has been inferred from the inability to visualize the antipodal cells under light microscopy. The exception is the study by Murgia et al. who used transmission electron microscope (TEM) to observe the cell structure of the antipodals in a starchless *Arabidopsis* mutant and concluded that they “degenerate by the time of pollination” in the embryo sacs (Murgia et al. 1993). Thus, the current understanding in *Arabidopsis* is that the life of antipodal cells ends with the maturation of female gametophyte, when they undergo programmed cell death (PCD). Consequently, it is considered that the *Arabidopsis* female gametophyte prior to pollination, at stage FG7, consists of four cells (Christensen et al. 1997).

However, there is ambiguity in the literature concerning the antipodal life span in a mature female gametophyte, and it has been suggested that the antipodals might still exist at maturity. For example, “If the female gametophyte is unfertilized, the antipodal cells eventually disappear or undergo cell death; however, at the time of fertilization, the female gametophyte most likely is a seven-celled structure (i.e., the antipodal cells are present)” (Drews and Koltunow 2011). Nevertheless, due to lack of direct evidence,

there is no consensus in the present literature in favor of this suggestion.

Here, we have studied the expression of fluorescent reporter genes in the *Arabidopsis* embryo sac and early seed development and report that the antipodal cells do not degenerate prior to fertilization, but instead persist through early stages of seed development, indicating that the *Arabidopsis* embryo sac should properly be considered as seven-celled.

Materials and methods

Plant materials and growth conditions

For all observations, except the one noted below, we used wild-type *Arabidopsis* plants of the ecotype Columbia, transformed with different fluorescent reporters as described. All plants were grown in growth chambers with a 16-h light and 8-h dark cycle at 22 °C. For observations of unfertilized embryo sacs, flowers were manually emasculated 1 day before anthesis to ensure maturation of the female gametophytes.

Plasmids

CDC123::H2B:YFP is a gift from Dr. Weicai Yang (Institute of Genetics and Developmental Biology, CAS, Beijing). For DD65::NLSmCherry plasmid construction, we fused SV40 Nucleus Localization Signal (NLS) with mCherry fluorescent protein in frame and then insert the fusion protein under the control of DD65 promoter (Steffen et al. 2007). To generate the DD65::NUF2:GFP construct,

DD65 promoter was cloned into pCambia1300-GFP-NOST vector termed as “1300-DD65-GFP-NOST,” and then, NUF2 was amplified by PCR and ligated into the 1300-DD65-GFP-NOST vector in frame with GFP protein. For DD65::GFP:CENH3, we amplified GFP:CENH3 fusion protein from CENH3::GFP:CENH3 plasmid (Ravi and Chan 2010) and then cloned it into a pCambia1300-based vector DD65pro-pCambia1300 to generate DD65::GFP:CENH3. To obtain DD1::NLSGFP, we first amplified DD1 promoter by using primers described previously (Steffen et al. 2007), and then, we cloned it into a modified gateway-based pGreen II vector (a gift from Mily Ron, UC Davis) carrying the promoterless NLS:3XGFP reporter to generate the fusion.

Microscopy

For cleared whole-mount ovules, we used Hoyer solution (Liu and Meinke 1998) and observations were performed using a Zeiss Axioskop 2 microscope under DIC optics. Images were captured on an AxioCam HRC CCD camera (Zeiss) using the Axiovision program.

Observations of fluorescent reporters were performed using Zeiss LSM 710 confocal microscope, with software Zen lite 2011. Photoshop and ImageJ were used to process the images.

Results

In a mature stage embryo sac, antipodals display strong fluorescent signals using a female gametophyte reporter

In typical visual observations by DIC following clearing, cellularized embryo sacs (beyond FG5, Christensen et al. 1997) clearly show seven cells. When compared with the synergid, egg and central cells, the antipodal cell size is smallest, and organelles such as the vacuole are usually undetectable. These factors all make the antipodals less easily detectable than the rest of cell types by this method, and at later maturation, these cells can be undetectable (Christensen et al. 1997). Figure 1a shows a 1-day emasculated ovule where the cells at the micropylar end are clearly detectable, whereas the antipodals at the other end are not as obvious.

We used the reporter CDC123::H2B:YFP, which expresses in all the nuclei of the embryo sac but not in the surrounding nucellar cells (Rotman et al. 2005), to investigate the cells of a mature female gametophyte. As expected, the YFP reporter marks the synergid and egg cells at the micropylar end, the central cell and the antipodals at the chalazal end. However, unlike the DIC observations, the antipodal cells can be visualized as

clearly as the other cells using this reporter (Fig. 1b). This suggests the antipodals are still present at FG7 stage. To determine whether the signal in the antipodal diminishes as the ovule continues to mature, we examined the reporter in 3-day emasculated ovules as well. The signals, while becoming weaker in the synergid and egg, stay strong in the central cell and the three antipodals (Supplementary Fig. 1s). This indicates that the antipodal cell nuclei are at least as stable as the other three cell types. Therefore, we conclude that rather than disappearing, the three antipodals still exist in the mature stage embryo sac.

Cellular activity and integrity of the antipodal cells at the mature stage of the female gametophyte

In order to further ascertain the activity and potential viability of the antipodal cells in the fully matured embryo sac, we used the promoter fusion reporter DD1::NLS:GFP to observe the antipodal cells 1 day after emasculatation. The antipodal cells showed clear expression of the reporter at this stage (Fig. 2a). DD1 is an antipodal cell-specific promoter (Steffen et al. 2007), so that the detected GFP signal implies either active DD1 transcription or ongoing translation of stored transcripts within the antipodal cells. The endogenous gene driven by the DD1 promoter encodes a ubiquitin-conjugating enzyme, a component of the cellular ubiquitination pathway. Therefore, DD1 expression suggests that essential cellular activities may be still maintained in the antipodal cells at this stage.

Next, we used a plasma membrane reporter DD65::NUF2:GFP (“Materials and methods”) to observe embryo sacs 1 day after emasculatation. The membranes of the antipodal cells, along with the central cell, are labeled by this reporter and displayed intact cell shapes (Fig. 2b). Since this reporter should reflect the integrity of the cell by labeling the plasma membrane, these observations further imply that the antipodal cells still maintain the normal framework of intact cells.

Persistent signals in the antipodal cells after fertilization suggest their late degeneration

Next, we utilized the CDC123::H2B:YFP construct which persists in the endosperm in early stages after fertilization but is not expressed in the surrounding sporophytic cells. When we checked the post-fertilized seed at various developing stages including 2-nuclei, 4-nuclei and 8-nuclei endosperm stage, we detected three extra nuclei at the chalazal pole expressing the reporter, at the positions where the antipodals are usually located (Fig. 3a, b). Since the endosperm nuclei divide synchronously at these stages, this indicates the three extra nuclei, which are smaller than the typical endosperm nuclei, might be the antipodals. They

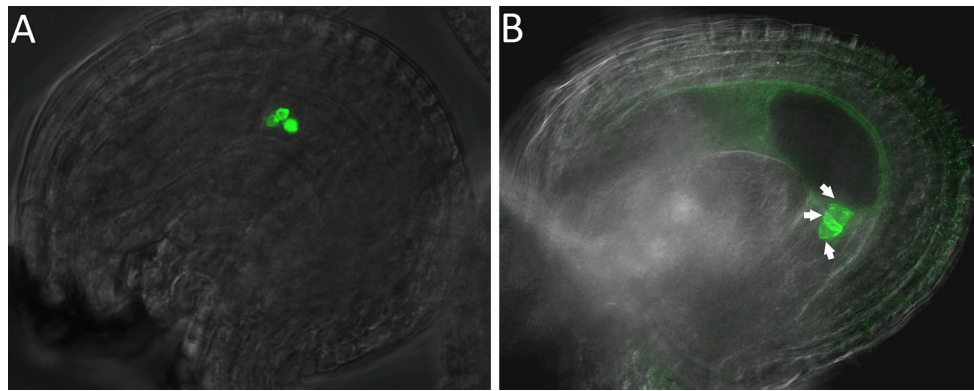


Fig. 2 Antipodal cells exhibit features of viable cells in mature female gametophytes after emasculation. **a** Expression of antipodal-specific reporter DD1::NLSGFP in embryo sacs 1 day after emasculation, indicative of active transcription in these cells.

b DD65::Nuf2::GFP, a membrane localization reporter, indicates that antipodal cell membranes (*white arrows*) 1 day after emasculation are intact and not collapsed

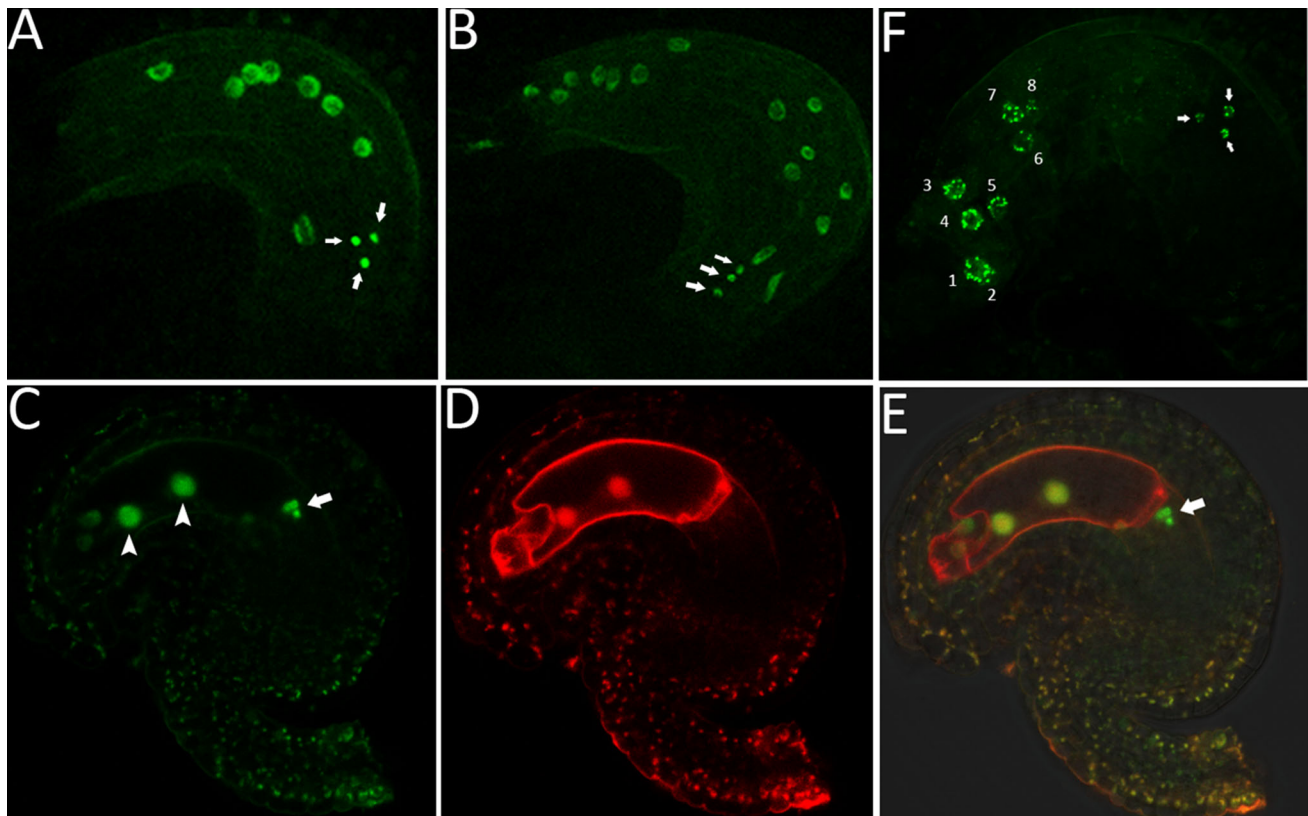


Fig. 3 Antipodal cells persist in early developing seeds of *Arabidopsis*. **a, b** Expression of CDC::H2B::YFP reporter observed after fertilization, at the 8-nuclear (**a**) and 16-nuclear (**b**) endosperm stages. Three antipodal-like nuclei (*white arrows*) are clearly present. Note that these nuclei are much smaller than the endosperm nuclei. **c–e** Cross-pollinated seed at 2-nuclear endosperm stage after crossing of two reporter lines. Maternal, CDC::H2B::YFP reporter. Paternal, DD65::NLSmCherry reporter. **c** CDC::H2B::YFP reporter labels the two endosperm nuclei (*white arrowhead*) and the three antipodal-like nuclei (*white arrow*). **d** DD65::NLSmCherry from pollen labels only the two endosperm nuclei that are products of double fertilization. Therefore, the group of the three nuclei at the chalazal pole must

correspond to the antipodal cells of the female gametophyte. **e** Overlay of the *green* (**c**) and the *red* (**d**) fluorescent images. **f** Haploid nuclei indicated by a centromere-specific reporter further confirm the antipodal cell identity. A seed from a plant carrying the DD65::CENH3::GFP fusion, at the 8-nuclear endosperm stage, with the three antipodal nuclei (*white arrow*) each showing five centromeric dots, consistent with the haploid genome of the antipodal cell. In contrast, the eight endosperm nuclei (numbered from 1 to 8) are triploid. Most of the nuclei are not showing all the 15 dots in this single plane image, but they are clearly not haploid. Nuclei 1 and 2 are overlapping. Nucleus 3 is displaying 13 dots out of the 15 centromeres corresponding to the triploid endosperm

are observed frequently at endosperm 16-nuclei stage as well, but less frequently at 32-nuclei stage. Supplementary Fig. 2s shows the frequencies of antipodal cell persistence at these various stages. This suggests the antipodal cells can persist until the 16 nuclear stage of the endosperm. However, the size of the three nuclei becomes smaller after fertilization as the seed develops, suggesting that degeneration of the antipodals might have been initiated at or prior to this stage.

To confirm that this group of three cells are indeed persistent antipodals and are not derived from the chalazal endosperm nuclei, we used pollen from plants carrying a reporter DD65::NLSmCherry that expresses in the central cell and early endosperm. After crossing with this pollen donor, if the three nuclei at the chalazal end are all derived from fertilized endosperm, they should express both CDC123::H2B:YFP and DD65::NLSmCherry signals, whereas if they are truly antipodal cells, they should only display the CDC123::H2B:YFP signal. Figure 3c–e shows at an early stage after fertilization, when the two endosperm nuclei are expressing both the maternal CDC123::H2B:YFP and paternal DD65::NLSmCherry signals, whereas the three nuclei in the corner express the maternal YFP reporter only. Supplementary Fig. 3s shows same results at a later (four endosperm nuclei) stage. These observations exclude the possibility that these three cells are fertilization products and suggest that they are indeed the persisting antipodal cells from the female gametophyte.

Furthermore, we used a centromere-localized reporter DD65::GFP:CENH3, which marks the number of chromosomes of the cell, to further confirm the gametophytic antipodal cell identity. DD65::GFP:CENH3 has expression in the central cell, antipodal and endosperm nuclei. In *Arabidopsis*, the haploid nuclei have five chromosomes which can be visualized as five centromeric dots using this reporter, whereas the fertilized triploid endosperm nuclei can be visualized as 15 centromeric dots. At the eight endosperm nuclei stage following fertilization, we observed the expected fifteen dots in the endosperm nuclei, while the three nuclei at the chalazal pole contained only five dots (Fig. 3f, Supplementary Fig. 4s), suggesting these cells are the intact antipodals. Later on, at the 16-nuclei stage, the five dots gradually clustered together and became hard to count. This might be due to the start of degradation of the antipodal nuclei at this time. These results are consistent with the above observations of the existence of antipodal cells in post-fertilized seeds.

Taken together, the above results lead us to conclude that antipodal cells persist even after fertilization and that they continue to occupy the space in the chalazal pole even when all other cell types in the unfertilized embryo sac are no longer present.

Discussion

In this study, we have used several female gametophyte and fertilization markers to demonstrate the persistence of antipodal cells through fertilization. Based on studies using fixed samples (Murgia et al. 1993; Christensen et al. 1997), it has been generally assumed that the antipodals undergo PCD during embryo sac maturation (reviewed in Heydlauff and Gross-Hardt 2014). Typically, PCD includes membrane protrusion or blebbing, DNA fragmentation, proteolysis and the final removal of cell remnants (reviewed in Pennell and Lamb 1997). The observations of Murgia et al. (1993) reporting that the antipodals are completely absent, i.e., no membranes, cytoplasm or nuclei are visible at maturity, are consistent with the final outcome of PCD. In contrast, we show here that the antipodal cells are physically present through fertilization and beyond, that their nuclei are clearly detectable as shown by the H2B:YFP reporter, their chromosomes are still present as shown by the GFP::CENH3 reporter and that they are contained within defined plasma membranes as shown by the NUF2::GFP reporter. These observations are not consistent with the completion of PCD at maturity. We do find that antipodal cells become progressively difficult to identify after the 8–16 nuclear endosperm stage, suggesting that they might undergo PCD later in seed development. However, we cannot exclude that the antipodals might have initiated PCD around the time of fertilization and that the effects of this process are only manifested later. If this is the case, then they are unlike the synergid cells which disappear completely soon after the pollen tube invasion, consistent with active and rapid PCD. Our observations suggest that regardless of time of initiation, antipodal degeneration is rather a very slow process, with the cells physically present and displaying apparent gene expression activity at the mature FG7 stage, and persisting after fertilization.

Standard light microscopy observation methods might have been insufficient for visualizing the persistence of antipodal cells due to limited resolution and difficulties distinguishing the antipodal cells from the similar-looking nucellar cells. Furthermore, the eventual small size of a mature antipodal cell might contribute to the difficulty of detection under light microscopy. However, the TEM method used by Murgia et al. (1993) provides very high magnification and resolution, and therefore, the differences between their conclusion and those of this study require explanation. A possibility is that the methods of fixation used for TEM preparation might have caused collapse of the antipodal cells, whereas our observations were conducted without fixation. Another factor is the small size of the antipodal cells, which might require a very large number of sections to get an accurate observation of a cell

at different developmental stages by TEM. A further possibility is that the study of Murgia et al. (1993) was conducted in a starchless mutant of *Arabidopsis*, as compared to our study which uses wild-type *Arabidopsis* plants. While our findings discussed above are in Columbia ecotype of *Arabidopsis* plants, which is the same ecotype used by Murgia et al. (1993), we also reciprocally crossed the CDC123::H2B:YFP reporter in Columbia background with wild-type Landsberg *erecta* plants. The progeny show the same results as those in the Columbia ecotype at the mature stage of female gametophytes (Supplementary Fig. 5s), suggesting that antipodal cell persistence in mature female gametophytes is not restricted to specific ecotypes.

In conclusion, we suggest that the mature female gametophyte of *Arabidopsis* should properly be considered to have seven cells including the antipodal cells, and not four cells. The persistence of the antipodals raises the question of whether they contribute functionally to the embryo sac or the endosperm in *Arabidopsis*. In cereals, antipodal cells proliferate during embryo sac maturation and fertilization and have been proposed to act as transfer cells for nutrient intake from the maternal tissues of the ovule (Diboll and Larson 1966; Dong and Yang 1989; Maeda and Miyake 1997). It is possible that the antipodal cells in *Arabidopsis* perform a similar function during early endosperm development. This possibility is supported by the movement of reporter proteins between antipodals and nucellar cells suggestive of symplastic connections; however, no such movement was detected between the antipodal cells and the central cell (Lawit et al. 2013). Therefore, while it is possible that *Arabidopsis* antipodals can communicate with the nucellus, there is presently no definitive evidence that they can act as a conduit for movement of nutrients to the central cell or endosperm. Further studies using mutants or cell ablation might determine whether they might have specific functions in embryo sac or seed development. The results from this study also have implications for the interpretation of several gametophytic mutants described in *Arabidopsis*. For example, several candidate genes related to the antipodal cell life span have been discussed based on the assumption that antipodal cells degenerate at the mature stage in a wild-type embryo sac (reviewed in Heydlauff and Gross-Hardt 2014). These include genes proposed to directly affect antipodal life span such as SYCO ARATH (Kägi et al. 2010), GAMETE CELL DEFECTIVE1 (Wu et al. 2012) and others such as LACHESIS, CLOTHO and ATROPOS which result in enlarged and mis-positioned antipodal-like cells when mutated (Kägi et al. 2010; Moll et al. 2008). For many of these genes, the possibility that the visibility of antipodal cells in the mutant embryo sacs might not be due to extension of the antipodal life spans, but arise from cell fate specification defects, should be

considered. The observations reported in this study that the antipodal cells are present in mature female gametophytes, and early seed development in *Arabidopsis* will assist in the formulation of conclusions about gene functions and facilitate a comprehensive understanding about the female gametophyte and antipodal cell development.

Author contribution V.S. and X.S. designed the research. X.S. and L.Y. performed experiments. V.S. and X.S. analyzed data. X.S. and V.S. wrote the article.

Acknowledgments The authors are grateful to Wei-Cai Yang, Institute of Genetics and Development, for the CDC123::H2B:YFP fusion, to Maruthachalam Ravi for the CENH3::GFP fusion, and to Scott Russell (U. Oklahoma), Marissa Simon and Shai Lawit (Pioneer), and the members of the Sundaresan and Gasser laboratories at the University of California-Davis for helpful comments and suggestions. This research was supported by a National Science Foundation Grant IOS-1051951 to V.S. X.S. was partially supported by the Elsie Taylor Stocking Memorial Research Fellowship.

Conflict of interest The authors declare that no conflict of interest exists.

References

- Christensen CA, King EJ, Jordan JR, Drews GN (1997) Megagametogenesis in *Arabidopsis* wild type and the *Gf* mutant. Sex Plant Reprod 10:49–64
- Diboll AG, Larson DA (1966) An electron microscopic study of the mature megagametophyte in *Zea mays*. Am J Bot 53:391–402
- Dong J, Yang HY (1989) An ultrastructural study of embryo sac in *Oryza sativa* L. Acta Bot Sin 31:81–88
- Drews GN, Koltunow AM (2011) The female gametophyte. The Arabidopsis Book 9:e0155. doi:10.1199/tab.0155
- Heydlauff J, Gross-Hardt R (2014) Love is a battlefield: programmed cell death during fertilization. J Exp Bot 65:1323–1330
- Higashiyama T, Kuroiwa H, Kawano S, Kuroiwa T (1998) Guidance in vitro of the pollen tube to the naked embryo sac of *Torenia fournieri*. Plant Cell 10:2019–2031
- Kägi C, Baumann N, Nielsen N, Stierhof Y, Groß-Hardt R (2010) The gametic central cell of *Arabidopsis* determines the lifespan of adjacent accessory cells. PNAS 107:22350–22355
- Lawit SJ, Chamberlin MA, Agee A, Caswell ES, Albertsen MC (2013) Transgenic manipulation of plant embryo sacs tracked through cell-type-specific fluorescent markers: cell labeling, cell ablation, and adventitious embryos. Plant Reprod 26:125–137
- Leshem Y, Johnson C, Wuest SE, Song X, Ngo QA, Grossniklaus U, Sundaresan V (2013) Pollen tube entry into the synergid cell of *Arabidopsis* is observed at a site distinct from the filiform apparatus. Plant Reprod 26:93–99
- Liu CM, Meinke DW (1998) The titan mutants of *Arabidopsis* are disrupted in mitosis and cell cycle control during seed development. Plant J 16:21–31
- Ma H, Sundaresan V (2010) Development of flowering plant gametophytes. Curr Top Dev Biol 91:379–412
- Maeda E, Miyake H (1997) Ultrastructure of antipodal cells of rice (*Oryza sativa*) before anthesis with special reference to concentric configuration of endoplasmic reticula. Jpn J Crop Sci 66:488–496
- Maheshwari P (1950) An introduction to the embryology of angiosperms. McGraw-Hill, New York

- Moll C, von Lyncker L, Zimmermann S, Kagi C, Baumann N, Twell D, Grossniklaus U, Gross-Hardt R (2008) CLO/GFA1 and ATO are novel regulators of gametic cell fate in plants. *Plant J* 56:913–921
- Murgia M, Huang BQ, Tucker SC, Musgrave ME (1993) Embryo sac lacking antipodal cells in *Arabidopsis thaliana* (Brassicaceae). *Am J Bot* 80:824–838
- Pennell RI, Lamb C (1997) Programmed cell death in plants. *Plant Cell* 9:1157–1168
- Randolph LF (1936) Developmental morphology of the caryopsis in maize. *J Agric Res* 53:881–916
- Ravi M, Chan SW (2010) Haploid plants produced by centromere-mediated genome elimination. *Nature* 464:615–618
- Rotman N, Durbarry A, Wardle A, Yang WC, Chaboud A, Faure JE, Berger F, Twell D (2005) A novel class of MYB factors controls sperm-cell formation in plants. *Curr Biol* 15:244–248
- Schneitz K, Hulskamp M, Pruitt RE (1995) Wild-type ovule development in *Arabidopsis thaliana*: a light microscope study of cleared whole-mount tissue. *Plant J* 7:731–749
- Sprunck S, Gross-Hardt R (2011) Nuclear behavior, cell polarity, and cell specification in the female gametophyte. *Sex Plant Reprod* 24:123–136
- Steffen JG, Kang IH, Macfarlane J, Drews GN (2007) Identification of genes expressed in the *Arabidopsis* female gametophyte. *Plant J* 51:281–292
- Sundaresan V, Alandete-Saez M (2010) Pattern formation in miniature: the female gametophyte of flowering plants. *Development* 137:179–189
- Wu JJ, Peng XB, Li WW, He R, Xin HP, Sun MX (2012) Mitochondrial GCD1 dysfunction reveals reciprocal cell-to-cell signaling during the maturation of *Arabidopsis* female gametes. *Dev Cell* 23:1043–1058
- Yadegari R, Drews GN (2004) Female gametophyte development. *Plant Cell* 16(Suppl):S133–S141