

The peri-germ cell membrane: poorly characterized but key interface for plant reproduction



Pollen is the male gametophyte of flowering plants, the typically haploid generation of the plant life cycle that produces the male gametes.

Upon germination, pollen grains extend a pollen tube to transport the male gametes (sperm cells) towards the ovules for double fertilization of the female gametes harboured by the female gametophyte or the embryo sac. Pollen grains exhibit a unique ‘cell(s) within a cell’ organization. After meiosis, each single-celled microspore divides highly asymmetrically to give rise to a vegetative cell and a generative cell. The generative cell is subsequently engulfed by the vegetative cell, separates from the external pollen wall and thus becomes enclosed within the large vegetative cell while migrating to the centre of the pollen grain. Later in development, the generative cell divides into a pair of sperm cells. As a result of this developmental process, a unique membrane surrounds the male germ cells (the generative cell and subsequently the two sperm cells). Here we propose to standardize and unify the name of this membrane as the peri-germ cell membrane (Fig. 1a).

Over the past 50 years, only a few studies have examined the peri-germ cell membrane. Nonetheless, it has been assigned multiple names. For example, the peri-germ cell membrane was identified in 1969 (ref. 1) as the ‘generative cell envelope’. Subsequent ultrastructural work in the 1980s led to new names, including ‘internal plasma membrane of the vegetative cell’, ‘inner plasma membrane of the pollen grain’ and ‘inner vegetative cell plasma membrane’^{2–6}. Another study referred to it as the ‘pollen tube inner plasma membrane’ when considering the pollen tube⁷. When the *Arabidopsis* small GTPase RHO OF PLANTS 9 (*AtROP9*) was identified as the first protein reported to locate to this membrane, the authors named it the ‘invasinated pollen tube plasma membrane’⁸. More recently, when the maize NOT-LIKE-DAD protein (NLD; also known as MATRILINEAL (MTL) or PHOSPHOLIPASE-A1 (*ZmPLA1*)) was found

to localize exclusively to the peri-germ cell membrane, the authors named it the ‘pollen endo-plasma membrane’⁹.

This diverse nomenclature causes confusion and calls for the implementation of a consensus on terminology. For example, the term ‘generative cell envelope’ is too restrictive, as the peri-germ cell membrane also encloses the two sperm cells. Similarly, the use of ‘pollen tube’ in the nomenclature is not appropriate because this membrane is also present before pollen germination. Furthermore, using ‘plasma membrane’ could lead to confusion with the classical plasma membrane (PM) of the generative cell, sperm cells or vegetative cell. Additionally, the term plasma membrane is misleading, given that the peri-germ cell membrane may differ in protein and lipid composition from the classical PM.

In Fig. 1, we show that three PM marker proteins expressed in the vegetative cell do not localize to the peri-germ cell membrane (3×mNG-SYP121, 3×mNG-SYP132, and tdTomato-LTI6b in Fig. 1c–e; see Supplementary Methods). Conversely, NLD (Fig. 1b), the engineered Lyn24 probe (a chimeric protein that associates with detergent-resistant membranes in metazoan cells)⁸ (Fig. 1c), *AtROP9*⁸, *AtLARP6C* (La-related proteins)¹⁰ and the glutamate receptor like channels (*AtGLR3.3*)¹¹ all localize to the peri-germ cell membrane and either are absent from the PM of the vegetative cell or only weakly label the vegetative cell PM at pollen tube tips (in the cases of Lyn24 and *AtROP9*)⁸ (Fig. 1c). Last, lipid sensors differentially mark the vegetative cell PM and the peri-germ cell membrane, suggesting a distinct lipid signature of the peri-germ cell membrane⁹ that may be responsible for the selective sorting of membrane proteins. Together, these data underscore the importance of avoiding the term plasma membrane when referring to this membrane. To reach a consensus on terminology and facilitate integration of future independent studies in the field of sexual plant reproduction, we propose to designate the membrane enveloping the generative cell and subsequent sperm

cells that represent the flowering plants’ male germ cells as the peri-germ cell membrane. We opted for the prefix peri-, inspired by examples from cell biology where structures are enveloped by plant membranes. For instance, in arbuscular mycorrhiza symbiosis, the fungal hyphae located within the plant cell are encircled by a plant membrane known as the periarbuscular membrane¹², and in the symbiotic association between *Rhizobium* bacteria and roots, rhizobial symbionts are enclosed by a membrane derived from the plant PM, referred to as the peribacteroid membrane¹². We encourage the research community to adopt the term peri-germ cell membrane for future use, to clarify and unify the nomenclature. To avoid confusion, we advise the community to not use abbreviations as much as possible, but in case an abbreviation is needed, then use PGCm.

As we move forward, it is essential that we deepen our insight into the function and importance of the peri-germ cell membrane. Previous work indicated that the peri-germ cell membrane may have a structural role in pollen grains, as sperm cells not enclosed within the peri-germ cell membrane do not remain connected as a pair⁷. Moreover, this ‘cell(s) within a cell’ organization implies that if there is any form of communication between the vegetative cell and the germ cells (either generative or sperm cells), it must involve the peri-germ cell membrane. In this context, it is also important to note that the inter-membrane space between the peri-germ cell membrane and the PM of germ cells should be considered as an apoplast with unique features due to its origin, the extremely close proximity of the two membranes (–20–50 nm)^{1,9,13} and the mobility of the germ cells within the vegetative cell’s cytoplasm. Furthermore, the peri-germ cell membrane attaches the germ cells to the neighbouring vegetative nucleus in a structure named the male germ unit^{3,13,14} (MGU; Fig. 1a–c,e). Consequently, all male gametophytic nuclei move as a unit within the pollen tube to reach the embryo sac. Upon delivery of the MGU to the female gametophyte,

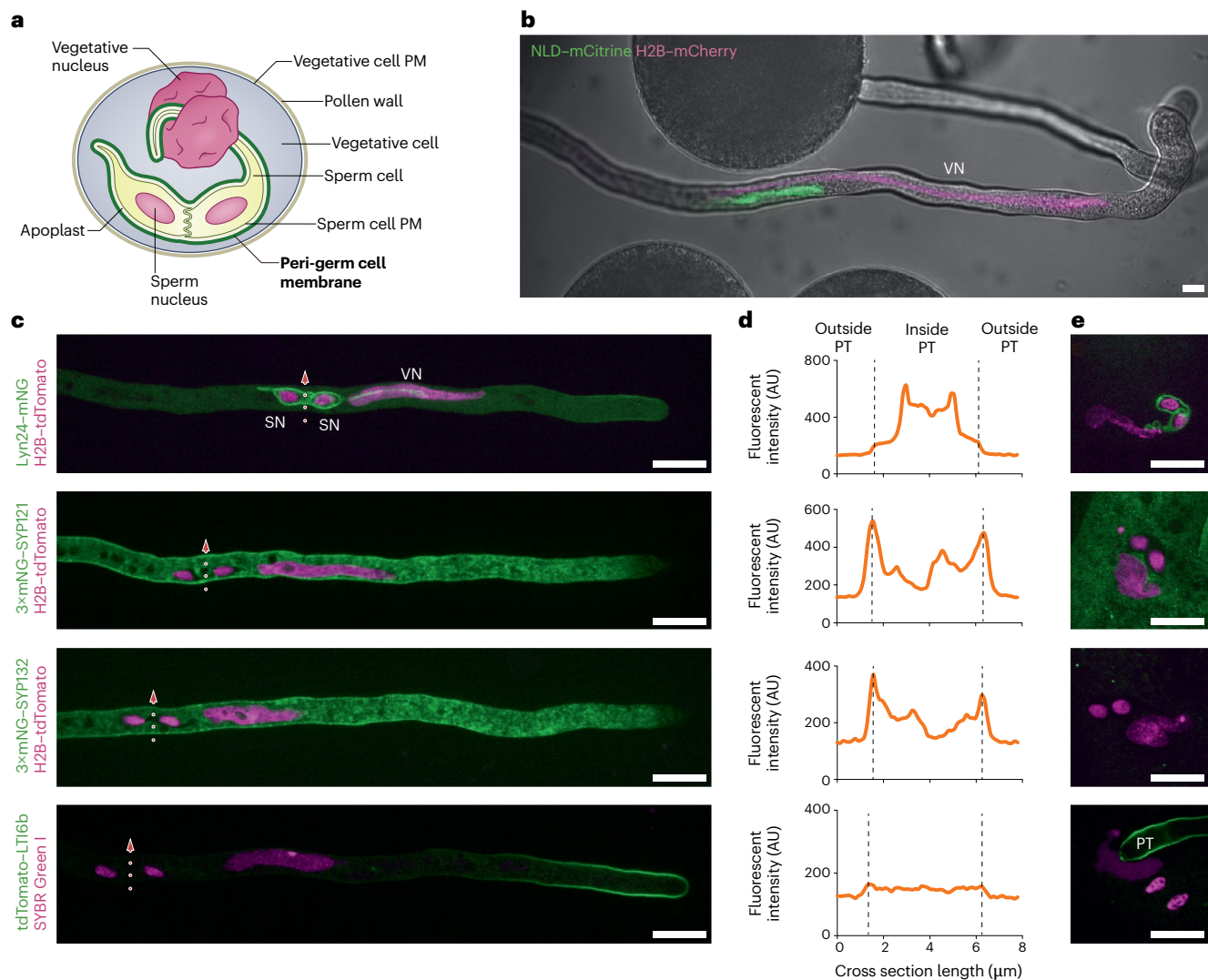


Fig. 1 | Peri-germ cell membrane labelling differs from canonical PM makers.

a, Diagram highlighting the peri-germ cell membrane in a pollen grain. **b**, Representative confocal images of a maize pollen tube expressing the peri-germ cell membrane-localized protein NLD-mCitrine and a vegetative nucleus (VN)-localized H2B-mCherry under the control of the maize vegetative cell-specific *NLD* promoter. Scale bar, 10 μm . **c**, Representative confocal images of pollen tubes expressing the plasma membrane marker proteins 3xmNG-SYP121, 3xmNG-SYP132 and tdTomato-LTI6b under the control of the vegetative cell-specific *LATS2* promoter. The marker Lyn24-mNG, expressed under the control of the vegetative cell-specific *ACA3* promoter, is used as a marker of the

peri-germ cell membrane. Sperm and vegetative nuclei were labelled with SYBR Green I in the tdTomato-LTI6b marker line or with the ubiquitous H2B-tdTomato nuclear marker in the other three lines. Scale bars, 10 μm . **d**, Plots of fluorescence intensities measuring transverse pollen tube (PT) sections at sperm cell connections indicated by red dotted arrows in **c**. The x axis represents the length along these arrows, and zero indicates the starting point of the section. Dashed lines indicate limits between outside and inside of the pollen tube. **e**, Confocal images of male germ units (sperm cells and vegetative nuclei) immediately after discharge from the pollen tubes after their rupture. Scale bars, 10 μm . SN, sperm nucleus.

the peri-germ cell membrane is removed to allow gamete fusion¹³. Recent cell biological studies have visualized peri-germ cell membrane breakdown upon fertilization^{9,15}, but the mechanism(s) that regulate these phenomena are unknown. Deciphering the roles of the peri-germ cell membrane during both pollen development and fertilization will be

challenging, but will also be pivotal for advancing plant reproductive biology.

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Published online: 15 October 2024

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Acknowledgements

We thank H. Ikeda and H. Kakizaki for technical support; Y. Mizuta for *Arabidopsis* seeds containing the *pLAT52:tdTomato-LT16b* transgene (YMv075); and D. Kurihara for Dkv278. We acknowledge the PLATIM imaging facility of the SFR Biosciences (Université Claude Bernard Lyon 1, CNRS UAR3444, Inserm U8, ENS de Lyon). This work was supported by JSPS KAKENHI (JP20H05778 and JP20H05781 to D.M.; JP23K14214 and JP24KJ0184 to N.S.); the Nagahisa Science Foundation (G2023-01 to N.S.); the French National Research Agency (ANR) (ANR-19-CE20-0012 to T.W.); the Région Auvergne-Rhône-Alpes (grant 'HD-INNOV' from the 'pack ambition recherche' to T.W.); and the DIVEDIT project of the Sélection Végétale Avancée research program, and benefited from government funding managed by the National Research Agency under the France 2030 program, reference ANR-22-PSV-003 to T.W.; A.R.M.C. is currently supported by a PhD fellowship from the Association Nationale de la Recherche Technique (ANRT) (Grant Cifre no. 2023/1284).

Author contributions

T.W. and D.M. took the lead on the renaming project and wrote the manuscript with the help of M.M.-B. N.S. performed the analysis of *Arabidopsis* pollen tubes and prepared figure panels. A.R.M.C. and N.M.A.J. observed pollen tubes in maize. All authors participated in the discussion regarding the term peri-germ cell membrane, contributed to the critical reading of the manuscript and approved the manuscript submission.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41477-024-01818-5>.