3. Bull, M.J., and Plummer, N.T. (2014). Part 1: The human gut microbiome in health and disease. Integr. Med. (Encinitas) *13*, 17–22.

4. Davy, S.K., Allemand, D., and Weis, V.M. (2012). Cell biology of cnidarian-dinoflagellate symbiosis. Microbiol. Mol. Biol. Rev. *76*, 229–261.

5. Oldroyd, G.E., Murray, J.D., Poole, P.S., and Downie, J.A. (2011). The rules of engaguement in the legume-rhizobial symbiosis. Annu. Rev. Genet. *45*, 119–144.

6. Zehr, J.P. (2015). EVOLUTION. How single cells work together. Science *349*, 1163–1164.

7. Kräutler, B. (2005). Vitamin B_{12}: chemistry and biochemistry. Biochem. Soc. Trans. *33*, 806–810.

8. Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J., and Smith, A.G. (2005). Algae acquire vitamin B_{12} through a symbiotic relationship with bacteria. Nature *438*, 90–93.

9. Wagner-Döbler, I., Ballhausen, B., Berger, M., Brinkhoff, T., Buchholz, I., Bunk, B., Cypionka, H., Daniel, R., Drepper, T., Gerdts, G., *et al.* (2010). The complete genome sequence of the algal symbiont *Dinoroseobacter shibae*: a hitchhiker's guide to life in the sea. ISME J. *4*, 61–77.

10. Bonnet, S., Webb, E.A., Panzeca, C., Karl, D.M., Capone, D.G., and Sanudo-Wilhelmy, S.A. (2010). Vitamin B_{12} excretion by cultures of the marine cyanobacteria *Crocosphaera* and *Synechococcus*. Limnol. Oceanogr. *55*, 1959–1964.

11. Helliwell, K.E., Lawrence, A.D., Holzer, A., Kudahl, U.J., Sasso, S., Kräutler, B., Scanlan, D.J., Warren, M.J., and Smith, A.G. (2016). Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B_{12}. Curr. Biol. *26*, 999–1008.

12. Croft, M.T., Warren, M.J., and Smith, A.G. (2006). Algae need their vitamins. Eukaryot. Cell *5*, 1175–1183.

13. Sañudo-Wilhelmy, S.A., Cutter, L.S., Durazo, R., Smail, E.A., Gómez-Consarnau, L., Webb, E.A., Prokopenko, G., Berelson, W.M., and Karl, D. (2012). Multiple B-vitamin depletion in large areas of the coastal ocean. Proc. Natl. Acad. Sci. USA *109*, 14041–14045.

14. Helliwell, K.E., Scaife, M.A., Sasso, S., Araujo, A.P.U., Purton, S., and Smith, A.G. (2014). Unraveling vitamin B_{12}-responsive gene regulation in algae. Plant Physiol. *165*, 388–397.

Plant Reproduction: AMOR Enables Males to Respond to Female Signals

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The pollen tube of flowering plants undertakes a long journey to transport two sperm cells for double fertilization. New work on pollen tube guidance has identified an arabinogalactan-derived ovular factor that primes tubes to respond to female gametophyte-secreted attraction signals.

Fertilization in flowering plants (angiosperms) is the most complex among all organisms, because it involves the generation and function of multicellular gametophytes [1]. After two mitotic cell divisions, male gametophytes — consisting of the haploid pollen tube cell and two enclosed sperm cells — are formed after meiosis from microspores. Female gametophytes — consisting of two female gametes, the haploid egg and di-haploid central cell, and accessory cells at both poles — are generated in the ovule from megaspores after three mitotic nuclei divisions (Figure 1) [2]. After sperm cell release and cell fusions, the fertilized egg cell or zygote develops into a diploid embryo, and the fertilized central cell produces the triploid endosperm. Together with the sporophytic maternal tissues of the ovule, they form the seed. One of the major developments in the angiosperm lineage is that the ovules are enclosed within a carpel — a structure consisting of the stigma, where pollen tubes germinate, a style and the ovary, harboring one or many ovules.

It has been a long-standing question as to how pollen tubes make their way from the stigma, through the transmitting tract of the style and ovary, and into the micropylar opening of the ovule(s). The past decade has shown that during their journey, tubes communicate extensively with the surrounding sporophytic maternal tissues, and important peptide-mediated signaling processes have been discovered that regulate germination and growth support, guidance, sperm cell release and activation [3–5]. However, these studies also revealed that fully *in vitro*-germinated and -grown pollen tubes of the eudicot model plants *Torenia fournieri* (wishbone flower) and *Arabidopsis thaliana* (thale cress) are not capable of growing into ovules and nor do they react to agarose beads releasing the peptide attractant(s) [5]. Therefore, a SIV (semi-*in vivo* or semi-*in vitro*) assay was established for both species in which pollen tubes germinate on the stigma, enter the transmitting tract of the style and exit the tract to grow towards excised ovules exposed in the vicinity of the cut styles [6,7]. In conclusion, these previous findings indicated that during their journey, pollen tubes acquire competence to respond to female attraction signals secreted from the ovule and embryo sac, respectively. Indeed the gene expression patterns of pollen tubes of both species change significantly after growing through the sporophytic tissues of the style [8,9],

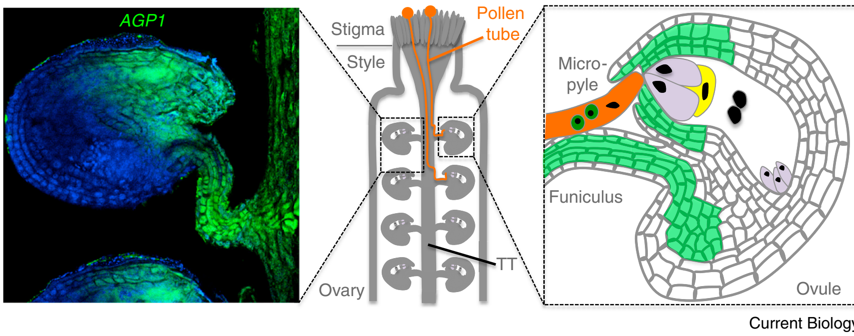

Figure 1. AMOR primes the pollen tube.
In the wishbone flower, the arabinogalactan AMOR primes pollen tubes to respond to female gametophyte-derived attraction signals. AMOR is derived from side chains of arabinogalactan proteins (AGPs). In *Arabidopsis*, AGP genes are expressed all along the pollen tube pathway that is depicted in the scheme in the middle. *AGP1*, *4*, *12* and *15* are expressed in papilla cells of the stigma and in the style, *AGP1* and *4* in transmitting tract cells of the style and ovary, while *AGP1*, *4*, *9*, *12* and *15* are expressed in ovules, which develop into seeds after fertilization. The left image shows a fluorescent *in situ* labeling of *AGP1* transcripts (green) in the ovule, funiculus and placenta. The scheme at the right shows a pollen tube (in orange) harboring two sperm cells (in green) arriving at the micropylar opening of the ovule. The female gametophyte consists of the egg cell (in yellow), the large di-haploid central cell and accessory cells (in grey) at both poles. Ovular cells expressing *AGP1*, *4*, *9* and *12* are indicated in transparent green. Left image reproduced with permission from Oxford University Press.

indicating that unknown sporophytic factor(s) activate and prime tubes to respond to female attraction signals.

A new study on pollen tube guidance, published in this issue of *Current Biology*, now reports the discovery of AMOR, a sporophytic ovular factor that induces pollen tube competency in the wishbone flower to respond to female signals [10]. The symbolic name is very well chosen after Amor, or Cupido, the Roman god who inflames love between male and female partners. After tedious isolation procedures using various purification and staining methods, Mizukami *et al.* finally discovered a non-proteinaceous competence factor(s), which seems to act in a non-species-preferential manner. First, the authors found that, after passing through the cut style of a pistil, pollen tubes are not immediately competent to respond to the attraction signal. However, within 2–6 h in ovule-containing medium, they gradually acquire competence. Surprisingly, the AMOR factor was also released into culture medium from leaves and pistils, although the highest concentration was found in ovules and in the placenta of pistils. Cultivation of ovules from related *Lindernia* species also resulted in competence acquisition, indicating that AMOR does not act in a species-specific manner, and may represent a general competency molecule. Finally, the authors identified AMOR activity as a methyl-glucuronosyl arabinogalactan (AG) polysaccharide. Moreover, they found that a chemically synthesized disaccharide, the β-epimer of methyl-glucuronosyl galactose (4-Me-GlcA-β-(1→6)-Gal or 4Me-GlcA-Gal) showed full AMOR activity. The activity of its α-epimer was significantly reduced, while the disaccharide lacking the methyl group was completely inactive.

The disaccharide 4Me-GlcA-Gal occurs exposed on the side chains of arabinogalactan proteins (AGPs), highly glycosylated cell-wall proteins that have been implicated in various aspects of pollen tube–pistil and –embryo sac interactions, although their precise mechanistic role remained unclear [11]. AGPs are a family of diverse hydroxyproline-rich plant-specific cell wall proteins defined by the presence of extensive AG polysaccharides that reside mainly at the plasma membrane–cell wall interface and in plant exudates. AG polysaccharides are added to proteins using *O*-glycosylation onto hydroxyproline residues in the AGP backbone — the addition of AG polysaccharides results in a glycoprotein whose interactive molecular surface is largely composed of carbohydrate. There are different subfamilies of AGPs, including classical AGPs, lysine-rich AGPs, AG peptides, fasciclin-like AGPs and other chimeric AGPs, and most contain a glycosylphosphatidylinositol (GPI) plasma membrane anchor sequence that tethers the extracellular AGPs to the cell surface. Notably, AGPs are very abundant along the pollen tube pathway and occur in the transmitting tract, placenta, funiculus and the micropylar opening of the ovule in *Arabidopsis* (Figure 1) [12]. Previous work has already shown that AGPs are important for pollen tube growth and guidance — in tobacco, the transmitting tract-specific AGP TTS displays a gradient of increasing glycosylation from the stigma to the ovary and is required to support pollen tube growth towards the ovule, and attract tubes in a SIV culture system [13,14]. In *Arabidopsis* it was recently shown that AGP4 plays a pivotal role in pollen tube guidance into the pistil. *agp4* mutant ovules attract more than one pollen tube into their embryo sacs [15]. By using Yariv staining, Mizukami *et al.* [10] demonstrate in this issue that AGPs accumulate especially at the sporophytic micropylar region of wishbone flower ovules, the entry site of the pollen tube. Using so-called JIM antibodies, the authors showed that AGP genes are expressed in the same region of *Arabidopsis* ovules (Figure 1). The observation that an ovular methyl-glucuronosyl arabinogalactan (AMOR) induces competency of the pollen tube to respond to ovular attractants now presents a big step forward, towards our understanding of both AGP function and the complexity of pollen tube guidance.

It will now be important to find out to what extent the above finding can be generalized, how bioactive AMOR is generated or released from AGPs, and how it acts at the mechanistic level. SIV *Arabidopsis* pollen tubes, for example, appear almost competent without ovules and thus may not need priming by AMOR. However, the tubes of this species grow through AGP-rich tissues of the transmitting tract and may already acquire competence before arriving at ovules (Figure 1). Pollen tubes of other species, such as the monocot model crop plant maize, don't require activation or priming at all and grow straight towards the attractant in *in vitro* assays [16]. Moreover, in the wishbone flower and *Arabidopsis*, the step of growth through the style cannot be fully replaced by high AMOR concentrations, pointing towards the existence of additional factor(s) involved in pollen tube activation. Future experiments should now include more detailed analysis of AGP mutants as well as mutants defective in AMOR biosynthesis (and especially its release from AGPs). AGPs are also expressed in the pollen tube [11] and it thus has to be clarified why pollen tubes don't acquire self-competence.

Although it remains unclear how AMOR acts at the mechanistic level at the pollen tube surface, the identification of the disaccharide 4Me-GlcA-Gal will allow the identification of its receptor and the downstream signaling pathway(s), possibly including lectin receptor-like kinases, which are known to be activated by saccharide signals and which are expressed in stylar tissues [17]. Sugar signaling by disaccharides such as trehalose-6-phophate play key roles during vegetative development and stress responses, and a number of downstream players have been identified [18]. The exciting findings about the role of the disaccharide 4Me-GlcA-Gal during reproduction not only add to the players of sugar signaling, but moreover significantly add to our understanding of pollen tube guidance, and elucidate another player in the complex fertilization process in flowering plants.

REFERENCES

1. Dresselhaus, T., Sprunck, S., and Wessel, G.M. (2016). Fertilization mechanisms in flowering plants. Curr. Biol. *26*, R125–R139.
2. Schmidt, A., Schmid, M.W., and Grossniklaus, U. (2015). Plant germline formation: common concepts and developmental flexibility in sexual and asexual reproduction. Development *142*, 229–241.
3. Dresselhaus, T., and Franklin-Tong, N. (2013). Male-female crosstalk during pollen germination, tube growth and guidance, and double fertilization. Mol. Plant *6*, 1018–1036.
4. Qu, L.J., Li, L., Lan, Z., and Dresselhaus, T. (2015). Peptide signalling during the pollen tube journey and double fertilization. J. Exp. Bot. *66*, 5139–5150.
5. Higashiyama, T., and Takeuchi, H. (2015). The mechanism and key molecules involved in pollen tube guidance. Annu. Rev. Plant Biol. *66*, 393–413.
6. Higashiyama, T., Kuroiwa, H., Kawano, S., and Kuroiwa, T. (1998). Guidance in vitro of the pollen tube to the naked embryo sac of *Torenia fournieri*. Plant Cell *10*, 2019–2032.
7. Palanivelu, R., and Preuss, D. (2006). Distinct short-range ovule signals attract or repel *Arabidopsis thaliana* pollen tubes in vitro. BMC Plant Biol. *6*, 7.
8. Qin, Y., Leydon, A.R., Manziello, A., Pandey, R., Mount, D., Denic, S., Vasic, B., Johnson, M.A., and Palanivelu, R. (2009). Penetration of the stigma and style elicits a novel transcriptome in pollen tubes, pointing to genes critical for growth in a pistil. PLoS Genet. *5*, e1000621.
9. Okuda, S., Suzuki, T., Kanaoka, M.M., Mori, H., Sasaki, N., and Higashiyama, T. (2013). Acquisition of LURE-binding activity at the pollen tube tip of *Torenia fournieri*. Mol. Plant *6*, 1074–1090.
10. Mizukami, A.G., Inatsugi, R., Jiao, J., Kotake, T., Kuwata, K., Ootani, K., Okuda, S., Sankaranarayanan, S., Sato, Y., Maruyama, D., *et al*. (2016). The AMOR arabinogalactan sugar chain induces pollen-tube competency to respond to ovular guidance. Curr. Biol. *26*, 1091–1097.
11. Pereira, A.M., Pereira, L.G., and Coimbra, S. (2015). Arabinogalactan proteins: rising attention from plant biologists. Plant Reprod. *28*, 1–15.
12. Pereira, A.M., Masiero, S., Nobre, M.S., Costa, M.L., Solis, M.T., Testillano, P.S., Sprunck, S., and Coimbra, S. (2014). Differential expression patterns of arabinogalactan proteins in *Arabidopsis thaliana* reproductive tissues. J. Exp. Bot. *65*, 5459–5471.
13. Cheung, A.Y., Wang, H., and Wu, H.M. (1995). A floral transmitting tissue-specific glycoprotein attracts pollen tubes and stimulates their growth. Cell *82*, 383–393.
14. Wu, H.M., Wang, H., and Cheung, A.Y. (1995). A pollen tube growth stimulatory glycoprotein is deglycosylated by pollen tubes and displays a glycosylation gradient in the flower. Cell *82*, 395–403.
15. Pereira, A.M., Nobre, M.S., Pinto, S.C., Lopes, A.L., Costa, M.L., Masiero, S., and Coimbra, S. (2016). "Love is strong, and you're so sweet": JAGGER is essential for persistent synergid degeneration and polytubey block in Arabidopsis thaliana. Mol. Plant *9*, 601–614.
16. Márton, M.L., Fastner, A., Uebler, S., and Dresselhaus, T. (2012). Overcoming hybridization barriers by the secretion of the maize pollen tube attractant ZmEA1 from Arabidopsis ovules. Curr. Biol. *22*, 1194–1198.
17. Vaid, N., Macovei, A., and Tuteja, N. (2013). Knights in action: lectin receptor-like kinases in plant development and stress responses. Mol. Plant *6*, 1405–1418.
18. Tsai, A.Y., and Gazzarrini, S. (2014). Trehalose-6-phosphate and SnRK1 kinases in plant development and signaling: the emerging picture. Front. Plant Sci. *5*, 119.

Spatial Cognition: Finding the Boundary in the Occipital Place Area

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A new study using transcranial magnetic stimulation and a virtual reality navigation task has shown that we need the brain's occipital place area to accurately remember where objects are located in relation to boundaries, but not in relation to landmarks.

Trying to remember where you parked your car among a large number of other parked cars can be frustrating. One solution is to remember where you parked relative to the boundaries of the parking area; another is to locate your car relative to a prominent landmark. Neuroimaging evidence suggests that these two strategies engage different brain circuits. Using a landmark to find a location appears to engage the dorsal striatum, whereas using a boundary recruits the hippocampus [1]. These findings have been used with other discoveries to argue that the hippocampus provides a cognitive map of the environment that is used to store long-term spatial and episodic memories, and that the striatum associates actions with discrete stimuli, such as landmarks [2,3]. Increasingly sophisticated experiments have helped characterise these functional circuits, but there is still uncertainty about how these systems receive the information needed to form memories and associations. In the case of the hippocampal circuit, there