

## Plant Evolution: What does it take to be an egg?

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Two recent studies in the liverwort *Marchantia polymorpha* shed light on the conserved function of a RKD transcription factor as a key regulator of egg cell fate in the land plant lineage.

When we think of plants, most likely a flowering plant (angiosperm) comes to our mind first. Yet, angiosperms are one of the most recent lineages in the course of land plant evolution. The first colonizers of land were Bryophytes approximately 500 Mya. The liverwort *Marchantia polymorpha* and the moss *Physcomitrella patens* are among their extant relatives [1, 2]. Bryophytes inherited from their green algae ancestors (Charophyceae) a dominant gametophytic life cycle, but gametes are produced within protective male (antheridia) and female (archegonia) multicellular gametangia. Like their ancestors reproduction is largely dependent on water, as flagellated sperm must swim to reach and fertilize a single, non-motile egg in the archegonia. The resulting zygote is retained in archegonia and does not undergo immediate meiosis, but proliferates to form an embryogenic diploid sporophyte that is protected and nourished during development. This innovation is a hallmark of all land plants (embryophytes), and heralded the appearance of a progressively dominant sporophytic life cycle, so that in angiosperms haploid gametophytes are reduced to just a few cells. Here each male (pollen) and female (embryo sac) gametophyte produces two pairs of gametes. This was paired with the invention of a unique mode of reproduction called double fertilization: One sperm fertilizes the egg forming a diploid zygote, while the second sperm fertilizes the central cell producing a triploid endosperm that nourishes the developing embryo.

In the past decade considerable progress has been made in identifying genes required for gametophyte development [3, 4], but the genetic networks that promote germ cell

specification and patterning within male and female gametophytes remain poorly understood [5, 6]. One of the best characterized genetic pathways involves the *MYB* transcription factor *DUO1* as a central regulator of cell cycle progression and sperm cell differentiation in *Arabidopsis thaliana* [7, 8]. However, few genes are known to be specifically expressed in the egg [9, 10] or to cause misspecification of egg cell fate [11, 12].

In this issue of *Current Biology*, two independent studies [13, 14] show that the *Marchantia polymorpha* transcription factor *MpRKD* conserved an ancestral function in the regulation of germ cell fate throughout land plant evolution. *RKD* factors are a monophyletic group belonging to the plant-specific *RWP-RK* transcription factor family. In *Arabidopsis*, *RKD* enriched expression in the egg cell [15] and *RKD2*'s ability to ectopically activate an egg cell-like transcriptome [10], made them primary candidates for specification of egg cell fate. Yet, definite evidences for a central regulatory control was still lacking due to an apparent functional redundancy. In a phylogenetic reconstruction, Koi *et al.* and Rövekamp *et al.* identified *Marchantia* homologues of the plant-specific *RWP-RK* transcription factor family, but just one (*MpRKD*) belongs to the land plant *RKD* clade, providing the opportunity to unfold its ancestral reproductive function.

Using transgenic reporters, the two groups showed that the transcriptional activity of *MpRKD* is initiated in developing eggs and sperm precursors (Fig. 1). The egg cell expression is maintained until maturation and disappears after fertilization. Both studies also report a lower expression in the edges of gemmae cups that develop in the haploid gametophyte plant body. These structures are dedicated to dispersal and protection of gemmae, the vegetative sprouts that develop by asexual reproduction (Fig. 1). Koi *et al.* and Rövekamp *et al.* then used different genetic strategies to investigate *MpRKD* function. Consistent with the observed expression patterns, *MpRKD* deletion lines [14] and transgenic lines expressing artificial *microRNAs* (*amiRNA*) targeting *MpRKD* transcripts [13] showed a predominant defect in egg cell development resulting in sterile female plants. The phenotype observed was similar; presence of an abnormal number of cells within the mature archegonia.

Remarkably, these observations led to two distinct interpretations. A detailed structural and cytological analysis by Koi *et al.* showed that cells within the mature archegonia of deletion mutants are highly vacuolated and abnormally surrounded by cell walls. These cells are conspicuously different from the spherical shape and electron dense cytoplasmic content of a typical mature egg cell protoplast. In addition, Koi *et al.* observed that archegonia from deletion mutants fail to attract sperm cells. Thus they concluded that *MpRKD* has a central

regulatory function in egg cell differentiation in *Marchantia*. On the other hand, Rövekamp *et al.* proposed that the abnormal number of cells present in mature archegonia result from a failure of the egg cell to maintain a transcriptional quiescent state. In this condition the egg cell initiates mitotic divisions in the absence of fertilization (parthenogenesis). This hypothesis is based on the observation that the egg cell precursor develops normally, and that the cell divisions in follow a cleavage type, usually associated to zygotes that undergo rapid cell cycles with no intermediate cell growth. However, the atypical cell division patterning, ultimately leads to embryo abortion. Rövekamp *et al.* concluded that *MpRKD* is thus required for regulation of gametophyte-to-sporophyte transition in *Marchantia* by suppressing parthenogenesis.

In addition to the egg cell phenotype, Koi *et al.* observed asynchronous cell divisions of sperm cell precursors in antheridia (Fig.1). Therefore, and although some viable sperm cells were still produced, *MpRKD* lack of function also affects spermatogenesis. Phenotypical defects also extended to gemma cup formation (Fig. 1). Here Rövekamp *et al.* reported the complete absence of gemma cup development in *amiRNA* lines. Although the production of functional gemmae was not affected, the vegetative growth of these asexual gametophytes was significantly reduced. Taken together the results from both teams support that the *Marchantia MpRKD* transcription factor acts specifically in the gametophytic generation and predominantly in reproductive structures (sexual and asexual).

Recent comparative genomic approaches have fostered the idea of “a basic molecular toolkit” [16], where ancestral regulatory genes are frequently preserved throughout land plant evolution and their ancestral functions repurposed to control the development of novel structures, sometimes with similar regulatory functions. For several reasons the *RWP-RK* family seems to be part of such a basic toolkit. The *RKD* clade in particular did not undergo extensive duplication during land plant evolution, suggesting that their functions could be related in bryophytes and angiosperms. In this context, it is also notable that the *Physcomitrella patens PpRKD* homolog is preferentially expressed in antheridia and archegonia [17 and unpublished results]. In *Arabidopsis AtRKD2*'s reprogramming potential to an egg cell-like fate [10, 15] and *AtRKD4* key function as a regulator in early stages of embryogenesis [18] acting downstream of the YDA-dependent signaling pathway [19] also support this hypothesis. *AtRKD5* however is more broadly expressed, possibly reflecting a progressive transition of *RKD* ancestral reproductive functions in the gametophytic generation to broader functions in the dominant sporophyte of flowering plants.

The noteworthy findings reported by these two studies [13, 14] open exciting new questions. First and foremost, is *MpRKD* a central regulator of egg cell differentiation or of

gametophyte-to-sporophyte transition? In this regard, the hypotheses proposed by these two studies may not be mutually exclusive. If the egg cell precursor is correctly determined in *Marchantia* deletion and amiRNA lines, then *MpRKD* could be potentially involved in two distinct developmental pathways: the first activating a final egg cell differentiation program, while a second pathway would maintain the mature egg cell in a quiescent state preventing initiation of mitotic proliferation in the absence of fertilization. Parthenogenetic development is a fairly rare event in plants, but in *Arabidopsis* a *MULTICOPY SUPPRESSOR OF IRA 1 (MSII)* mutant is able to develop nonviable embryos from haploid egg cells in the absence of fertilization, though the regulatory mechanisms underlying this process are not yet understood. The introgression of egg cell and embryo specific markers and identification of *MpRKD* targets in *Marchantia* can now be tackled and will help to clarify potential evolutionary genetic pathways controlling egg cell fate. The second question relates to *MpRKD*'s possible ancestral function in the differentiation of male and female gametes. Indeed, *MpRKD* deletion mutants show defects in both female and male gametogenesis, though less pronounced in the latter. The best link to a possible ancestral function of *RKD* factors in sex determination is found in the differentiation of complementary mating types in the unicellular algae *Chlamydomonas reinhardtii*. Upon nitrogen depletion, the *MINUS DOMINANCE (MID)* gene is activated in the *minus* gamete. In the green algae *Volvox carteri* *MID* function was conserved in male gametes. Interestingly, *MID* has close homology to the plant *NLP* clade of the *RWP-RK* gene family with functions in nitrogen-responsive processes. Although a relation of *RKD* factors and potential *MID*-targets remains unexplored in basal land plants, the expression pattern of a known male-specific *MID*-target in *Physcomitrella patens* egg cells is of special note [20]. This raises the question whether *RKD* or related ancestral *RWP-RK* genes were somehow involved in the morphological differentiation of sexual gametes (anisogamy) in close ancestors of Bryophytes, like in extant Charophycean algae members.

In summary, these two studies open new avenues of research into the genetic pathways leading to egg cell differentiation and fate determination. This knowledge will have a critical impact on future efforts aiming to manipulate reproductive development in our favor to meet the ever-increasing agricultural demands.

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## FIGURE LEGEND

**Figure 1:** *MpRKD* expression and function in the liverwort *Marchantia polymorpha*.

The dominant phase in the life cycle of *Marchantia* is the haploid gametophyte with male and female plants, on which stalked, umbrella-like reproductive structures will develop (a and g). Within these structures a single egg cell (d) and numerous flagellate sperm (j) are produced within multicellular female (archegonia) (b-e) and male (antheridia) (h-j) gametangia. Upon release the sperm cells swim to the archegonia and fertilize the mature egg cell. The diploid zygote is retained in the archegonia and quickly proliferates, developing into an embryogenic sporophyte (e). Vegetative reproduction also occurs in male and female plants through the formation of asexual sprouts or gemma within fringed cups (gemma cups) (f). *MpRKD* expression in archegonia is initiated in the egg cell (b-d) and in some cells in the archegonia neck canal, in sperm cell precursors (h-j) and in the edges of gemma cups (f). In *MpRKD* *amiRNA* and deletion lines the egg cell precursor proliferates in the absence of fertilization by atypical mitotic divisions (c'-d'), forming a multicellular embryo-like structure that later aborts (e'). In addition, spermatogenesis is asynchronous (j'), but antheridia still produce some functional sperm cells. Gemma cups are absent or reduced, leading to gemma with reduced vegetative growth (f'). Dashed rectangles highlight main phenotypes of *MpRKD* *amiRNA* and deletion lines.

Figure 1

