



ACUTE VIEW

Alternation of generations – unravelling the underlying molecular mechanism of a 165-year-old botanical observation

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ABSTRACT

Characteristically, land plants exhibit a life cycle with an ‘alternation of generations’ and thus alternate between a haploid gametophyte and a diploid sporophyte. At meiosis and fertilisation the transitions between these two ontogenies take place in distinct single stem cells. The evolutionary invention of an embryo, and thus an upright multicellular sporophyte, in the ancestor of land plants formed the basis for the evolution of increasingly complex plant morphologies shaping Earth’s ecosystems. Recent research employing the moss *Physcomitrella patens* revealed the homeotic gene *BELL1* as a master regulator of the gametophyte-to-sporophyte transition. Here, we discuss these findings in the context of classical botanical observations.

ALTERNATION OF GENERATIONS

The German 19th century botanists Hofmeister (1851, 1862) and Strasburger (1894) recognised that all land plants alternate between two phases with different body plans (ontogenies): the haploid gametophyte and the diploid sporophyte. Hofmeister (1851) coined the term *Generationswechsel* for these fundamental phase transitions. This term was insufficiently translated into ‘alternation of generations’, although in a strict biological sense, a generation is not an ontogeny. According to morphological (Graham *et al.* 2000) and molecular (Karol 2001; Ruhfel *et al.* 2014; Zhong *et al.* 2014) evidence all land plants are monophyletic; with the alternation of generations as an autapomorphic feature (Graham 1993). In molecular terms, each single plant genome encodes two different developmental programmes (ontogenies), posing the fundamental question of how the switches from one ontogeny to the other are controlled at the genetic level (Friedman 2013). It is evident that under normal conditions, fertilisation and meiosis are the checkpoints between the two developmental programmes. However, haploidy and diploidy accompany these developmental switches, but are not their prerequisites, as for example haploid sporophytes can be obtained from pollen of flowering plants (Kimber & Riley 1963) and diploid gametophytes can be obtained by protoplast fusion in mosses (Hohe & Reski 2005; Schween *et al.* 2005).

A SPOROGENIAL FACTOR UNDER EPIGENETIC CONTROL

Gametophytes and sporophytes of non-flowering plants can be easily observed and manipulated. Therefore, they traditionally have been employed for studies of developmental processes underlying the alternation of generations (Edwards & Kenrick 2015). Among these, mosses like *Physcomitrella patens* are early-diverging land plants and therefore well-suited models to infer the origin and evolution of developmental pathways (evo-devo; Floyd & Bowman 2007; Menand *et al.* 2007). In mosses, meiosis can be circumvented by mechanically injuring sporophytic vegetative tissue; this causes the development of diploid tissue with gametophyte morphology (apospory; Pringsheim 1876). In several fern species, the development of sporophytes from vegetative gametophytic cells (apogamy) is a common mechanism (Farlow 1874; Dyer *et al.* 2012). Although apogamy is not a feature of regular moss development, it can occur in old cultures of several moss species (Lal 1984). Bauer (1959) found that once apogamy was established, gametophytic cells gave rise to further apogamous sporophytes. However, the gametophytic tissue lost this capacity once it was mechanically separated from already established apogamous sporophytes. Therefore, Bauer (1959) suggested that a mobile self-replicating ‘sporogonial factor’ is produced in moss sporophytes that can induce the development of further sporophytes from

gametophytic cells. Ripetsky (1985) succeeded in establishing a gametophyte moss culture *via* aposporous regeneration of sporophytic cells. This diploid gametophyte continuously gave rise to apogamous sporophytes, even in the absence of previously established sporophytes. Based on these observations, Ripetsky (1985) postulated that the hypothetical sporogonial factor is under epigenetic control and is switched on in sporophytes.

A SINGLE GENE TRIGGER FOR ASEXUAL REPRODUCTION

In recent years, genes controlling the transition from one generation to another have been identified in *P. patens*. *MKN6*, coding for a member of the class 2 KNOTTED1-LIKE HOME-ODOMAIN (KNOX) transcription factors, was identified as a repressor of apospory: targeted deletion of the gene led to the development of gametophytic (but diploid) protonemata from sporophytic cells (Sakakibara *et al.* 2013).

Conversely, targeted deletion of either POLYCOMB REPRESSIVE COMPLEX 2 (PRC2) member *FERTILISATION INDEPENDENT ENDOSPERM (FIE)* or *CURLY LEAF (CLF)* resulted in apogamy; the formation of structures that resembled sporophytic tissue in gene expression and morphology instead of adult gametophytic tissue (leafy gametophore shoots; Mosquna *et al.* 2009; Okano *et al.* 2009). At the morphological level this transition is characterised by a switch from three-faced meristematic cells in the leafy gametophore shoot to two-faced apical cells in the sporophyte, including the embryo (see Reski 1998 for a review on moss morphology). This reveals that in the gametophyte sporophyte-specific developmental programmes are repressed *via* epigenetic control of sporophyte-specific gene expression exerted by the PRC2. In plants, animals and humans this protein complex regulates development by silencing transcription factor genes *via* trimethylation of lysine 27 on histone H3 (H3K27me3; Simon & Kingston 2009; Margueron & Reinberg 2011). Known PRC2 targets include *KNOX* genes as well as genes coding for the dimerisation partners of KNOX proteins, the BELL proteins (Zhang *et al.* 2007; Lodha *et al.* 2013).

The *P. patens* genome (Zimmer *et al.* 2013) encodes four *BELL* genes, of which *BELL1* has a particularly intriguing expression pattern: translational *BELL1*:GUS fusion proteins accumulate in the egg cell and in the sporophyte. In addition, its transcription is induced in *P. patens* PRC2 knockout

mutants. Deletion of *BELL1* sufficed to suppress the *PRC2* knockout phenotype, resulting in the reversion to leafy gametophyte development. The ectopic overexpression of *BELL1* in the *P. patens* gametophyte caused the development of fully functional sporophytes without fertilisation (apogamy) from vegetative gametophytic tissue (Fig. 1), making *BELL1* the first identified single gene trigger for asexual reproduction in plants (Horst *et al.* 2016). In several flowering plant species asexual reproduction can occur (apomixis; Winkler 1908), but a trigger for this phase transition has not yet been identified (Radoeva & Weijers 2014). A model for the control of the *P. patens* *Generationswechsel* is depicted in Fig. 2.

The *BELL1* protein can interact with all five *P. patens* KNOX proteins, suggesting activity of *BELL1*-KNOX dimers at the developmental switch from gametophyte to sporophyte. As *BELL1* is epigenetically regulated by the PRC2 (Widiez *et al.* 2014), and a *BELL* mRNA can be mobile (Banerjee *et al.* 2006), the discovery of the role of *BELL1* in moss development (Horst *et al.* 2016) provides mechanistic insights into the predictions of Bauer (1959) and Ripetsky (1985) of a mobile sporogonial factor under epigenetic regulation that is produced in the sporophyte and can induce the formation of further sporophytes.

THE INVENTION OF THE EMBRYO

Land plant evolution led to an increasing dominance of the sporophyte over the gametophyte. Extant members of streptophyte algae, the sister group of land plants, have a multicellular gametophyte and a single diploid stem cell, the zygote, which undergoes meiosis. A delay of meiosis and intercalation of mitotic divisions resulted in the formation of a multicellular embryo that gives rise to the multicellular sporophyte (Bower 1908), a fundamental innovation in land plant evolution (Niklas 1997; Graham *et al.* 2000). This innovation enabled the development of complex tissues and thus the complex ecosystems in which we live. The genetic network governing this new plant body was recruited from algal genes (Niklas & Kutschera 2010). Part of it was co-opted from genes originally determining the multicellular gametophyte (Menand *et al.* 2007; Yip *et al.* 2016). However, the specification of the diploid life stage by *BELL*-KNOX heterodimers was already established prior to the emergence of land plants: in the unicellular haplontic alga *Chlamydomonas* a single KNOX protein and a BELL-related protein specify the zygote (Lee *et al.* 2008). The findings of Horst *et al.* (2016) now suggest that altered expression of an algal *BELL* gene facilitated the development of a multicellular embryo. Subsequent multiplications of *KNOX* and *BELL* genes (Mukherjee *et al.* 2009) then provided the molecular basis



Fig. 1. A regular sporophyte derived from fertilisation on the apex of a leafy *Physcomitrella patens* gametophyte (A). An apogamous sporophyte derived from asexual reproducing gametophytic filamentous cells (B). Scale bars: 500 µm in A, 200 µm in B.

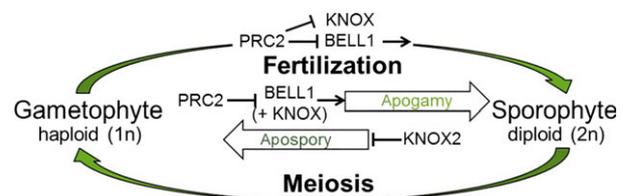


Fig. 2. Model for the molecular regulation of the alternation of generations in *Physcomitrella patens*.

for growing sporophyte complexity in land plants (reviewed in Hay & Tsiantis 2010; Tsuda & Hake 2015).

CONCLUSIONS

The combinatorial control by a network of BELL and KNOX homeodomain transcription factors of the diploid phase of plants has deep evolutionary roots. The identification of the homeobox gene *BELL1* as a master regulator of gametophyte-to-sporophyte transition, embryo development and asexual

reproduction by Horst *et al.* (2016) provides mechanistic insights into the evolutionary origin of the land plant life cycle. It solves the question of how a plant stem cell can switch from one ontogeny to another, both encoded in the same genome. Moreover, if this gene trigger is not only evolutionary conserved between algae and mosses, it will be instrumental to control apomixis in flowering plants (Spillane *et al.* 2004), and thus contribute to improved crop production.

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