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Quantitative moss cell biology

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Research on mosses has provided answers to many fundamental questions in the life sciences, with the model moss *Physcomitrella patens* spearheading the field. Recent breakthroughs in cell biology were obtained in the quantification of chlorophyll fluorescence, signalling via calcium waves, the creation of designer organelles, gene identification in cellular reprogramming, reproduction via motile sperm and egg cells, asymmetric cell division, visualization of the actin cytoskeleton, identification of genes responsible for the shift from 2D to 3D growth, the structure and importance of the cell wall, and in the live imaging and modelling of protein networks in general. Highly standardized growth conditions, simplicity of most moss tissues, and an outstandingly efficient gene editing facilitate quantitative moss cell biology.

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Introduction

Research on bryophytes (comprising mosses, liverworts and hornworts) has contributed towards major breakthroughs in biology. Among them are the first description of non-Mendelian inheritance, the continuity of chromosomes through mitosis, sex chromosomes in plants (reviewed in [1]), and the efficient use of gene targeting to determine plant gene functions by reverse genetics; in this case the first eukaryotic organelle division protein [2]. From the about 20,000 bryophyte species, the moss *Physcomitrella patens*, henceforth named Physcomitrella, has developed into a model species for plant evo-devo studies with a fully sequenced genome [3•], for biotechnology, e.g., as an efficient cell factory for recombinant biopharmaceuticals [4] and for synthetic biology with the first commercial products on the market [5].

In evolutionary terms, mosses bridge the gap of approx. one billion years between single-celled green algae and seed plants [6], although the exact phylogeny of the land plants is still not solved [7]. Unlike seed plants, the dominant phase of moss development is the haploid gametophyte, while the diploid sporophyte is short-lived and dependent on nutrients provided by the gametophyte (Figure 1). Because most moss tissues are only one cell layer thick this plant group is especially amenable to advanced cell biology techniques. Moreover, it can be grown in highly controlled environmental conditions on pure mineral media, thus enhancing the reproducibility of biological observations. In the following, I will review some of the progress that has been made in the last three years employing the combination of advanced live cell imaging and the creation of mutants by highly precise genome engineering.

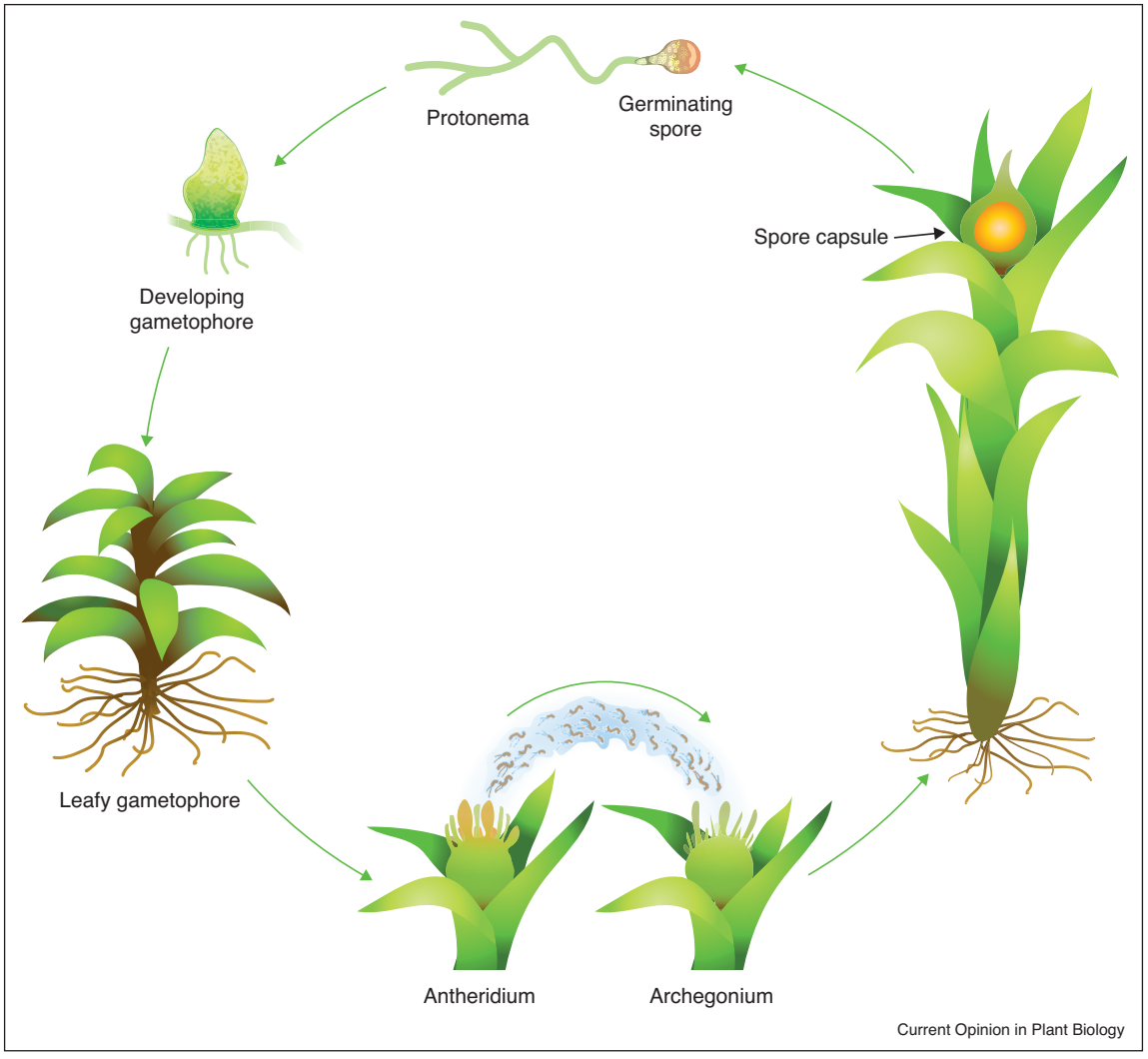
Chlorophyll fluorescence

To maintain the efficiency of photosynthesis under varying light conditions and salt stress, photosystems I and II are dynamically reorganized [8]. Using live-cell imaging it became evident that the fine thylakoid structures in Physcomitrella chloroplasts are flexible in time [9]. High-speed 3D laser scanning microscopy in combination with high-sensitivity multi-channel detection revealed the excitation energy dynamics in such plastids within 1.5 s intervals, thus reflecting the energy dynamics between light-harvesting antenna proteins and the photosystems. These results reveal the energetically active nature of photosynthetic proteins in thylakoid membranes [10••].

Designer organelles

One characteristic of eukaryotic cells is their extensive compartmentalization to separate incompatible reactions and processes, and to store high concentrations of products potentially harmful to the cell. In Physcomitrella peroxisomes, the PEX5 receptor protein interacts with different natural PTS1 amino-acid sequences to control organellar protein import. In a combinatorial mutagenesis approach, this protein-protein interaction was changed, resulting in the downregulation of normal peroxisomal functions and the establishment of an alternative protein import pathway. These alterations are regarded as an essential step towards a designer organelle, valuable for synthetic biology and biotechnology [11••].

Figure 1



The Physcomitrella lifecycle is an alternation of heterophasic and heteromorphic generations. Life cycle starting at top and proceeding counter-clockwise. Haploid spores germinate under the influence of light and water to give rise to the filamentous haploid protonema. Some protonema cells give rise to buds (developing gametophores), which proliferate via three-faced apical cells to give rise to the haploid rhizoids, stems and leaves (phyllids), the adult gametophyte, which is also called the leafy gametophore. It carries the male (antheridium) and female (archegonium) sexual organs (gametangia). Buds, stems and leaves are covered by a phenol-enriched cuticule that reduces water loss and enables erect growth. The antheridium generates bi-flagellated sperm that actively swim in a film of water to the archegonium for fertilization. The diploid zygote is the stem cell that gives rise to the embryo, the sporophyte. Embryo and sporophyte grow via two-faced apical cells. The spore capsule (sporangium) is covered by an epidermis that contains, after reprogramming of the epidermal cells, stomata that facilitate gas exchange and provide a fitness advantage. The spore capsules contain spore mother cells, which after meiosis give rise to the haploid spores that are covered by sporopollenin. Physcomitrella spore capsules do not have specific structures for spore release but simply break open under suitable environmental conditions to release the spores. Taken from [20].

Cell reprogramming

In some eukaryotes, differentiated cells can reprogram to pluripotent stem cells under specific physiological conditions. Mosses are special in this respect, as most differentiated cells reprogram directly into a stem cell that mimics the spore and regenerates into a protonema (Figure 1), when isolated from their neighbouring cells; even sporophytic cells behave like this. In contrast, cells from vascular plants undergo a callus phase upon stimulation and need subsequent hormone treatments to reprogram into differentiated cells. Interestingly, callus formation was not observed in mosses, contrary to the situation in vascular plants. When two adjacent Physcomitrella leaf (phyllid) cells are isolated as a cell pair from their leaf tissue by laser ablation, normally only one reprograms into a stem cell, while the other does not divide. The same is true for a triplet of three adjacent cells outside the leaf tissue. In this scenario, the middle cell does not divide,