

Title: 450 million years between the two DELLA-mediated green revolutions

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Summary

Plant hormone signaling pathways have diversified during plant evolution. A new study revealed a conservation of DELLA functions in growth and environmental stress responses across land plants.

Text:

In the mid 50's the Green Revolution that ultimately saved millions of lives from starvation was mediated by the development of semi-dwarf crop varieties that encode variants of the DELLA proteins (DELLAs) [1]. DELLAs belong to the GRAS family proteins and are transcription regulators originally identified in flowering plants as central negative regulators in the plant hormone Gibberellic Acid (GA) signaling pathway [1]. *Arabidopsis thaliana* DELLAs knock-out mutants display constitutive growth, delayed floral transition and are more sensitive to diverse stresses such as cold, salinity or pathogens attack [2]. Almost all known GA-mediated processes are repressed by DELLAs through sequestration of transcription factors. For example, DELLAs mediate hypocotyl elongation through PIFs transcription factors sequestration. Besides this first described roles as negative regulators, DELLAs have been also determined as transcriptional co-activators in multiple developmental processes such as seed germination, root development and arbuscular mycorrhizal symbiosis [2,3,4]. Phylogenetic analyses covering the entire plant lineages [5,6] indicated that DELLA proteins are restricted to the land plants, and thus have evolved 450 million years ago, around the time where the first land plants colonized lands, which may be considered as the first green revolution. In between this first Green Revolution and the second one in last century, DELLAs have been maintained in plant genomes, leading to their presence in extant species from the two main land plant clades: the vascular plants that encompasses ferns, conifers or flowering plants, and the Bryophytes, which are non-vascular plants including the hornworts, liverworts and mosses.

However, only vascular plants possess the complete GA signaling pathway upstream of DELLA proteins, such as the GA receptor *GID1* and the E3 ligase *GID2/SLY1* [5,7,8]. As for any other trait, to be considered ancestral a function of DELLA should be conserved in both

the vascular plants and the Bryophytes [9], thus GA signalling cannot be considered as the ancestral function of DELLA.

In this issue of *Current Biology*, Hernandez-Garcia *et al.* [10] performed the functional characterization of the single DELLA in the liverworts *Marchantia polymorpha* (MpDELLA) to determine which trait, if any, regulated by DELLA in flowering plants is also regulated in Bryophytes, and thus to infer the ancestral function of this protein in plants.

MpDELLA plays a role in growth inhibition and stress responses.

Hernandez-Garcia *et al.* found that overexpression in *M. polymorpha* of the unique MpDELLA ortholog (MpDELLAox) leads to growth inhibition involving the inhibition of cell proliferation in the meristematic regions. This is reminiscent of the flowering plant *Arabidopsis thaliana* whose DELLAs repress growth by interfering with two mechanisms: cellular expansion but also cell-cycle progression. Using a combination of reverse genetics, protein-protein interaction screens and transcriptome sequencing, the authors described another role for DELLA proteins in stress responses. In flowering plants, DELLA's role in regulating developmental processes mostly rely on the sequestration of transcriptional regulators such as the transcription factors AtPIFs that are sequestered by DELLAs to prevent hypocotyl elongation in the dark [1]. MpDELLAox and Mppif show similar phenotypes under stress induced by far-red light, they interact together and both lines display transcriptional upregulation of genes belonging to specialized metabolites, especially phenylpropanoids and flavonoids biosynthesis pathway. Altogether, these results show that MpDELLA can modulate developmental processes through inhibition of PIF transcription factors, as their flowering plant counterparts do. Hernandez-Garcia *et al.* demonstrate that the role of DELLA in regulating growth inhibition and stress responses is conserved between *Marchantia polymorpha* and flowering plants, and thus was likely already present in their last common ancestor. It will be fascinating to explore the function of DELLA in the other bryophyte clades. In mosses, *della* loss-of-function mutants have been actually already generated in *Physcomitrium patens*, but they were not affected in growth or cell-cycle progression [11]. However, whether overexpression of DELLA in *P. patens* leads to growth phenotypes remains to be tested. Similarly, with the recent development of hornworts transformation [12], such an experiment could be also conducted in the third clade of the Bryophytes.

Post-translational degradation of DELLA is a conserved mechanism

In flowering plants, DELLAs role is almost restricted to the negative regulation of downstream targets, which is released upon DELLA degradation. In vascular plants, DELLA homeostasis involves post-translational modifications mediated by the receptor GID1 that interact with

DELLA upon GA perception. This interaction leads to the degradation of DELLAs through the 26S proteasome after polyubiquitylation by the E3 ligase GID2/SLY1, and the release of the sequestered targets such as PIF [2]. In Bryophytes, GID1 orthologs have not been reported [5,7,8]. *M. polymorpha* encodes for an SLY1 ortholog but MpSLY1 involvement in MpDELLA degradation remains to be confirmed. In addition to the absence of cognate GA receptors, Bryophytes seem to be insensitive to GA treatment, suggesting that DELLA degradation must be mediated by another mechanism [13]. Furthermore, biochemical studies have showed that across land plants only vascular plants seem able to synthesize bioactive GA [14]. This suggests that integration of GAs in DELLAs growth inhibition pathway followed the emergence of vascular plants. The role of GAs in growth repression evolved by exaptation of the DELLA-PIF module.

A second scenario could be that bryophytes display a diverged trait and that vascular plants actually maintained the ancestral state. Indeed, the lack of GID1 in Bryophytes could well be the result of a single loss before the radiation of this lineage instead of a gain in vascular plants. Similarly, the lack of the final steps in the GA biosynthetic pathway might be the result of gene loss. It has even been proposed by recent *in silico* analyses that Bryophytes, with the exception of *P. patens*, might have the complete enzymatic package to produce some bioactive GAs [15]. In that scenario, an ancestral GA-GID1-DELLA-PIF signaling module could have existed in the most recent common ancestor of land plants, before diverging into a GA/GID1-independent signaling pathway in Bryophytes.

Are growth repression and regulation of stress response the only ancestral functions of DELLA?

In flowering plants, DELLA proteins also play a role in arbuscular mycorrhizal symbiosis (AMS) [3]. By contrast with the GA-mediated induction of responses (through the degradation of the negative effect imposed by DELLA) DELLAs act in coordination with positive regulators of AMS, called CCaMK and CYCLOPS, to control arbuscules development through the activation of downstream symbiotic transcription factors [16]. AMS and the symbiosis-specific regulators CCaMK and CYCLOPS have been lost in *M. polymorpha*. By contrast, the closely related species *Marchantia paleacea* maintained AMS and is suitable for reverse genetic analyses [17]. Using this species, it would be possible to determine whether, in addition to the negative regulation of PIF discovered by Hernandez-Garcia *et al.*, the role of DELLA as positive regulator is also an ancestral feature of land plants.

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Figure 1 : Evolution of DELLA proteins and signaling pathway in plants.

A. DELLAs emerged with land plants and were involved in both growth inhibition and stress responses. Biosynthesis of bioactive GAs and perception by GID1 followed the emergence of vascular plants. **B.** Alternative scenario for the evolution of the DELLA signaling pathway. The DELLA-GA-GID1-PIF module was already present in the last common ancestor of land plants, both GA biosynthesis and GID1 were lost in Bryophytes. The origin of the DELLAs function in the Arbuscular Mycorrhizal Symbiosis remains to be determined.