Viewpoints

Xyloglucan evolution and the terrestrialization of green plants

Summary

Xyloglucan (XyG) is the major noncellulosic nonpectic matrix polysaccharide in cell walls of most land plants. Initially thought to be restricted to land plants, the last decade has seen the detection of XyG and the discovery of synthesis and modification/degradation genes in charophycean green algae (CGA). Recently, a totally new function of XyG was discovered as a potent soil aggregator released by roots and rhizoids of all major groups of land plants. In this Viewpoint, I show the presence of a complex XyG genetic machinery in most CGA groups. I discuss the context of XyG evolution in light of the terrestrialization of early CGA that gave rise to embryophytes and its possible role in early soil formation.

Introduction

The colonization of land masses by plants is certainly one of the most important events in the history of life on Earth, with a global-scale impact on terrestrial life and the environment. Although land surfaces were available soon after life evolved, the first fossil record of terrestrial plants (Embryophyta) are spores related to extant liverworts from the mid-Ordovician (*c.* 470 million yr ago (Ma); Strother *et al.*, 1996; Wellman *et al.*, 2003; Rubinstein *et al.*, 2010). Molecular evidence suggests that land plants could have evolved much earlier, *c.* 700 Ma (Heckman *et al.*, 2001). Embryophyta is a monophyletic group that is phylogenetically more related to two groups of CGA: Zygnematophyceae and Coleochaetophyceae (Laurin-Lemay *et al.*, 2012). Many common traits have been found across land plants and charophytes, one of which is the cell wall hemicellulosic polysaccharide XyG (Ikegaya *et al.*, 2008; Sorensen *et al.*, 2010, 2011; Herburger *et al.*, 2018). XyG is the major noncellulosic nonpectic matrix polysaccharide in the cell walls of most land plants and consists of a β(1→4)-linked D-glucan backbone that is further substituted with xylosyl residues. Xylosyl residues can be further substituted with other glycosyl (mainly galactosyl and fucosyl) and nonglycosyl residues that can vary across plant groups (Pauly & Keegstra, 2016). A typical unit of XyG is shown in Fig. 1(a) with the associated enzymes required for its synthesis, modification and degradation.

XyG was first thought to be absent from CGA (Popper & Fry, 2003) and primarily involved in the mechanical properties of the cell walls of eudicots and noncommelinid monocots by interacting with cellulose (Thompson & Fry, 2000). This view of XyG was broadened and somewhat challenged during the last decade. Ikegaya *et al.* (2008) detected a XyG-like polysaccharide in the CGA *Spyrogira* (Zygnematophyceae) and proposed a role for it in cell–cell attachment. Cavalier *et al.* (2008) were able to produce *Arabidopsis* plants deficient in XyG by disrupting two XyG xylosyltransferases (*XXT1* and *XXT2*). The double mutant *xxt1 xxt2* displayed only minor phenotypic alterations, suggesting that XyG is not essential for the fundamental mechanical properties of cell walls. We wrote a paper during my PhD (Del Bem & Vincentz, 2010) suggesting a model for the emergence of XyG-related genes showing that two essential enzymes in XyG synthesis and modification – β-Glucan synthase from Cellulose Synthase-Like subfamily C (CSLC) and XyG endotransglucosylase/hydrolase (XTH) – most likely originated in charophytes, before the emergence of embryophytes. Now new work (Galloway *et al.*, 2018) has extended the understanding of XyG, showing that it is a potent soil particle aggregator released by the roots and rhizoids of all major groups of embryophytes. They detected XyG in a variety of soils suggesting that it is involved in soil formation and its properties.

Here I extend this idea a little further by proposing that XyG originated during the terrestrialization of early Streptophyta as a key adaptation, allowing unicellular CGA to interact and modify early soils and substrates.

Searching for the origin of xyloglucan genetic machinery

To better understand the origin of XyG, I used a combination of several CGA transcriptomes obtained from the OneKP project (http://sites.google.com/a/ualberta.ca/onekp/), NCBI EST database (http://www.ncbi.nlm.nih.gov/nucest) and Sequence Set Browser (http://www.ncbi.nlm.nih.gov/Traces/wgs/), and the complete genome of the klebsormidiophycean *Klebsormidium nitens* (Hori *et al.*, 2014) to update our previous work from 2010. I searched all available datasets with the same well-characterized genes involved in XyG synthesis and modification/degradation that I used before (Fig. 1a; Del Bem & Vincentz, 2010) looking for possible homologs with a stringent blast criterion (BLASTP *e*-value < e^{-20}). I was surprised to find that almost all XyG processing enzymes occurred in very early diverging Streptophyta belonging to Klebsormidiophyceae. Cells from the soil alga *Klebsormidium nitens* have homologs of most of the XyG-related genes first discovered in angiosperms, including all known enzymes required for XyG synthesis (Fig. 1b). Some of these enzymes are essential for XyG synthesis, like CSLC which produces the β(1→4)-linked D-glucan backbone (Cocuron *et al.*, 2007), and XXT that adds the xylose residues (Faik *et al.*, 2002). These enzymes probably act together in the form of a protein complex

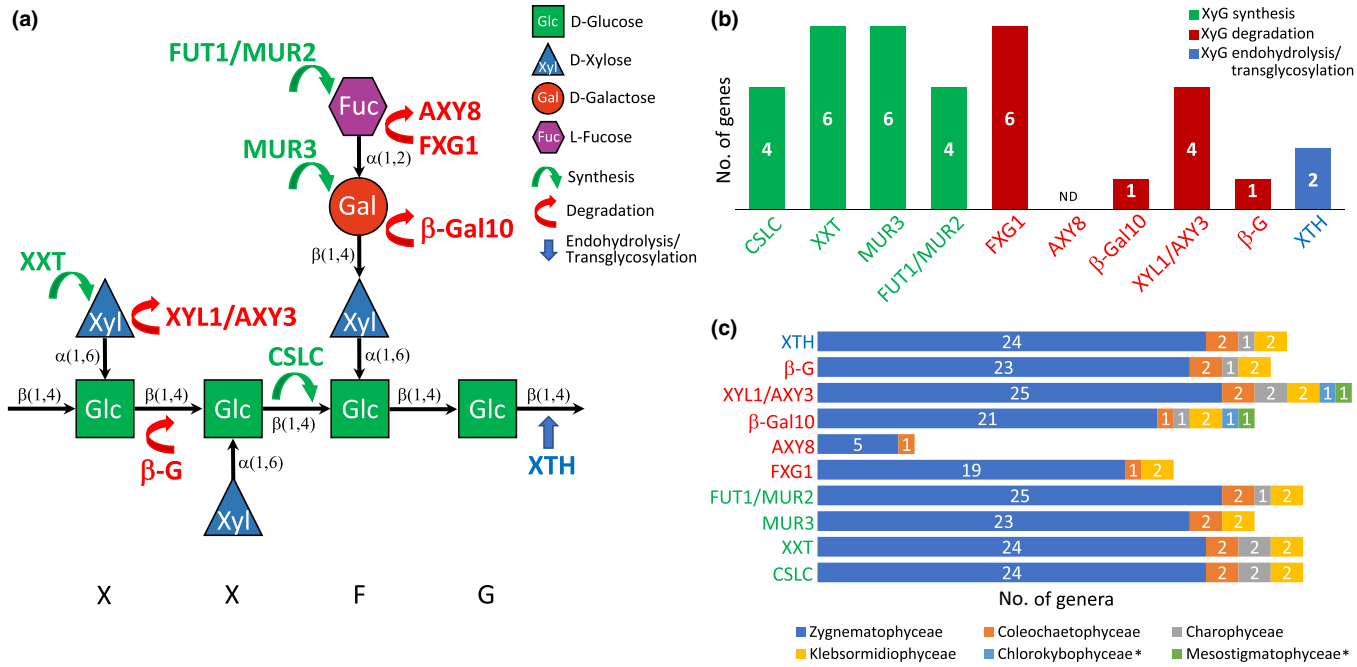

Fig. 1 Xyloglucan (XyG)-related genes and their presence across charophycean green algae (CGA) lineages. (a) A typical XyG subunit (XXFG) showing its molecular structure and the enzymes required for XyG synthesis (glycosyl transferases, green), degradation (glycosyl hydrolases, red) and endohydrolysis/transglycosylation (XTH, blue). (b) The number of XyG-related genes detected by BLASTP (*e*-value < e^{-20}) in *Klebsormidium nitens* NIES-2285 genome v.1.0 (http://www.plantmorphogenesis.bio.titech.ac.jp/~algae_genome_project/klebsormidium/). Homologs of *AXY8* were not detected (ND) even with an *e*-value cutoff of e^{-4}. (c) The number of CGA genera with positive BLASTP hits (*e*-value < e^{-20}) in transcriptomic data for each XyG-related gene. The assembled transcriptomes were obtained from the OneKP project (http://www.onekp.com/public_data.html) for the genera *Penium*, *Cosmarium*, *Staurodesmus*, *Phymatodocis*, *Bambusina*, *Pleurotaenium*, *Micrasterias*, *Gonatozygon*, *Staurastrum*, *Euastrum*, *Onychonema*, *Xanthidium*, *Desmidium*, *Roya*, *Mesotaenium*, *Spirotaenia*, *Zygnema*, *Zygnemopsis*, *Nucleotaenium*, *Cylindrocystis*, *Netrium*, *Closterium*, *Mougeotia*, *Planotaenium* and *Spyrogira* (Class Zygnematophyceae); *Coleochaete* and *Chaetosphaeridium* (Coleochaetophyceae); *Chara* (Charophyceae); *Klebsormidium* and *Entransia* (Klebsormidiophyceae); and *Mesostigma* (Mesostigmatophyceae). From the NCBI EST database (https://www.ncbi.nlm.nih.gov/nucest/?term=chlorokybus) for the genus *Chlorokybus* (Chlorokybophyceae; 23 716 unique sequences) and the NCBI Sequence Set Browser (https://www.ncbi.nlm.nih.gov/Traces/wgs/) for the genera *Coleochaete* (Coleochaetophyceae, accession GBSL01) and *Nitella* (Charophyceae, accession GBST01). *, Classes composed of a single genus.

during XyG synthesis in the Golgi apparatus (Pauly & Keegstra, 2016). Another important enzyme that incorporates newly synthesized XyG into the cell wall and promotes cell wall loosening during cell expansion is XTH (Rose *et al.*, 2002). XTH proteins have two known activities: XyG endotransglucosylase (XET) resulting in the nonhydrolytic cleavage and ligation of XyG chains, and XyG endohydrolase (XEH) that catalyzes irreversible chain shortening (Eklöf & Brumer, 2010). XET activity was recently shown to be abundant in young filaments of *Klesormidium* (Klebsormidiophyceae) and *Zygnema* (Zygnematophyceae), associated with the construction and growth of longitudinal cell walls (Herburger *et al.*, 2018). Young *Chara* (Charophyceae) cells predominantly incorporate XyG oligosaccharides into their cell walls, suggesting that XET activity is involved in cell growth (Herburger *et al.*, 2018).

All XyG-related genes were detected in dozens of other genera from different lineages of CGA (Fig. 1c) further supporting the idea that XyG genetic machinery evolved in CGA before the emergence of embryophytes. The only exceptions are *Mesostigma* (Mesostigmatophyceae) and *Chlorokybus* (Chlorokybophyceae), which most likely belong to the earliest diverging groups of the Streptophyta and probably diverged before the origination of XyG (Fig. 1c). These lineages only have homologs of the *Arabidopsis* α-xylosidase *XYL1/AXY3* (Sampedro *et al.*, 2010; Günl & Pauly, 2011) and β-galactosidase *AtBGAL10* (Sampedro *et al.*, 2012), which probably act on substrates other than XyG. Both enzymes may have been recruited for XyG degradation after the divergence of these early groups of CGA. Except for homologs of *XYL1/AXY3*, chlorophytes completely lack XyG genetic machinery (Fig. 2; Del Bem & Vincentz, 2010), suggesting that XyG is a true adaptation of the CGA that eventually gave rise to embryophytes.

Xyloglucan evolved during the colonization of land by charophytes

Most biologists tend to believe that terrestrialization by plants started with early embryophytes that emerged from a group of multicellular aquatic CGA evolving in the margins of drying pools (Kenrick & Crane, 1997; Raven & Edwards, 2001; Willis & McElwain, 2002). However, Stebbins & Hill (1980) proposed a very different view that helps to explain why CGA have genes associated with cell wall adaptations to live on land. They proposed that embryophytes emerged from unicellular CGA that colonized land environments long before this

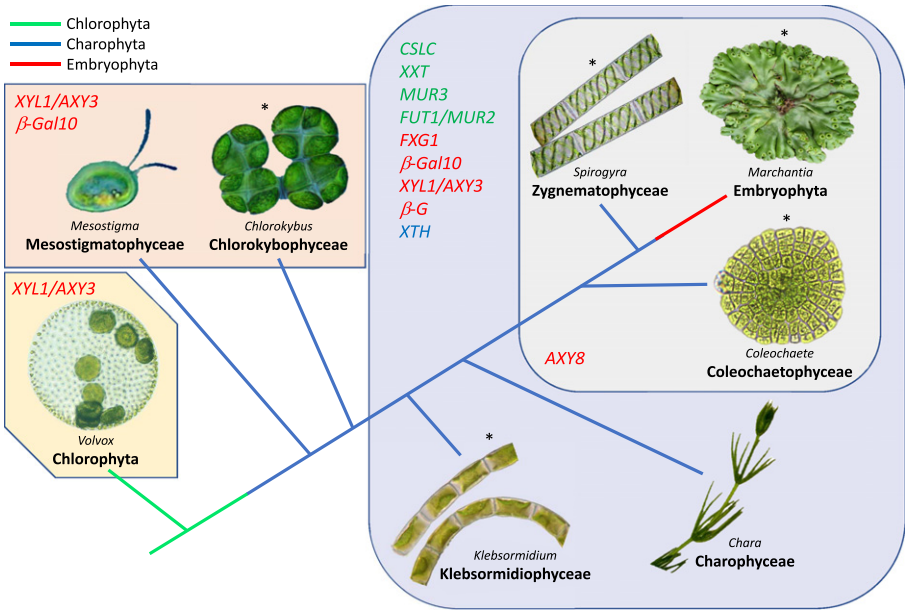

Fig. 2 Model for the emergence of xyloglucan (XyG) genetic machinery in Streptophyta. The boxes separate the major Viridiplantae groups based on the presence of particular sets of genes. All enzymes responsible for XyG synthesis emerged in the last common ancestor (LCA) of Klebsormidiophyceae, Charophyceae, Coleochaetophyceae, Zygnempatophyceae and Embryophyta. Homologs of the glycosyl hydrolase *XYL1/AXY3* and β-*Gal10* originated before the emergence of XyG synthetic machinery and were most likely later co-opted for XyG degradation. The homologs of the α-fucosidase *AXY8* emerged later in the LCA of Coleochaetophyceae, Zygnematophyceae and Embryophyta. The color scheme for enzyme function in XyG synthesis, degradation and modification are the same as those in Fig. 1. The phylogenetic relationships between Viridiplantae groups are based on Turmel *et al.* (2007), Timme *et al.* (2012) and Wickett *et al.* (2014). *, Groups with terrestrial or facultative terrestrial species.

time. They also proposed that multicellular CGA like *Chara* and *Nitella* (Charophyceae) are secondarily aquatic. This view has been further supported recently by plant cell wall studies (Harholt *et al.*, 2016). XyG likely evolved during the process of land colonization by CGA as a cell wall molecule involved in cell expansion and in the modification of soils when secreted or present in the biomass of soil crusts, making soils more suitable for the early CGA and other terrestrial photosynthetic organisms. The early accumulation of CGA biomass containing XyG and other cell wall polysaccharides possibly had a role in soil formation and its properties. Soil is one of the key components of the biosphere that drives terrestrial ecosystem composition and has a major role in carbon, nitrogen and nutrients cycles (Scharlemann *et al.*, 2014; Lehmann & Kleber, 2015; Paustian *et al.*, 2016; Leake & Read, 2017).

Many CGA, like *Klebsormidium* and *Zygnema*, are frequently found growing in soil and other moist substrates, fulfilling numerous important ecological functions as components of biological soil crusts (Elbert *et al.*, 2012). It is likely that the CGA that first colonized land environments were also part of early biological soil crusts. Thus, the key selective pressures in XyG evolution were possibly happening on land habitats associated with soil crusts. The emergence of XyG perhaps allowed early terrestrial CGA to aggregate soil particles around cells, creating a better microenvironment and improved cell wall properties related to cell growth and expansion. It might have helped in the successful colonization of land by CGA, a process that ultimately led to the evolution of embryophytes, their most notable terrestrial descendants.

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See also the Commentary on this article by Raven, **219**: 1139–1142.