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Mgr. Milan Horňák

Land plant terrestrialization – New insights from genomes
of charophyte algae
Přechod rostlin na souš – nové poznatky z genomů charofytních řas

Bachelor's Thesis

Supervisor: RNDr. Martina Pichrtová, Ph.D.
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Prohlášení

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Poděkování

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Abstract:

The terrestrialization of land plants was an important event in the history of life on Earth. Genomic and transcriptomic studies published in the last decade shed light on many aspects of this process. This work presents an overview of the traits facilitating the conquest of land by land plants. It focuses on streptophyte algae to elucidate what properties relevant to life on land the ancestors of terrestrial plants possessed and in what form, primarily using recent genomic and transcriptomic data.

Keywords: Terrestrialization, Streptophyta, Land plants, Adaptation, Transciptomes, Genomes

Abstrakt:

Přechod rostlin na souš byl důležitou událostí v dějinách života na Zemi. Genomické a transkriptomické studie publikované během posledního desetiletí ozřejmily mnoho aspektů tohoto procesu. Tato práce shrnuje vlastnosti, které umožnily kolonizaci souše vyššími rostlinami. Soustředí se přednostně na data získaná z genomů a transkriptomů streptofytních řas a ukazuje vlastnosti napomáhající přežití na souši, jež byly přítomné v předcích vyšších rostlin, a take jakou podobu tyto vlastnosti nejspíše měly.

Klíčová slova: Přechod na souš, Streptofyta, Vyšší rostliny, Adaptace, Transkriptomy, Genomy

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Abbreviations

KCM	Klebsormidiophyceae, Chlorokybophyceae, Mesostigmatophyceae
CSLD	Cellulose synthase-like D
ZCC	Zygnematophyceae, Coleochaetophyceae, Charophyceae
HG	Homogalacturonan
RG-I	Rhamnogalacturonan I
RG-II	Rhamnogalacturonan II
AGP	Arabinogalactan protein
CEF	Cyclic electron flow
ROS	Reactive oxygen species
NDH	NADH dehydrogenase-like complex
NPQ	Non-photochemical quenching
ABA	Abscisic acid
EGT	Endosymbiotic gene transfer
cpDNA	Chloroplast DNA
PAP	Phosphoadenosine-5'-phosphate
PEP	Plastid-encoded RNA polymerase
HGT	Horizontal gene transfer
SA	Salicylic acid
JA	Jasmonic acid
SLs	Strigolactones
GA	Gibberellins
BR	Brassinosteroids
RLK	Receptor-like kinase
MAG	DNA-3-methyladenine glycosylase
PRR	Pattern-recognition receptors
R proteins	Resistance proteins
NLR, NBS-LRR	Nucleotide binding-site leucine-rich repeat
2D	Two-dimensional
3D	Three-dimensional
PPB	Preprophase band
TF	Transcription factor
TR	Transcriptional regulator

Introduction

The colonization of land by plants was a crucial event in the history of life on Earth. It changed the composition of both soil and air and eventually allowed for terrestrialization of various animal species (Donoghue *et al.* 2021). Unlike terrestrial animals, land plants constitute a single monophyletic clade, which is in turn nested deep within Streptophyta (Leliaert *et al.* 2012).

Streptophyta belong to the clade of green plants (Viridiplantae) together with Chlorophyta and Prasinodermophyta (Li *et al.* 2020). Apart from land plants (Embryophyta), Streptophyta include unicellular Mesostigmatophyceae, sarcinoid packets forming Chlorokybophyceae, filamentous Klebsormidiophyceae, unicellular or filamentous Zygnematophyceae, parenchymatous Coleochaetophyceae, and finally macrophyte Charophyceae (Fürst-Jansen *et al.* 2020).

For some time, this was the presumed branching order of the group, one where evolution corresponded with rising morphological complexity (Timme *et al.* 2012). Moreover, Charophyceae were confirmed as sisters to land plants by contemporary analyses (Karol *et al.* 2001; Qiu *et al.* 2006).

However, new data, both from transcriptomes (Wickett *et al.* 2014; One Thousand Plant Transcriptomes Initiative 2019) and plastid genomes (Ruhfel *et al.* 2014; Zhong *et al.* 2014) showed in recent years that it is in fact the morphologically much simpler Zygnematophyceae who constitute the most probable sister group to land plants.

These studies also showed further grouping within the streptophyte algae, with Klebsormidiophyceae, Chlorokybophyceae a Mesostigmatophyceae forming a basal branching KCM group, while Zygnematophyceae, Coleochaetophyceae and Charophyceae make up the more derived ZCC group (de Vries *et al.* 2016). The ZCC and Embryophyta together constitute the clade Phragmoplastophyta (Buschmann, Zachgo 2016).

While the colonization of land was a pivotal moment, dating it has proved difficult, mainly due to a poor and ambiguous fossil record (Harholt *et al.* 2015; Strother, Foster 2021). While recent study by Su *et al.* proposed a much earlier date (Su *et al.* 2021), the most probable datation puts the appearance of crown Embryophyta in the range of 515 – 470 MYA (Morris *et al.* 2018), still somewhat earlier than previously expected (Kenrick, Crane 1997; Becker, Marin 2009).

This work will look at the most important pre-adaptations and adaptations of the streptophyte algae that eventually allowed their embryophyte relatives to conquer the *terra firma* through the lens of recent genomic and transcriptomic studies. The position of Zygnematophyceae as the closest relatives to land plants was not the only surprise that the omics-based new methods brought.

They also showed that many of the traits that were considered hallmarks of Embryophytes were, in fact, shared with their relatives (Hori *et al.* 2014; Turmel *et al.* 2005), and therefore presumably with their common ancestors. This work will therefore explore the areas where our understanding was enhanced by the new methods the most.

The first two chapters of the work will focus on two important loci active in response to stresses that characterize the terrestrial environment, that is the cell wall and the plastid. The third chapter will describe the nascent hormonal pathways. The next sections will talk about the interactions with microbiotic agents, and finally the last one will look at the traits of streptophyte algae that allowed for complex body plans of the Embryophytes.

As the focus of this work are the pre-adaptive traits in the light of genomic and transcriptomic studies of streptophyte algae, it will not talk directly about the traits of the last common ancestor of land plants derived from comparative studies of embryophyte genomes. It will also not discuss adaptations of extant streptophyte algae directly, as they are relevant to this topic only when they extend our understanding based on the omics methods.
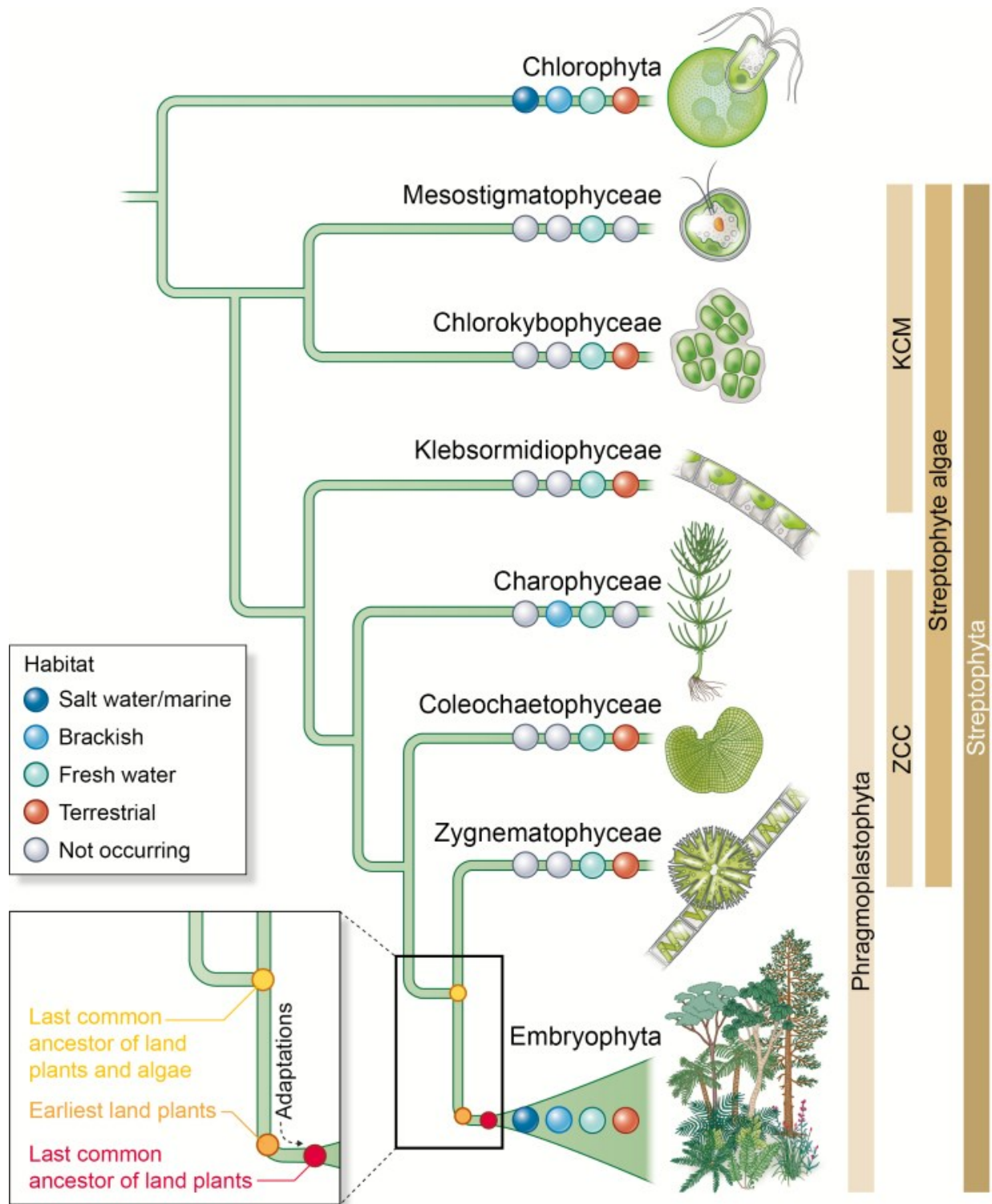
Figure 1: Cladogram showing the relations among Streptophyta together with their habitats (Fürst-Jansen *et al.* 2020).

Cell wall

The plant cell wall is one of the key adaptations to terrestrial life. It provides body support in absence of water buoyancy and enables the growth of tissues specialized for conducting water or nutrients (Harholt *et al.* 2015). Cell walls can also guard the plant from stresses like dehydration or UV radiation (de Vries, Archibald 2018b; Harholt *et al.* 2015). The plant cell wall itself consists of cellulose scaffolding connected to hemicellulose polysaccharides embedded in the pectin matrix, accompanied by glycoproteins (Jiao *et al.* 2020).

Since plant cell walls are such an important aspect of terrestrialization and many adaptations were necessary in this process, it is key to study the streptophyte cell wall to elucidate their evolution (Sørensen *et al.* 2011). What these studies have so far shown is that a significant amount of the new streptophyte genes evolved after the split from Chlorophyta are tied to the increasing complexity of the cell wall (Bowles *et al.* 2020). What moreover became apparent is that the cell walls of streptophyte algae are similar to those of land plants both structurally (Harholt *et al.* 2015) and in their contents (Holzinger, Pichrtová 2016).

This does not mean that this is true for every streptophyte species. Indeed, the Mesostigmatophyceae do not even produce cell walls, instead being covered by scales (Domozych *et al.* 2012). The other members of the KCM group, Chlorokybophyceae and Klebsormidiophyceae, have cell walls, but they differ significantly from their embryophyte equivalents in their polysaccharide content (Sørensen *et al.* 2011). It is chiefly the Zygnematophyceae and Coleochaetophyceae which produce cell walls like those of Embryophyta, with Charophyceae showing similarity but also important differences (Domozych *et al.* 2012).

It may come as a surprise that supposedly aquatic organisms such as the algal ancestors of land plants would possess as many terrestrial pre-adaptations. To respond to this conundrum, Harholt *et al.* proposed, in line with older preposition made by Stebbins and Hill (Stebbins, Hill 1980), that the bulk of streptophyte evolution actually happened already on land (Harholt *et al.* 2015), making the modern day brackish and freshwater ZCC algae (see Fig. 1) only secondarily aquatic.

However, there may also be a simpler explanation. Likely in contrast with their chlorophyte relatives, the early streptophyte were freshwater organisms, possibly the very first eukaryotic freshwater algae (Becker, Marin 2009). As the adaptations required for life on land and in ephemeral bodies of water are quite alike (Donoghue, Paps 2020; Leliaert *et al.* 2012), it is perhaps less of a surprise that the Streptophytes came to the more xeric environments already prepared.

Important cell wall components

Cellulose

Cellulose, constituting the plant cell wall scaffolding, has been found in streptophyte algae, above all in the ZCC clade (Domozych *et al.* 2012), which is probably not that surprising since it is also found in multiple other types of algae and also in bacteria (Mikkelsen *et al.* 2014). Nevertheless, it is not found in *Mesostigma*, which is lacking in cell walls (Wang *et al.* 2020). In the rest of the lower branching KCM group cellulose is found, although in relatively low amounts (Sørensen *et al.* 2011).

The cellulose in the higher Streptophyta is also similar to its embryophyte equivalent by the way it is produced. While a plethora of organisms produces cellulose using linear terminal complexes (Mikkelsen *et al.* 2014), the Streptophyta are the only group producing cellulose in hexametric rosette terminal complexes (Mikkelsen *et al.* 2014; Umen 2014).

The first time the land plant type synthesis of cellulose arose is, however, uncertain. Although the synthases of Klebsormidiophyceae or Charophyceae have been described as close to the land plant ones (Mikkelsen *et al.* 2014; Nishiyama *et al.* 2018), it seems that the only group of streptophyte algae possessing true land plant-like cellulose synthases are the Zygnematophyceae (Fitzek *et al.* 2019), although it is possible that even the common ancestor of Chlorokybophyceae and land plants employed some rosette-forming cellulose synthase (Mikkelsen *et al.* 2021).

Moreover, proteins related to the plant-like cellulose synthase, called CSLDs (cellulose synthase-like D), which in land plants play role in cell differentiation and tip growth, also originated in streptophyte algae (Mikkelsen *et al.* 2014). Interestingly, one cellulose synthase and one cellulose synthase-like gene was found in *Mesostigma*, even though it lacks cell walls as well as many genes needed for its synthesis (Liang *et al.* 2020).

Xylan

Xylan is the second most abundant land plant cell wall polysaccharide (Fitzek *et al.* 2019). It interacts with cellulose and is crucial for cell wall strength and vessel development (Herburger *et al.* 2018). Earlier tests detected the presence of its constituting block, xylose, in streptophyte algae (Mikkelsen *et al.* 2014), and xylan itself has been found in *Klebsormidium* and *Zygnema* (Jensen *et al.* 2018; Herburger *et al.* 2018), where it could be found in thickened cell wall areas, reflecting its role in some land plants (Herburger *et al.* 2018).

Significant portion of the pathway for xylan synthesis is already present in the genome of *Mesostigma viride*, even though xylan probably evolved later (Wang *et al.* 2020), most likely in the common ancestor of Klebsormidiophyceae and the ZCC clade, as *Klebsormidium* already shows a functioning xylan synthase (Jensen *et al.* 2018). Interestingly, the genome of *Chara* (Charophyceae) does not show orthologs of any of the genes of the plant xylan synthesis pathway, perhaps developing a unique way of synthetizing it (Nishiyama *et al.* 2018; Fitzek *et al.* 2019).

Mannan

Mannans are present in cell walls of many different organisms from the red algae to angiosperms with varying quantity, function and complexity (Franková, Fry 2021). They are present over the whole diversity of Streptophyta, including the wall-less Mesostigmatophyceae (Sørensen *et al.* 2011; Wang *et al.* 2020). Nevertheless, their quantity in Chlorokybophyceae and Coleochaetophyceae is very low, similarly to land plants (Franková, Fry 2021).

Mannan therefore serves rather to show alternatives than direct precedents to embryophyte solutions. In *Mesostigma*, mannan may work as an alternative to cellulose in the zygote (Wang *et al.* 2020), and more importantly it functions as a main target for cell wall remodeling in the streptophyte algae, followed by xylan, providing an alternative solution to embryophyte cell wall remodeling targeting xyloglucan (Franková, Fry 2021).

Xyloglucan

Xyloglucan is one of the most common elements of non-commelinid angiosperm cell walls (Herburger *et al.* 2018). It has been proposed as specific to Embryophyta (Popper, Fry 2003), where it has a role in cell expansion and cell wall architecture (Mikkelsen *et al.* 2021). The absence of xyloglucan has been confirmed for lower branching Streptophyta (Wang *et al.* 2020), but they have since been found in various higher branching lineages (Herburger *et al.* 2018; Mikkelsen *et al.* 2021).

That being said, only streptophyte algae with significant amounts of xyloglucan were from the clade of Zygnematophyceae, with other clades containing only low amounts (Franková, Fry 2021). This might be due to only a local or situational role of xyloglucan (Franková, Fry 2021), or composition differing from those of land plants (Sørensen *et al.* 2011; Mikkelsen *et al.* 2021). Interestingly, streptophyte algae possess transglycanase activities targeting xyloglucan typical of land plants, which suggest these mechanisms are highly conserved given the limited presence of plant-like xyloglucan in most streptophyte algae (Franková, Fry 2021).

Moreover, recent studies were also able to detect fucosylated xyloglucans in *Mesotaenium caldariorum* (Zygnematophyceae) (Mikkelsen *et al.* 2021; indirect evidence in Pfeifer *et al.* 2022). This feature is most prominent in Spermatophyta and has a relevance

for vasculature (Mikkelsen *et al.* 2021), so their presence in algae may be an interesting pre-adaptation and also speaks to the xyloglucan diversity in earlier Streptophyta.

Another possible significance of xyloglucans for streptophyte algae and the terrestrialization of land plants was put forward by Del-Bem (Del-Bem 2018). Building on the ability of land plant xyloglucan to function as a powerful soil aggregator, he proposes that soil dwelling ancestors of land plants used xyloglucan to interact with surrounding soil particles, thus improving their microenvironment and cell wall properties. Given that some Zygnematophyceae are terrestrial (see Fig. 1), it could be interesting to test if their xyloglucan really can function in this way.
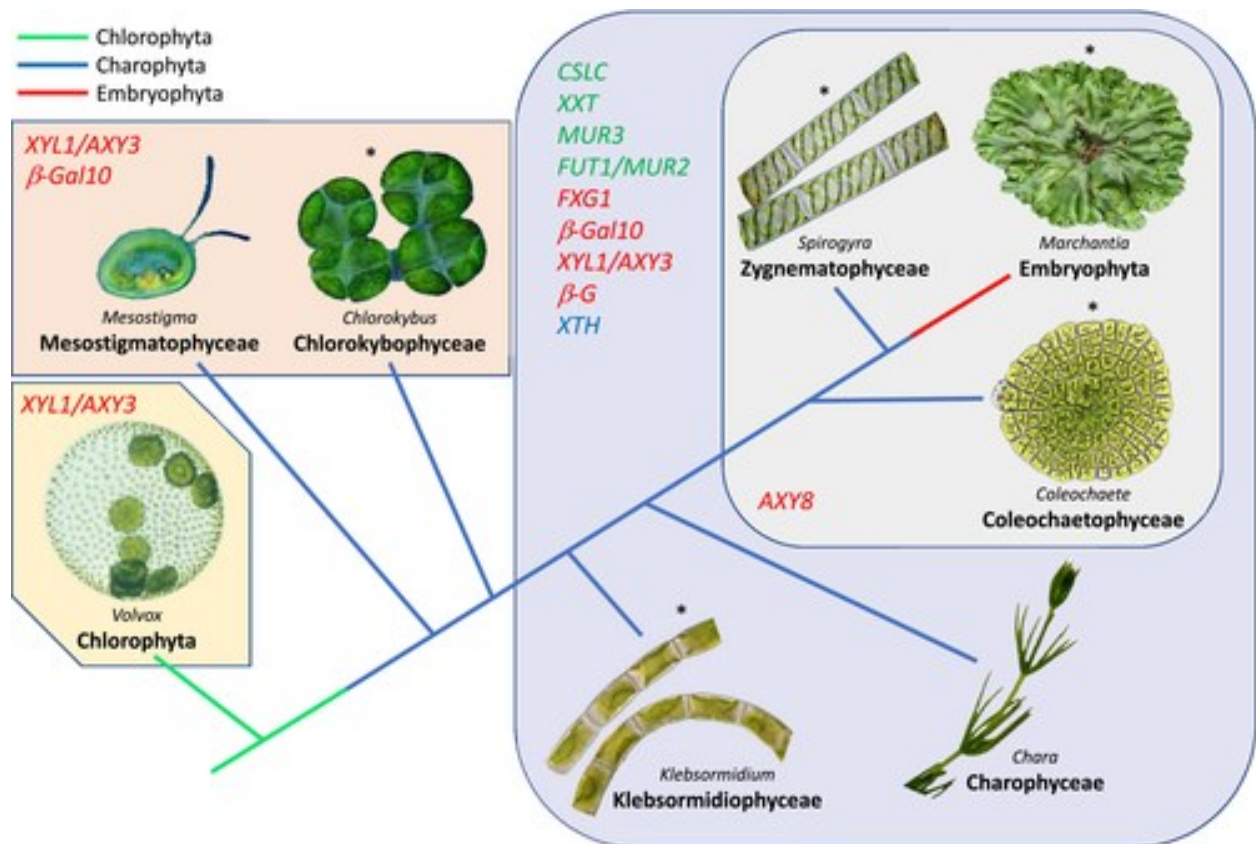

Figure 2: Evolution of xyloglucan genetic toolkit. Groups with terrestrial species marked by an asterisk (Del-Bem 2018).

Pectins

Pectins are a group of polysaccharides which have an important position in land plant cell walls. They are active in many tasks, such as structural, hydrating, lubricating and porosity defining (O'Rourke *et al.* 2015). They also play a role in resistance to abiotic stresses

(Domozych *et al.* 2021), making them particularly interesting for the question of plant terrestrialization. Pectins consist of three domains, homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II).

Pectins probably originated in Streptophyta (Jensen *et al.* 2018). HG especially seems like an ancient streptophyte characteristic (Domozych *et al.* 2012; O'Rourke *et al.* 2015). Their presence in the lower branching KCM clade is however somewhat uncertain. While one study could chemically detect putative pectins in *Chlorokybus* but not in *Klebsormidium* (O'Rourke *et al.* 2015), other one found them in neither (Sørensen *et al.* 2011) while the analysis of genomes found pectin lyases in *Klebsormidium*, but not in *Chlorokybus* (Wang *et al.* 2020*)*.

In the higher branching Streptophyta, things are markedly clearer, with pectins found both chemically (O'Rourke *et al.* 2015; Sørensen *et al.* 2011) and also in the so-far available genomes (Nishiyama *et al.* 2018; Jiao *et al.* 2020). RG-I is also to be found in the ZCC clade (O'Rourke *et al.* 2015), albeit only low amounts were detected (Sørensen *et al.* 2011). Therefore, from the three land plant pectin domains, only RG-II seems specific to Embryophyta, with some traceable parts of its biosynthesis in streptophyte algae (Sørensen *et al.* 2011; Harholt *et al.* 2015).

Zygnematophyceae, the sister clade of land plants, have considerably enlarged repertoire of pectin metabolism genes (Jiao *et al.* 2020). Significantly, pectins play an important role in stress resistance in Zygnematophyceae, be it by changes in the cell wall architecture (Jiao *et al.* 2020; Herburger *et al.* 2019), or by producing a protective mucilage layer (Holzinger, Pichrtová 2016). It is therefore quite possible that pectins played an important role in protecting the first Embryophyta.

Glycoproteins

Extensins are a group of cell-wall associated glycoproteins, found across the diversity of Viridiplantae, including most extant streptophyte clades (Mikkelsen *et al.* 2014; Domozych *et al.* 2012). However, they have not been found in Charophyceae, underscoring the difference of their cell walls to those of the Embryophyta compared to the two other ZCC clades (Domozych *et al.* 2012).

Another important group of glycoproteins are the arabinogalactan proteins (AGP), which in land plants play a role in response to drought and other stress situations and are therefore potential actors in acclimatization to terrestrial conditions (Pfeifer *et al.* 2022).

Using monoclonal antibodies to detect AG-epitopes, the presence of AGP in multiple streptophyte clades has been reported (Sørensen *et al.* 2011; Palacio-López *et al.* 2019), although these results were recently put into question (Pfeifer *et al.* 2022). Nevertheless, the same study was able to find AGPs at least in *Spirogyra* (Zygnematophyceae), proposing the last common ancestor of Embryophyta and Zygnematophyceae as an origin of these glycoproteins (Pfeifer *et al.* 2022).

Other cell wall polymers

Callose is a polysaccharide found in land plant cell walls, where it is involved in wide range of processes, including response to wounding, regulation of transport through plasmodesmata and cell division (Davis *et al.* 2020). Callose is found already in the KCM cell walls, where it forms a regular structural component (Sørensen *et al.* 2011). It also acts there during desiccation stress, contributing to *Klebsormidium*'s elevated desiccation resistance (Herburger, Holzinger 2015). In Zygnematophyceae, this function is less pronounced, although still present (Herburger, Holzinger 2015).

Lignin was a crucial element in the success of land plants due to its function in desiccation tolerance, structural integrity and decay resistance (Umen 2014). It is produced by the phenylpropanoid pathway, which is one of the most important in land plants (de Vries *et al.* 2021). In the case of lignin biosynthesis, this pathway rarely presents clear embryophyte orthologs in streptophyte algae and important parts of the canonical phenylpropanoid pathway seem missing there (Rieseberg *et al.* 2022).

This stands in contrast to the presence of phenylpropanoid-derived flavonoids in *Penium* (Zygnematophyceae) (Jiao *et al.* 2020) Moreover, low amounts of lignin-like polymers were found in Coleochaete (Sørensen *et al.* 2011), so it seems there are alternative pathways in play, but not enough data to claim the presence of lignin in the streptophyte algae.

Sporopollenin is a resilient material covering the spores of various Eukaryotes. In Embryophytes, it constitutes the coating of pollen (de Vries, Archibald 2018b). Sporopollenin, or sporopollenin-like compounds, has been found in streptophyte algae, where its helps with protection against abiotic stresses (Rieseberg *et al.* 2022; Holzinger, Pichrtová 2016).

Expansins are a group of land plant proteins active in cell wall loosening (Cosgrove 2015). Expansin-related genes were found in several streptophyte algae, including three members of Zygnematophyceae and one of Charophyceae, so it is likely that this method of cell wall remodeling originated before the advent of the first land plants (Cosgrove 2015).

Plastid

More than 1.9 billion years ago, a eukaryotic organism incorporated a cyanobacterium into its cell, giving birth to a lineage that eventually led to land plants (Delwiche, Cooper 2015; Han *et al.* 2019). This endosymbiotic event enabled this lineage to gain energy by the means of oxidative photosynthesis, using light energy to eventually create complex organic molecules (Delwiche, Cooper 2015).

Both the host and the bacterium were forever changed as a result of this endosymbiosis. The cyanobacterium turned into the plastid, now an organelle separated by two membranes with a greatly reduced number of genes compared to its free-living bacterial relatives. Correspondingly, many of these genes were transferred to the eukaryotic nucleus, giving it control over a large part of plastid-related processes (Delwiche, Cooper 2015).

A localization of this crucial event is not yet clear, but one theory situates the acquisition of the chloroplast to the freshwater environment, making, for example, the great variety of saltwater Chlorophyta only secondarily marine (Delwiche, Cooper 2015). Of course, this would be at odds with the theory stating that what helped the Streptophyta conquer the land was their role as the first group of freshwater algae (Becker, Marin 2009). Clearer situation of the primary endosymbiosis would therefore provide better insight on the environment of early Streptophyta.

Role in stress resistance

The plastid was also a place of important adaptation to life in the terrestrial environment. The reason is that the organelle is prone to photooxidative damage, which can be limited in water by adjusting the depth and therefore allowing for filtration of excessive light (de Vries *et al.* 2016). This is naturally not possible on land, giving rise to the need of adaptations (de Vries *et al.* 2016; Orton *et al.* 2020).

Moreover, the chemical composition of the environment is different, with elevated amounts, compared to water, of oxygen, a photosynthesis inhibitor, and simultaneously lower amounts of carbon dioxide, the final part of the reaction (Gerotto, Morosinotto 2013). Another important stressor is drought, as witnessed by the significant effect it has on the photosynthesis and photorespiration of extant terrestrial streptophyte algae (Holzinger *et al.* 2014; Rippin *et al.* 2017).

Resistance to irradiance

Irradiance may be countered in various ways, one of which is RNA editing. In an environment with elevated chances of deleterious mutation, a system of DNA repair, reverting back any non-synonymous changes, would provide a great benefit. Therefore, it is

possible that this system evolved in response to challenges brought by higher exposition to damaging UV radiation (de Vries *et al.* 2016).

Another way of coping with heightened light damage is the cyclic electron flow (CEF) activity, responsible for dealing with the reactive oxygen species (ROS) induced by stress conditions (de Vries *et al.* 2016). This activity is mediated by the NADH dehydrogenase-like complex (NDH) pathway or the PGR5 pathway in land plants (Hori *et al.* 2014).

While NDH seems to be lost in the Chlorophyta (Hori *et al.* 2014), its retention in Streptophyta may have proven an important advantage in terrestrialization (de Vries *et al.* 2016). Accordingly, the NDH pathway mediated CEF activity was detected in *Klebsormidium*, even though some genes of the canonical embryophyte pathway were missing (Hori *et al.* 2014).

One presumed effect of this CEF activity is the activation of non-photochemical quenching (NPQ) (Hori *et al.* 2014). A reaction to strong light, it serves to dissipate the excess energy in the form of heat (Gerotto, Morosinotto 2013). To work properly in vascular plants, it requires a protein called PSBS, which reorganizes the photosynthetic apparatus. Eukaryotic algae, in their turn, use LHCSR instead, a protein whose function is to bind pigment (Gerotto, Morosinotto 2013).

In the KCM Streptophytes, LHCSR is still the preferred option, however higher streptophyte algae, as well as mosses, seem to use both or even strictly prefer PSBS (Gerotto, Morosinotto 2013). One theory for this shift, which was completed by the loss of LHCSR in vascular plants, holds that PSBS performs better in environment with abrupt changes in illumination. LHCSR probably has to be accumulated only when it is needed to prevent unwanted dissipation of energy, and therefore it may take longer to mount a proper response (Gerotto, Morosinotto 2013).

Another option to mitigate high light stress is to use phenolic compounds to shield against the UV irradiance (Fürst-Jansen *et al.* 2020). While flavonoids and other phenolic compounds were detected in Zygnematophyceae (Jiao *et al.* 2020; Rieseberg 2022), the exact pathways for their production are still not clear (Rieseberg *et al.* 2022).

Flavonoids are produced by the phenylpropanoid pathway (de Vries *et al.* 2017), already mentioned in connection with lignin. However, it is not found in its entirety in the streptophyte algae (de Vries *et al.* 2021). Nevertheless, our models are biased toward the angiosperms, and the individual enzymes show high variety across the Streptophyta. Therefore, further studies could bring us much richer picture of this important pathway (de Vries *et al.* 2021).

On the other hand, the new flavonoids of Zygnematophyceae would probably not be of significant improvement in terms of UV-B protection (Nascimento, Tattini 2022), and therefore it is possible that they were selected for rather for their wide range of functions and other advantages, such as interaction with phytohormones, fungi or pathogens (Nascimento, Tattini 2022).

Plastid-based stress signaling

Drought stress in land plants leads to similar gene expression as high light stress (de Vries *et al.* 2016). One of the major elements in both of these responses is abscisic acid (ABA), which has been proposed to fulfill this role also in streptophyte algae (de Vries *et al.* 2016). The presence and role of ABA in streptophyte algae will be also discussed lower in the section concerning phytohormones.

Another pathway for stress response in land plants relies on the mediation by the 3′-phosphoadenosine-5′-phosphate (PAP), which is accumulated during drought and excess light conditions and stimulates drought tolerance and stomatal closure (Zhao *et al.* 2019).

The turnover of PAP is in turn mediated by the SAL1/FRY1 nucleotide phosphatase (Zhao *et al.* 2019). The discovery of transit peptides for SAL1 in higher branching streptophyte algae and a putative SAL1 transit peptide in *Klebsormidium* suggests that this desiccation response pathway already existed before the emergence of Embryophyta, although the exact function of SAL-PAP pathway there is unclear, as streptophyte algae lack guard cells (Zhao *et al.* 2019).

Overall, the higher branching streptophyte algae possess a stress response that is significantly plant-like and where plastids play a key role (de Vries *et al* 2018a; Jiao *et al.* 2020). It is likely that these adaptations were of considerable help to the Embryophyta when they first established themselves on land.

Endosymbiotic gene transfer

Alternative way how to protect the plastid genes from deleterious mutations caused by photooxidative damage is to keep those genes out of the plastid. Thusly they are at once better protected and also able to participate in sexual recombination, lowering the threat of Muller ratchet effect (de Vries *et al.* 2016).

Moreover, as mentioned above, the continuing endosymbiotic gene transfer (EGT) allows the nucleus more control over the plastid. The progressive pseudogenization of tufA in Streptophyta (Lemieux *et al.* 2016), a gene which plays a role in bacterial cell division, may be an example of the continual shift of power over plastid cell division towards the nucleus (de Vries *et al.* 2016).

Such losses of plastid autonomy may in turn allow multiplastidy, as witnessed in higher branching streptophyte algae, making the alga less dependent on division synchronization and providing more space for evolutionary experimentation (de Vries *et al.* 2016). Embryophyta followed this trend even further, gaining genes for plastid division control and eventually creating the multiple specialized plastid types known from vascular plants (de Vries *et al.* 2016).

The process of EGT can be seen on the evolution of the plastid genome of streptophyte algae. *Mesostigma* and *Chlorokybus* have chloroplast DNA (cpDNA) with the most genes, most resembling both the cpDNA of early branching Chlorophyta and the cyanobacterial genome. On the other hand, Coleochaetophyceae and Zygnematophyceae have cpDNA with features resembling that of land plants (Lemieux *et al.* 2016).

However, the cpDNA of streptophyte algae is very dynamic, unlike the one present in the plastid of land plants (Lemieux *et al.* 2016). This is especially pronounced in the case of Zygnematophyceae (Turmel *et al.* 2005; Civáň *et al.* 2014) but is true also for Klebsormidiophyceae and Coleochaetophyceae (Lemieux *et al.* 2016). The most likely explanation, that this was caused by nuclear encoded plastid targeted genes (Lemieux *et al.* 2016), only highlights the amount of control the nucleus has over the plastid.

Another way to control the plastid is to control its transcription. Plastid-encoded RNA polymerase (PEP) serves as the plastid RNA polymerase in streptophyte algae (de Vries *et al.* 2018a). In land plants, PEP is controlled by PEP-associated proteins, which are already present in streptophyte algae (Nishiyama *et al.* 2018), where they can for example modify plastid response to stresses (de Vries *et al.* 2018a).

Photorespiration and light perception

Dry land is an environment with markedly higher concentrations of oxygen, compared to water (Gerotto, Morosinotto 2013). In land plants, photorespiration serves as a response to oxygen-rich atmosphere (Nishiyama *et al.* 2018). The genome of *Chara braunii* already contains genes necessary for embryophyte-like photorespiration, including plant-like glycolate oxidase, which has also been find in *Klebsormidium*, but not in *Chlamydomonas* (Chlorophyta) (Nishiyama *et al.* 2018). This suggests that the plant-like photorespiration may have been a trait common to all Streptophyta, eventually aiding some of their members in coping with terrestrial conditions (Nishiyama *et al.* 2018).

Finally, to properly react to light in the environment, one must be able to sense it. The blue light signaling pathway, together with UV-A signaling, was probably present in the ancestor of both the red and green lineages, living in the deeper water where red light does not play a role (Han *et al.* 2019). The UV-B signaling pathway is common to the green lineage, likely originating as their ancestors moved to shallower water with a greater risk of UV damage (Han *et al.* 2019).

The red and far-red signaling pathway emergence probably coincided with the move to the surface of water, where this type of radiance is present. This likely happened in the common ancestor of Streptophyta, as they all possess the canonical land plant phytochrome (Han *et al.* 2019). Apart from the phytochrome itself, streptophyte algae also seem to share the related signaling pathway with land plants (Han *et al.* 2019).

Moreover, the seedling emergence signaling pathway and the canonical photomorphogenesis network may have originated in streptophyte algae (Han *et al.* 2019),

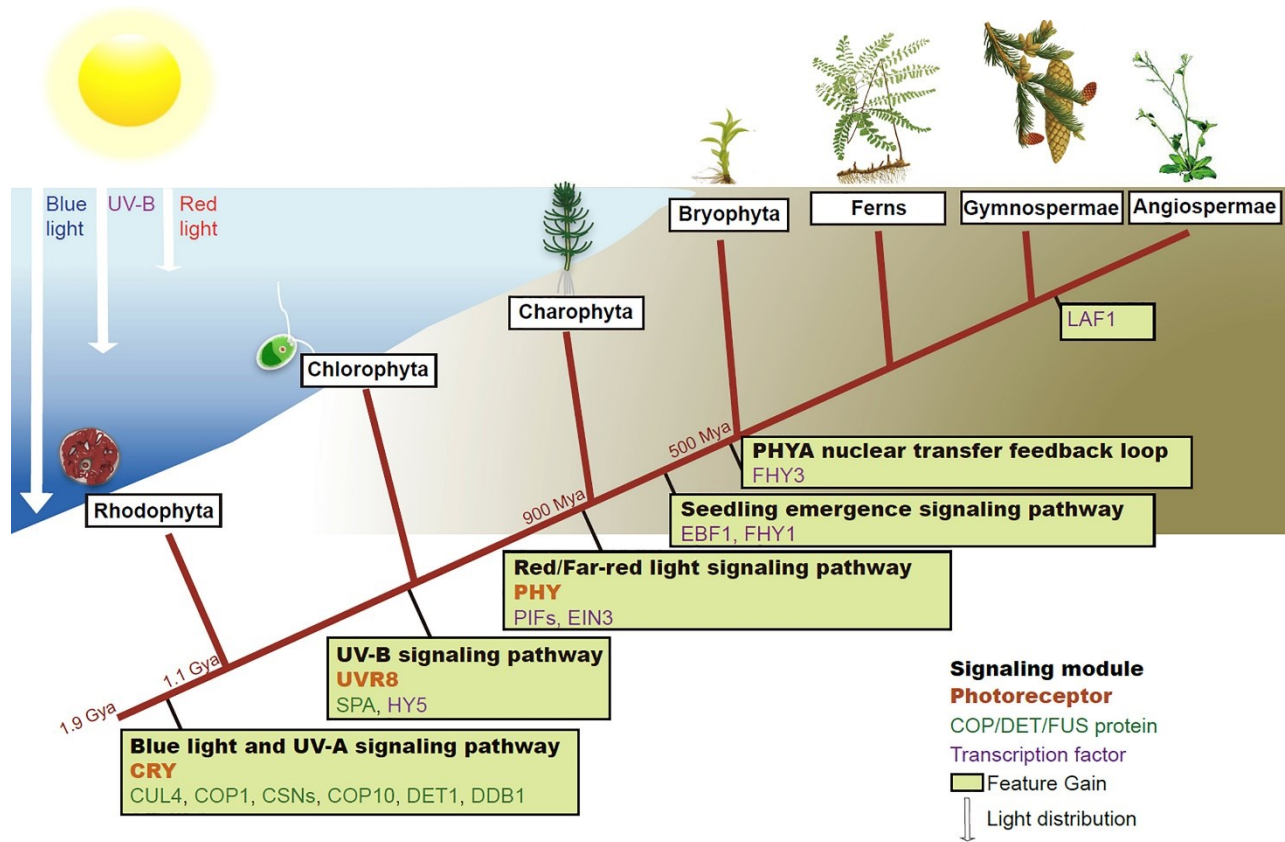providing the Embryophytes with a robust system of light perception from the very beginning.

Figure 3: Proposed origins of various constituents of land plant light perception (Han *et al.* 2019).

Phytohormones

As seen in the previous chapters, the move to terrestrial environments was characterized by a multitude of new stresses. Therefore, it comes as no surprise that land plants evolved many signaling pathways for dealing with them (de Vries, Archibald 2018b). These are in turn often mediated by various phytohormones (de Vries, Archibald 2018b).

Although plant hormones have a decisive role in growth, development and defense, many aspects of their evolution remain unclear (Wang *et al.* 2015). In recent years, several phytohormone systems previously thought land-plant specific were detected in streptophyte algae (Delwiche, Cooper 2015).

Moreover, a great part of the evolution of land plant phytohormones seem to have taken place before the advent of Embryophyta. While there are almost no orthologs for actors of the most important embryophyte phytohormone pathways in *Mesostigma*, with only the cytokinin pathway present (Liang *et al.* 2020; Wang *et al.* 2020), *Klebsormidium* already possesses certain types of primitive land plant hormone pathways (Hori *et al.* 2014).

Finally, the backbone of biosynthesis of all phytohormones had already evolved before the first land plants or only shortly after (Bowles *et al*. 2020). This chapter will therefore go over the various phytohormones and their pathways and examine in what state they were bequeathed to the land plants by their streptophyte ancestors.

Auxin

Auxin is the most famous phytohormone, critically responsible for various tasks connected to the plant body development, but also for an adjustment to stress (Fürst-Jansen *et al.* 2020). Auxin has been detected in streptophyte algae, which is not surprising since it has also been detected in other organisms ranging from distantly related algae to bacteria (Rieseberg *et al.* 2022). In some of those other algae, it has even been confirmed in its role in growth regulation (Ohtaka *et al.* 2017).

In land plants, the biosynthesis of auxin is facilitated by the TAA and YUCCA genes. While both of these are found in *Klebsormidium* (Hori *et al.* 2014), and YUCCA can be found even in *Mesostigma* (Wang *et al*. 2020), the *Chara* and *Penium* genomes lack them (Nishiyama *et al.* 2018; Jiao *et al.* 2020). As some form of auxin signaling is present in both, this suggest that the biosynthesis of auxin follows a pathway entirely different from that of land plants there (Nishiyama *et al.* 2018).

The function of auxin requires a creation of a concentration gradient within tissues (Viaene *et al.* 2013). This is achieved by polar auxin transport between cells, which mostly depends on PIN transporters (Viaene *et al.* 2013). These, and also other auxin transport genes are again already present in *Klebsormidium*, albeit with only one PIN sequence (Hori *et al.* 2014), and in *Mesostigma* with two PIN sequences (Wang *et al.* 2020). While the

genome of *Chara* lacks some of the transport proteins, it already has 6 PINs (Nishiyama *et al.* 2018).

The difference is moreover not only quantitative, as the single PIN homolog from *Klebsormidium* was not localized at the contact points between the cells of the filament, nor was it able to establish polarity when introduced in *Arabidopsis* (Rieseberg *et al.* 2022). In comparison, a PIN from *Chara* was able to establish polarity (Rieseberg *et al.* 2022), a trait that may have evolved convergently with Embryophyta (Fürst-Jansen *et al.* 2020).

Of the genes involved in auxin signaling in land plants, TIR1 has not been found in any streptophyte algae (Jiao *et al.* 2020). Other gene involved, AUX/IAA, has been found already in *Chara* (Nishiyama *et al* 2018), but it differs significantly from the canonical land plant protein. The canonical form was found in *Penium*, but not in the other sequenced Zygnematophyceae (Jiao *et al.* 2020). Accordingly, the auxin signaling in *Klebsormidium* was found to work independently of both TIR1 and AUX/IAA, in contrast to the land plant pathway (Ohtaka *et al.* 2017).

The last group of genes associated with auxin signaling in land plants, ARFs, are divided in three groups, A, B and C. Of those, C-ARF is already present in *Chlorokybus*, implying origin in the common ancestor of Streptophyta (Wang *et al.* 2020), while a common A/B-ARF homolog of the remaining groups was found only in the Zygnematophyceae (Jiao *et al.* 2020).

Overall, these ARFs act in algae in an auxin-independent manner, becoming dependent on auxin probably only after connecting with TIR1 and AUX/IAA in Embryophyta (Fürst-Jansen *et al.* 2020). Thus, this added regulatory mechanism turned an old gene regulatory cascade into a phytohormone-dependent cascade, probably in a time between the split from Zygnematophyceae and the last common ancestor of extant Embryophyta (Fürst-Jansen *et al.* 2020).

Abscisic acid

ABA is a prominent land plant stress hormone, active in dealing with stressors such as drought, salt or temperature (Fürst-Jansen *et al.* 2020). Moreover, it plays a role in UV resistance, already mentioned in the previous chapter, and may have aided the first land plants through its relationship with flavanols (Brunetti *et al.* 2019). The presence of ABA has been detected in various members of green algae (Sun *et al.* 2020), which is not that surprising given that they result from spontaneous degradation of carotenoids, which are evolutionarily conserved there (Rieseberg *et al.* 2022).

Despite this omnipresence of ABA in algae, only few of them were observed producing it in reaction to stress (Sun *et al.* 2020). Among those few was *Klebsormidium*, which moreover showed presence of majority of the canonical signaling pathway (Hori *et al.* 2014; Holzinger *et al.* 2014). Some parts of the ABA biosynthetic pathway are missing across all streptophyte algae (Jiao *et al.* 2020), but since there exist non-canonical routes

even in *Arabidopsis*, it is quite possible that the algae are also using some alternative (Rieseberg *et al.* 2022).

Of the major parts of the signaling pathway, consisting of the PYR/PYL/RCAR, PP2C, SnRK and AREB genes (Cuming 2019), only the first was not found in *Klebsormidium* (Holzinger *et al.* 2014). Furthermore, SnRK and AREB are already present in the genomes of the earliest branching Streptophyta (Wang *et al.* 2020). The homologous genes from *Klebsormidium* were also able to complement *A. thaliana* and *P. patens* mutants in experiments (Fürst-Jansen *et al.* 2020), showing probable conservation of ancient function.

The whole signaling pathway is present in Zygnematophyceae, as a PYL receptor was identified in *Zygnema circumcarinatum* (de Vries *et al* 2018a), and a PYR/PYL/RCAR homolog was also found in *Mesotaenium endlicherianum* (Cheng *et al.* 2019) and *Spirogyra pratensis* (Cuming 2019). The origin of this receptor is unclear, but a horizontal gene transfer (HGT) from soil-dwelling bacteria is a possible culprit (Cheng *et al.* 2019).

Before the discovery of PYL receptor in streptophyte algae, the GTG protein was proposed as a potential ABA receptor, but it does not seem to work in this way (Holzinger, Becker 2015). Indeed, although many various algae species contain ABA, few have any function associated therewith (Sun *et al.* 2020). Most do not even respond to treatment with high dosage of ABA, and those who do require dosage so high its effects could be rather ascribed to toxicity (Sun *et al.* 2020).

Perhaps surprisingly, the situation does not differ even for *Z. circumcarinatum*. While its PYL receptor indeed works as a part of the canonical ABA pathway, it does so in ABA-independent manner (Sun *et al.* 2019). One possible interpretation is that the first land plants modified an earlier pathway for desiccation tolerance into a more fine-tuned hormone-dependent drought response, more fitting their new complex body plans (Sun *et al.* 2019).

Ethylene

In land plants, ethylene plays a role in various processes, among those in cell elongation and division, cell death, fruit ripening and seed germination and also response to both biotic and abiotic stresses (Van de Poel *et al.* 2016). While the ethylene receptor is probably ancient result of EGT, the Chlorophyta seem to have lost it and neither them nor the cyanobacteria show ethylene binding activity (Ju *et al.* 2015). On the other hand, its function as a hormone is conserved across the Embryophyta (Van de Poel *et al.* 2016).

Parts of the ethylene signaling pathway are already present in the KCM clade (Wang *et al.* 2020; Hori *et al.* 2014). In the ZCC clade the pathway is already complete, suggesting that it emerged in the common ancestor of Phragmoplastophyta (Ju *et al.* 2015; Nishiyama *et al.* 2018).

As we have seen in the previous case, the presence of such pathway still does not necessarily imply the presence of a hormonal activity. In this case however, a hormonal function has been confirmed in *Spirogyra pratensis* (Ju *et al.* 2015). Not only does ethylene

induce cell elongation in this alga, but members of its pathway partially complement *Arabidopsis* mutants, suggesting functional conservation (Ju *et al.* 2015). In *Spirogyra*, ethylene regulates pathways connected to cell wall, photosynthesis and abiotic stresses, and may have therefore been an important factor in terrestrialization (Van de Poel *et al.* 2016).

Other phytohormones

Cytokinins play various roles in land plant growth and development (Wang *et al.* 2015). Their whole signaling pathway is already present in all branches of the KCM clade (Hori *et al.* 2014; Wang *et al.* 2020), with origin possibly going as far back as their common ancestor with Chlorophyta (Jiao *et al.* 2020). Cytokinin mediated regulation was detected in *Klebsormidium* (Holzinger, Becker 2015), although not all types of embryophyte cytokinins were found in algae (Holzinger, Becker 2015).

Salicylic acid (SA) is a hormone involved in land plant defense (Wang *et al.* 2015). Some homologs of SA-related genes can be found in Chlorophyta (Rieseberg *et al.* 2022) and its presence was identified in *Klebsormidium* (Hori *et al.* 2014). However, the signaling pathway does not seem complete in streptophyte algae (Jiao *et al.* 2020) and so its role in defense probably appeared after the establishment of Embryophyta (de Vries *et al.* 2018c).

Strigolactones (SLs) are carotenoid-derived plant hormones regulating root development as well as playing a role in establishing arbuscular mycorrhizal (AM) symbiosis (Delaux *et al.* 2012). Genes involved in their signaling pathway are completely missing in the earliest branching Streptophyta (Wang *et al.* 2020), and only parts of the canonical pathway are present in higher streptophyte algae (Nishiyama *et al.* 2018; Jiao *et al.* 2020).

Nevertheless, hormonal function of SLs was found in *Charophyceae*, where it controlled rhizoid elongation (Delaux *et al.* 2012). Given the sparsity of true orthologs of the land plant SL pathway, it is probable that SL biosynthesis and signaling differ in *Charophyceae* and land plants (Nishiyama *et al.* 2018), and the canonical SL pathway is an embryophyte invention (Rieseberg *et al.* 2022).

Jasmonic acid (JA) has, like SA, role in plant defense, but also in reproduction (Wang *et al.* 2015). In streptophyte algae, JA has a patchy presence (de Vries *et al.* 2018c). However, the genes of JA signaling pathway seem severely lacking in the published genomes (Hori *et al.* 2014; Wang *et al.* 2020; Jiao *et al.* 2020) and the role of JA as a phytohormone probably appeared later in the evolution of land plants (Rieseberg *et al.* 2022). The same is true for gibberellins (GA) and brassinosteroids (BR), other groups of plant hormones which emerge only deeper in embryophyte evolution (Wang *et al.* 2015).

Overall, it seems that the first land plants inherited some of the canonical phytohormone pathways already functioning as such, as in the case of ethylene or cytokinins. Moreover, as with auxin and ABA, they co-opted existing signaling pathways

into a hormone-controlled ones, gaining a fine-tuned response more appropriate for their increasingly complex bodies and processes.
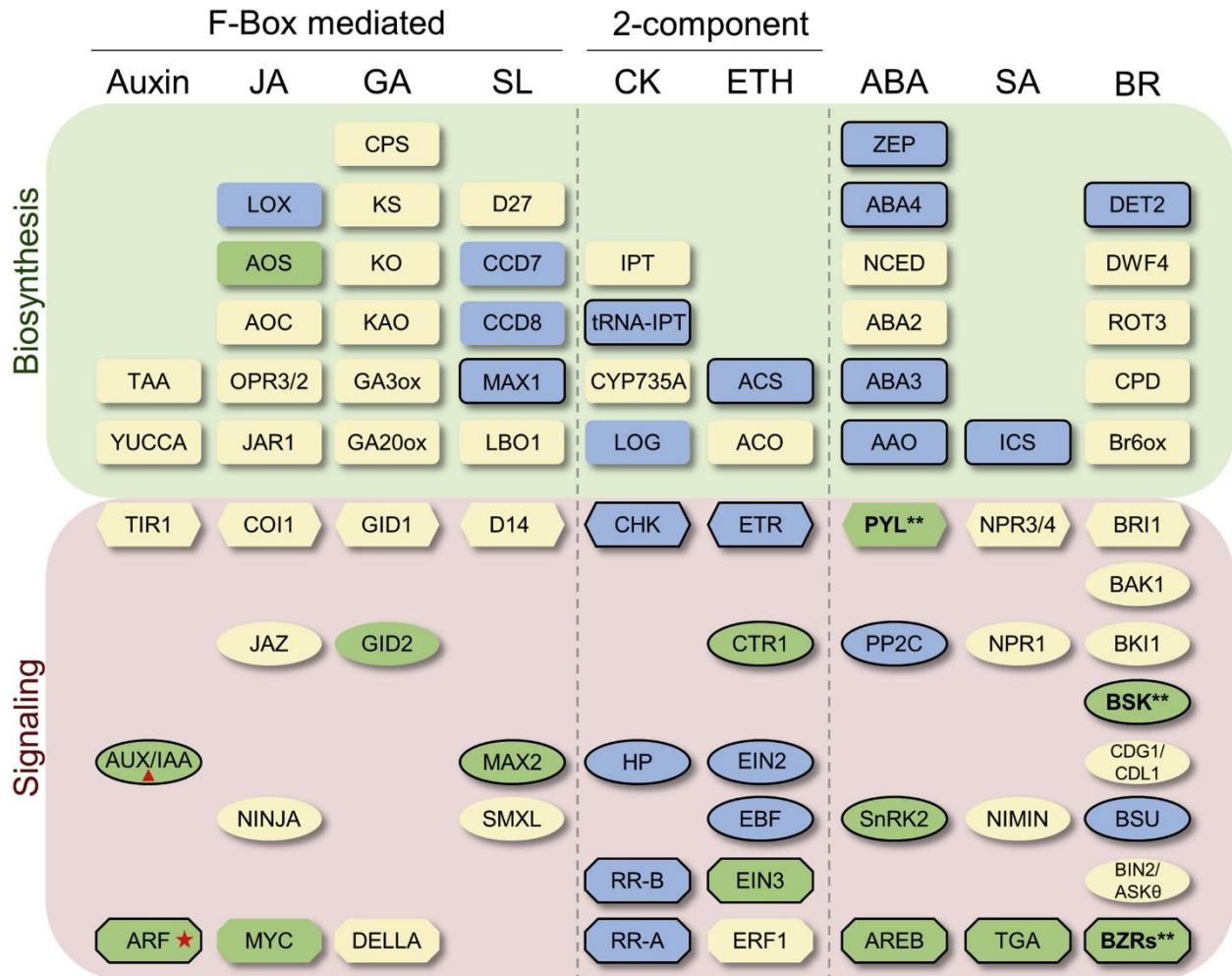

Figure 4: Genes involved in phytohormone biosynthesis and signaling. Blue signifies genes present in Chlorophyta and Streptophyta, green those appearing in Streptophyta including algae and pale yellow those present only in land plants (Jiao *et al.* 2020).

Interactions with microbiome

When the first Streptophyta established themselves on dry land, it was in no way a lifeless wasteland. They joined an ecosystem already populated by protists, bacteria or fungi (Kenrick, Crane 1997). Some of those helped the newcomers with facing the many new terrestrial stresses, while others became stressors themselves.

Arbuscular mycorrhizal symbiosis

A symbiotic relationship with fungi exists across land plant diversity, providing the plants with nutrients and possibly also helps with water uptake (Delwiche, Cooper 2015). Although evidence of this trait is identified deep within the embryophyte fossil record, it has never been detected in any streptophyte algae (Delwiche, Cooper 2015). Therefore, this symbiosis could have been the trait that set the Embryophyta apart and allowed the group to conquer land (Delwiche, Cooper 2015).

The formation of such AM association requires multiple steps. First, the fungus senses SL signals from the plant, responding with its own signal. The plant in turn senses this signal and allows for colonization by the fungus, which forms structures known as arbuscules therein (Delaux *et al.* 2015).

Main genes responsible for this communication on the land plant side are LysM-RLK and DMI2, which receive the fungal signal and activate DMI1. In turn, this potassium channel creates calcium signal recognized by CCaMK. This is followed by an activity of multiple downstream transcription factors, among those multiple GRAS sequences (Delaux *et al.* 2015). In Gymnosperms who lose the ability to form AM symbiosis, these genes are regularly lost, and so it seems they are AM-symbiosis specific (Delaux *et al.* 2015).

Interestingly, despite them not forming AM connections, the streptophyte algae harbor most genes of the upstream pathway, with only the status of DMI2 unsure (Delaux *et al.* 2015). Moreover, CCaMK originating from *Closterium* (Zygnematophyceae) was able to fully complement an embryophyte mutant, unlike related sequences from *Nephroselmis pyriformis* (Chlorophyta) or *Chlorokybus*. Moreover, several LysM-RLK sequences are present in *Chara braunii*, suggesting that this signaling pathway evolved in Phragmoplastophyta (Nishiyama *et al.* 2018).

On the other hand, the downstream path most likely only appeared in Embryophyta. While there are some GRAS sequences in higher streptophyte algae, the system active in land plant AM symbiosis probably emerged by duplication in land plants, just as the other components of this pathway (Delaux *et al.* 2015). It is therefore possible that an original signaling pathway, dedicated to interaction with other microorganisms, was co-opted by the first land plant to the nascent AM symbiosis (Delaux *et al.* 2015; Nishiyama *et al.* 2018).

Apart from this pathway, there are also other older streptophyte features that the early Embryophyta likely integrated to their new AM relationship. ARK1, a receptor-like

kinase (RLK), regulates the post-arbuscular development of the AM association in land plants. Phylogenetically related RLKs were found in *Klebsormidium*, suggesting that these sequences were probably also reassigned from older signaling pathways (Montero *et al.* 2021).

Finally, auxin is a central factor in AM symbiosis, frequently regulated by flavonoids (Nascimento, Tattini 2022). As mentioned above, flavonoids already appear in streptophyte algae, while the embryophyte auxin phytohormone signaling evolved on a foundation already laid out by streptophyte algae. As we have seen in this chapter, similar is true for the AM symbiosis, as this key element of land plant terrestrialization arose on a basis already present in their algal ancestors.

Symbioses and HGT

The lack of AM connections in extant streptophyte algae does not mean that there are no microbial species living in a symbiotic relationship therewith. Indeed, various microorganisms inhabit the microbiome of streptophyte algae (Knack *et al.* 2015). Moreover, this microbiome shows similarity to those of early-diverging land plants (Knack *et al.* 2015; Satjarak *et al.* 2022).

This may be significant, as there seems to be a connection between phylogenetical history of lineages and the microbiome composition (Knack *et al.* 2015). Furthermore, it is possible that the earliest land plants lived in community assemblies containing algae, bacteria or fungi, and so the modern symbioses between algae and microbes may exist in similar conditions as in those ancient communities (de Vries, Archibald 2018b).

Among those organisms living with today's streptophyte algae and Bryophyta are various diazotrophic microbes (Knack *et al.* 2015). This likely benefits the streptophyte algae, who typically occur in nitrogen-poor habitats, and also suggests that the link between Embryophyta and N-fixing cyanobacteria and rhizobialeans may go far back (Knack *et al.* 2015).

But a steady nitrogen supply is not the only thing one can gain in close interaction with microbiota. As was already mentioned, the ABA receptor was probably a result of a horizontal gene transfer from a soil bacterium. Although HGT from bacteria to eukaryotes is a controversial topic (Cheng *et al.* 2019; Donoghue, Paps 2020), studies of whole genomes point to widespread HGT in land plants (Bowles *et al.* 2020).

Other genes streptophyte algae possibly gained in this way include the aforementioned GRAS gene family (Cheng *et al.* 2019). Originally presumed a land plant invention, they were ultimately found present in Zygnematophyceae (Delaux *et al.* 2015; Wilhelmsson *et al.* 2017; Cheng *et al.* 2019). Both streptophyte GRAS and PYL sequences have a single origin while the rest of the respective gene families exists in bacteria, suggesting a HGT origin (Cheng *et al.* 2019).

Further genes of presumably HGT origin include the DNA-3-methyladenine glycosylase (MAG) gene (Fang *et al.* 2017). MAG serves in DNA repair, recognizing a

damaged sequence and initiating the base excision repair process (Fang *et al.* 2017). It is present in a wide range of land plants and in streptophyte algae, but it is missing from Chlorophyta (Feng *et al.* 2017). While it is also found in Archaea and a wide range of eukaryotes, the streptophyte gene shows a single origin nested within bacteria, suggesting a HGT origin (Feng *et al.* 2017). As was already discussed, UV radiation is an important stressor in shallow water and especially on dry land, so having another way to cope therewith may have provided another advantage for terrestrialization.

Immunity

Close contact with microbial life may naturally also bring dangers. It is likely that plants were in contact with various bacteria since the very beginning, as they arose from an interaction of their common ancestor and a cyanobacterium-like prokaryote (Han 2019). Some of these bacteria, just as various fungi, oomycetes or viruses, developed pathogenic relationship toward plants (Han 2019).

Of course, the relationships between plants and microbes do not divide into strict symbiont versus pathogen binary. Accordingly, the nature of the association might not always be clear, especially in the fossil record (Han 2019), and sometimes may switch in response to external factors (de Vries *et al.* 2018c). Moreover, pathogens may use plant systems developed for symbiosis, and conversely symbionts like the aforementioned AM fungi may have started as plant pathogens (Delaux, Schornack 2021).

Current land plants face diverse pathogen challenges and employ twofold interconnected system to answer them (Han 2019). The first one relies on cell surface pattern-recognition receptors (PRRs) which recognize microbe-associated molecular patterns to activate pattern-triggered immunity. The other utilizes disease resistance (R) proteins to counteract the effector proteins pathogens use to suppress plant immunity (Han 2019).

Naturally, our knowledge of plant pathogens comes mainly from angiosperms, whereas the immunity of algae is more obscure (Han 2019). Nevertheless, recent studies found homologs of actors in land plant immunity response in streptophyte algae. Overall, it seems that the ancestor of extant Streptophyta already expanded its arsenal of pathogen-related genes (Wang *et al.* 2020).

Moreover, RLKs, foremost group of the land plant PRRs, exhibit significant diversification in Streptophyta, especially after the split of Mesostigmatophyceae and Chlorokybophyceae (Gong, Han 2021). Furthermore, many RLK groups show signs of positive selection in streptophyte algae, chiefly in the extracellular regions, perhaps a testimony of an arms race between the algae and their pathogens (Gong, Han 2021).

Similar is also true for the main group functioning as R proteins, the nucleotide binding-site leucine-rich repeat (NLR or NBS-LRR) proteins. Originally proposed as a land plant specificity, they have since been detected in various other lineages of Streptophyta (Gao *et al.* 2018), and even in few members of Chlorophyta (Shao *et al.* 2018). They too

showed evidence of positive selection in streptophyte algae, suggesting an involvement in plant-pathogen interactions (Gao *et al.* 2018).

Overall, it is apparent that the ancestor of land plants inherited pre-adaptations not only for the crucial AM symbiosis but also for a whole scale of interactions with other organisms it was to meet after its emergence from among the streptophyte algae.
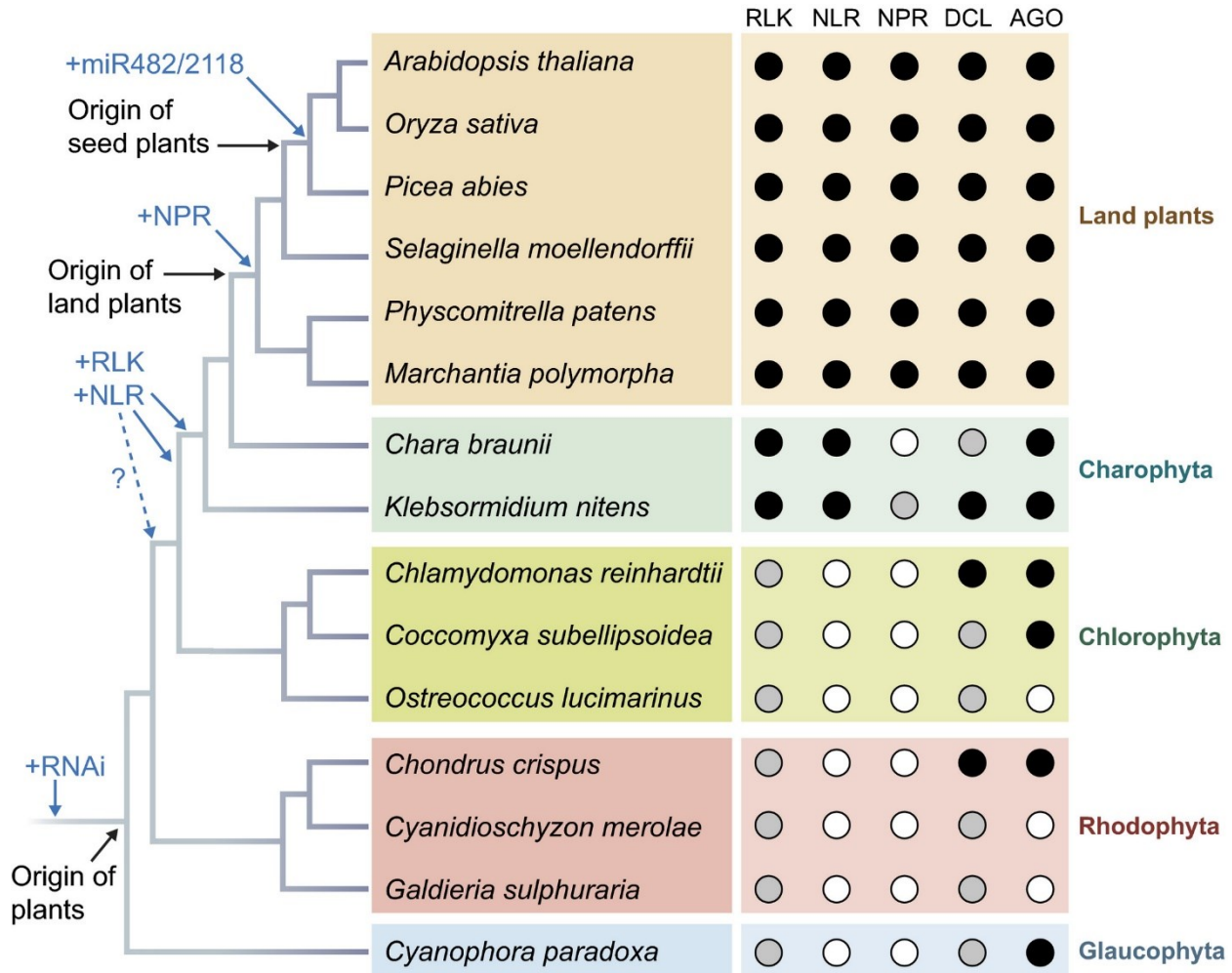

Figure 5: The evolution of immunity response across the Archaeplastida (Han 2019). Gray color signifies absence of canonical domain architecture. DCL and AGO act in anti-viral response and presumably pre-dated the last common ancestor of eukaryotes. NPRs are involved in SA signaling, which is discussed in the previous chapter (Han 2019).

Complexity

There are multiple species of photosynthetic organisms which are terrestrial, some of them even from the green lineage (Becker, Marin 2009; see Fig. 1 for terrestrial streptophyte algae). What really sets the Embryophyta apart is that they are the only macroscopic terrestrial lineage among these photoautotrophs (Becker, Marin 2009). While some algae are also macroscopic and some may even possess a true three-dimensional (3D) growth (Moody 2020), none of them has ever been able to replicate the embryophyte conquest of land (Becker, Marin 2009).

      Moreover, the macroscopic body plans could also be a factor that helped with terrestrialization, providing protection against stressors such as drought or strong light (Stebbins, Hill 1980). It is therefore natural to ask which traits of the streptophyte algae facilitated the evolution toward macroscopic terrestrial Embryophyta.

Body plan evolution

Multicellularity was a significant step towards a bigger body. Streptophyta took this path almost since their beginning, for while the ancestor of the green lineage was a unicellular organism, among the Streptophyta only Mesostigmatophyceae are strictly unicellular (Umen 2014).

      The first members of the Embryophyta likely had undifferentiated filamentous bodies, similar to those already found in Klebsormidiophyceae (Umen 2014). The predominant filamentous type of growth of Klebsormidiophyceae, while in some way only one dimensional (Buschmann 2020), probably brought some advantages over living separately, such as better anchoring, protection against predators or better space searching (Umen 2014).

      However, as member of the Phragmoplastophyta, the first land plants probably had access to at least two-dimensional (2D) body growth that is present in extant members of this clade (Buschmann 2020). The 2D growth likely provided new advantages, like enhancing access to chemical resources or maximizing photosynthesis (Buschmann 2020), and first appears already in some Klebsormidiophyceae (Mikhailyuk *et al.* 2018).

      2D growth in higher streptophyte algae may be realized by tip growth-facilitated filament branching, present in all members of the ZCC clade, or by division plane rotations, present for example in *Coleochaete* (Buschmann 2020). Among the streptophyte algae, Charophyceae especially show the most complex growth, which could be classified as 2D (Moody 2020), or even as 3D (Buschmann 2020).

      Interestingly, both apical cell growth and the ability to rotate division plane appeared together with the advent of Phragmoplastophyta (Buschmann 2020). Furthermore, their interconnectedness is suggested by the fact that several genes play a

role in both of these processes, and so it is likely that these important mechanisms of land plant growth originated together with the emerging phragmoplast (Buschmann 2020).

A structure related to apical growth is the rhizoid. They were one of the key adaptations to life on land and are already present in higher branching streptophyte algae (Fürst-Jansen *et al.* 2020). In Zygnematophyceae, they facilitate anchorage to a substrate, while the rhizoids of Charophyceae are involved in the modulation of gravitropism (Fürst-Jansen *et al.* 2020). The genetic mechanisms that regulate their development originated before the emergence of Embryophyta and are conserved with mechanisms responsible for root hair development in angiosperms (Harrison 2017).

Another trait shared across multiple phragmoplastophyte lineages are plasmodesmata, microscopic channels in cell walls that allow for movement of molecules between cells (Moody 2020; Brunkard, Zambryski 2017). They are present in majority of land plat cells, only absent in highly specialized ones (Brunkard, Zambryski 2017).

Despite their presence in the ZCC clade, the plasmodesmata there lack desmotubules, and therefore it is likely that they appeared only through convergent evolution and represent a property of parenchymatous multicellularity rather than an ancestral phragmoplastophyte trait (Brunkard, Zambryski 2017).

Phragmoplast

The emergence at the same of phragmoplast and a new way of branching is not coincidental. This array of microtubules plays a central role in cell division in Phragmoplastophyta, assembling a new cell wall between two nascent plant cells (Buschmann, Zachgo 2016). In land plants, this functionality is accompanied by the preprophase band (PPB), which predicts the plane of the upcoming cell division (Buschmann, Zachgo 2016).

This apparatus is not found in lower branching streptophyte algae, where cell division happens by centripetal cleavage and is aided by the centrosome, although *Klebsormidium* seems to already possess various genes for actors in phragmoplast and PPB mechanism (Buschmann, Zachgo 2016; Baquero Forero, Cvrčková 2019). Apart from the KCM clade, cleavage was reintroduced in some Zygnematophyceae with coinciding reduction or outright loss of the phragmoplast (Buschmann, Zachgo 2016).

After the development of the phragmoplast, the centrosome was mostly lost. It is present in *Coleochaete orbicularis*, where it regulates alternations between radial and circumferential cell division, but not in Charophyceae or Zygnematophyceae (Buschmann, Zachgo 2016). They are also possibly present in liverworts in a modified form called the polar organizer (Buschmann, Zachgo 2016).

While majority of land plant cell divisions utilizes PPBs, it is absent in Charophyceae and Coleochaetophyceae (Buschmann, Zachgo 2016). In contrast, their presence in Zygnematophyceae is not clear. While PPB was not detected in *Spirogyra* and *Zygnema*, it

seems to be present in *Mougeotia*, suggesting that this typical land plant structure originated before the rise of Embryophyta (Buschmann, Zachgo 2016).
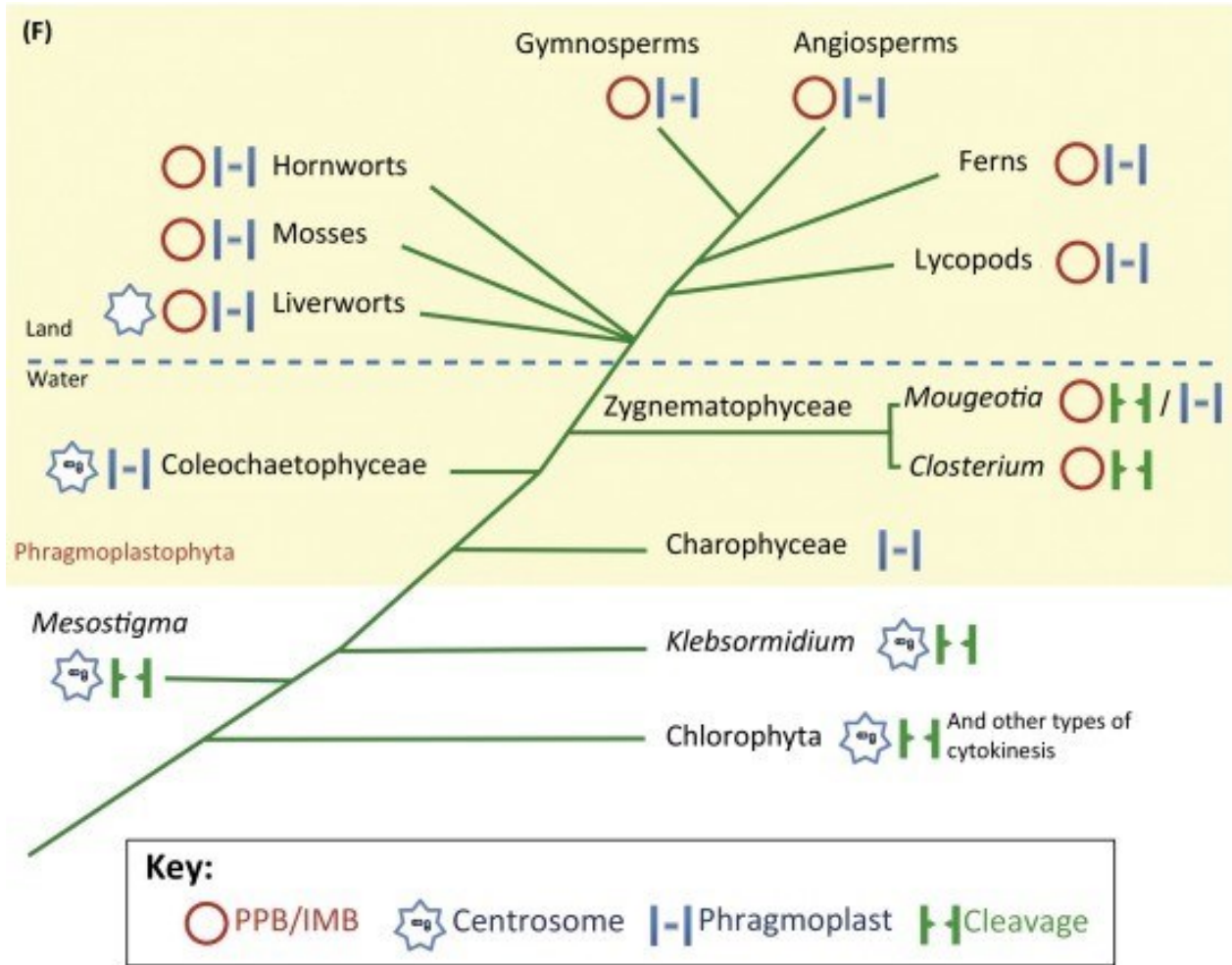
Figure 6: The evolution of streptophyte cytokinesis. The division between "Land" and "Water" separates Embryophyta from algae but ignores terrestrial members of streptophyte algae. IMB is a microtubule ring aiding cytokinesis in desmid algae (Buschmann, Zachgo 2016).

Life cycle

The land plant life cycle is marked by an alternation between two multicellular generations, that of the haploid gametophyte and of the diploid sporophyte (Rensing 2018). In contrast, in both chlorophyte and streptophyte algae the only diploid life stage is the unicellular

zygote (Rensing 2018). The sporophyte has been suggested as one of the main factors in land plant terrestrialization, giving the amphibious gametophyte a space for developing terrestrial adaptations (Harholt *et al.* 2015; for a review of early land plant life cycle evolution see Kenrick 2017).

On the other hand, oogamous sexual reproduction is a land plant characteristic that already appears in streptophyte algae, likely originating with the Phragmoplastophyta (Becker, Marin 2009; Wickett *et al.* 2014). Sexual reproduction was probably the original eukaryote mode of reproduction, accompanied by meiosis (Liang *et al.* 2020). It has been lost in early branching Streptophyta, who nonetheless still keep a large part of the meiosis toolkit (Liang *et al.* 2020; Wang *et al.* 2020). Furthermore, Zygnematophyceae lost their oogamy in favor of conjugation (Jiao *et al.* 2020).

While the streptophyte algae do not form sporophyte embryos, they still show a form of matrotrophy in retention of the zygote (Umen 2014). This was observed in *Coleochaete*, where the zygote remains associated with the vegetative thallus, which forms a strong connection therewith and provides the zygote with nutrients (Umen 2014).

Transcriptional regulation

Transcription is regulated by the transcription associated proteins, including transcription factors (TF) and transcriptional regulators (TR) (Wilhemsson *et al.* 2017). They play a key role in developmental processes, and indeed many typical land plant traits such as epidermis, roots or water conducting tissues are associated with characteristic TFs (Catarino *et al.* 2016).

The number of TFs seems to correspond with morphological complexity in plants and animals (Wilhelmsson *et al.* 2017). In Streptophyta, this rule mostly holds, with Mesostigmatophyceae containing the lowest amount of TFs (Liang *et al.* 2020) and *Chara* showing an increase from *Klebsormidium* (Nishiyama *et al.* 2018). However, Zygnematophyceae are an exception, as even the unicellular *Penium* has more TFs than most other streptophyte algae (Jiao *et al.* 2020).

Significant amount of TFs was acquired already in the common ancestor of Streptophyta (Bowles *et al.* 2020). Moreover, many of those TFs gained already in early streptophyte algae are associated with abiotic stresses in land plants and probably played a role during terrestrialization (Wang *et al.* 2020). For example, the NAC TF family active in stress response was originally considered land plant specific, but recent study detected it already in *Klebsormidium* with strongly conserved domains (Mougarny-Calès *et al.* 2016).

Stress related TFs were later significantly expanded in Zygnematophyceae (Jiao *et al.* 2020) and most land plant TF families were already present before the advent of Embryophyta (Wilhemsson *et al.* 2017; Catarino *et al.* 2016).

Overall, it is clear that many seeds of Embryophyte complexity were already planted in their algal ancestors. The rising morphological complexity was aided by the new cell

division employing phragmoplast and rising numbers of TFs, but also by previously mentioned traits such as increasing control over plastid or developing hormonal pathways.

Conclusion

The embryophyte conquest of land was a momentous chapter in the history of life on Earth. From simple beginnings among the streptophyte algae, land plants rose above the surface and ultimately covered the world. In recent years, new genomic and transcriptomic studies helped to partially elucidate this process, highlighting both unexpected continuity and persisting discontinuity.

The continuity manifested itself in the fact that streptophyte algae were found to be closer to land plants than previously expected, harboring genes that were considered characteristic to Embryophyta. In some areas the similarity is especially remarkable, for example in the cell wall. Indeed, *Penium margaritaceum* has even been proposed as a model organism to study plant cell walls (Rydahl *et al.* 2015). In this light it is not surprising that the view of land plants as always terrestrial was formulated by researchers studying streptophyte cell walls.

But these surprising commonalities should not obscure the innovations present in the last ancestor of land plants. Not only was its appearance marked by great genetical diversification (Bowles *et al.* 2020), but as we have seen over the course of this work, many traits of land plants were not present in the same form in their algal ancestors.

Thus, while there likely existed the whole signaling part of AM symbiosis pathway, the downstream path and the symbiosis itself only appeared in the first land plants. There was also a rise in the control over the plastid, but the diversity of plastid functional forms only appeared in vascular plants.

An example from the emerging hormonal pathways also show a possible pitfall of relying too much on omics studies, as not even the presence of the whole ABA signaling toolkit guarantees the same phytohormone function. This underlines the importance of designing good experimental verifications for hypotheses based on bioinformatical data, which may otherwise be misleading.

The land plants therefore built on the terrestrial adaptations present already in their algal ancestors, but innovated them according to the needs of their increasingly complex bodies. This provides an interesting possibility of comparison with their zygnematophyceous sisters, who in contrast found their place in the sun by reducing their bodily complexity.

Morphologically closest to their embryophyte relatives, the Charophyceae are another possible fascinating subject for comparison, as we have seen on these pages that they frequently found alternative solutions to the questions of bodily complexity than land plants, solution that were nonetheless based on the same genetic phragmoplastophyte toolkit.

While the relevance of Charophyceae for such comparisons may be limited by their aquatic lifestyle, this is not true for the Coleochaetophyceae, who seem relatively underexplored, given that they are the only lineage of streptophyte algae without a published genome, even though they are closer to land plants morphologically than

Zygnematophyceae and phylogenetically than Charophyceae. To appreciate the originality of streptophyte solutions to terrestrialization, comparison with other terrestrial algae should be enlightening, with *Draparnaldia* (Chlorophyta) recently proposed as a model organism (Caisová 2020).

To really understand the earliest land plants, discovery of older embryophyte fossils would be of utmost importance, but the likeliness of such event is questionable. But unlikely discoveries still sometimes happen, as was the case of recently discovered new streptophyte algal lineage (Mikhailyuk *et al.* 2018), so the next years may bring other impactful surprises.

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