A molecular atlas of plastid and mitochondrial evolution from algae to angiosperms

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Abstract

Algae and plants carry two organelles of endosymbiotic origin that have been co-evolving in their host cells for more than a billion years. The biology of plastids and mitochondria can differ significantly across major lineages and organelle changes likely accompanied the adaptation to new ecological niches such as the terrestrial habitat. Based on organelle proteome data and the genomes of 168 phototrophic (Archaeplastida) versus a broad range of 518 non-phototrophic eukaryotes, we screened for changes in plastid and mitochondrial biology across one billion years of evolution. Taking into account 331,571 protein families (or orthogroups), we identify 31,625 protein families that are unique to primary plastid-bearing eukaryotes. 1906 and 825 protein families are predicted to operate in plastids and mitochondria, respectively. Tracing the evolutionary history of these protein families through evolutionary time uncovers the significant remodeling the organelles experienced from algae to land plants. The analyses of gained orthogroups identifies molecular adaptations of organelle biology that connect to the diversification of major lineages and facilitated major transitions from chlorophytes en route to the global greening and origin of angiosperms.

Keywords: plastid evolution, plant mitochondria, terrestrialization, organelle proteomes

Introduction

Fewer natural phenomena have been as transformative to planet Earth as the global greening through plants [1,2]. The proliferation of plants on land rests on the emergence and expansion of the Chloroplastida, also referred to as the Viridiplantae or simply the green lineage. The Chloroplastida are made up of three phyla: chlorophytes, streptophytes and the prasinodermophytes that are thought to be the sister lineage to the two former [3]. Chloro- and prasinodermophytes are represented by algae only, whereas streptophytes are made up of algae and embryophytes, the latter uniting all land plants [3–5]. The list of key adaptations that fostered land plant expansion in a macroevolutionary context are multiple: roots, a mutualistic symbiosis with fungi, stomata, a cuticle, polyplastidy, and an expansion of many metabolite families such as flavonoids to name a few [1,3–10]. These innovations, evolving gradually in the common ancestor of land plants (LCA), provided a decisive fitness advantage over the non-terrestrial chloro-, prasinodermato- and streptophyte algal relatives [1,11].

The eponymous organelle of plants, the chloroplast, underwent various changes, too. It adapted in multiple ways to the challenges characterizing the habitat the LCA encountered. Improving stress response was necessary to deal for instance with increased levels of ultraviolet (UV) high light stress and to cope with temperature shifts that change rapidly on land in contrast to in water [12–14]. Polyplastidy, a phenomenon that separates plastid from nuclear division, leading to cells that can harbor more than one plastid per cell, was part of being able to develop larger body plans [12,15,16]. To communicate stress and the need for component biosynthesis, an elaborate retrograde signaling evolved on the basis of messenger proteins such as GUN1 and maybe WHIRLY [17,18]. In combination, these adaptations were decisive for the success of streptophytes, which is evident in the number of species they have evolved and the sheer biomass they produce [1,19].

Plastids do not operate autonomously, but are part of an intricate metabolic network and even physically interact with other compartments such as the endoplasmic reticulum and peroxisomes [20,21]. Marked metabolic and physical interactions of plastids also concern the only other compartment of ancient endosymbiotic origin: the mitochondrion. Plant mitochondria are much less in the focus of plant research. Next to their canonical functions, they are known to be involved in immunity, lipid metabolism and other (eco)physiological processes that are frequently in crosstalk with the photosynthetic organelle [22,23]. Like plastids, mitochondria were critical in the evolution and continued adaptation of important physiological traits, which characterize the green lineage. A notable example of preadaptation includes malate decarboxylation in the C4 photosynthetic pathway [24] – a trait of the green lineage [25] that improves plant photosynthetic efficiency in warm and dry habitats [26]. Similarly, some components of mitochondrial retrograde signaling also evolved in the land plants and likely contributed to its ROS and draught tolerance [27].

In spite of the importance of these two organelles of endosymbiotic origin in coordinating their duties, the evolution of components specific to chloroplast and mitochondrial biology has not been explicitly studied in light of streptophyte evolution or plant terrestrialization. Previous work has determined genes specific to certain plant clades and that are catalogued by valuable resources such as the “GreenCut” [28]. Such analyses, however, did not focus on organelle biology nor clustered protein families. They were also limited by a low number of archaeplastidal genomes and insufficient methods for orthology inference available at that time. Since then, genome assemblies of members from previously unsampled clades has increased manyfold [11,29–37] and more organelle proteomes and better functional annotations are available. Similarly, and concomitantly, the development of novel and accurate algorithms for orthology inference [38–41], along with advances in experimental biology allow to now identify critical evolutionary changes in an eco-evo context of plastid and mitochondrial biology that underpin the success of the Chloroplastida.

Here, we curate a database of protein families unique to the green lineage. We plot their evolution across the major splits in the evolutionary history of streptophytes, focusing on the biology of the two organelles of endosymbiotic origin. We report that the number of plastid- and mitochondria-associated protein families changes most significantly at two evolutionary bifurcations: firstly, at the green lineage itself and secondly at the split between Zygnematophyceae and embryophytes at the water to land transition. The newly recruited protein families influenced organellar processes such as carbon and lipid metabolism, information processing and organelle development. We provide an extensive catalogue of the changes the proteomes of plastid and mitochondria experienced throughout streptophyte evolution, which offers multiple angles from which to explore major evolutionary transitions such as the conquest of land and embryophyte diversification.

Results

Half of the chloroplastida protein families are unique to embryophytes

Out of a total of 12,862,035 proteins, 95% were categorized from 686 eukaryotes (Table S1A) and grouped into 331,570 orthogroups (Table S1B). From these, 31,650 were present only in chloroplastida, and classified as Green Ortho Groups (GOGs) (Fig. S1 and Table S1C-D). An examination of GOG distribution among green species revealed that around half of all GOGs were unique to terrestrial plants (Fig. 1A). Approximately 400 GOGs appeared in more than 90% of species, referred from here on to as the 'core GOGs' (Fig. 1B). For only 5% of all GOGs, a functional annotation could be identified (Fig. 1C, Table S1E). For embryophyte-specific GOGs the numbers were comparable, yet they maintained a consistent distribution of identified functions, except for an increased fraction of membrane trafficking proteins (Fig. 1D, Table S1F). Notably, for the core GOGs the number is higher. For 30% functional annotations covering photosynthesis, mitochondrial formation, trafficking, and information processing could be identified (Fig. 1E, Table S1G). The functions for a vast majority of the GOGs remain elusive (Table S1H), numbers that mirror those of previous studies [28], and they hence provide an excellent ground for experimental exploration.

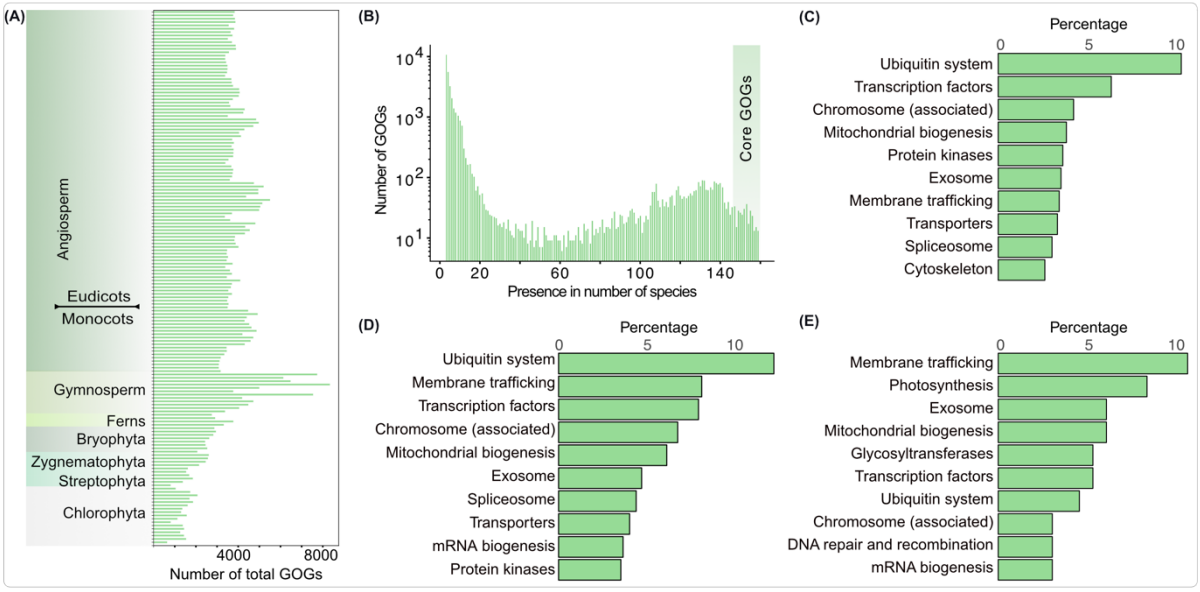

Fig. 1: Distribution and functional annotation of green orthogroups (GOGs). (A) Total number of GOGs present in each species from major Chloroplastida taxa. (B) Number of GOGs as a function of their presence

across 159 Chloroplastida species. Major functional categories of 4.71% of all GOGs (c), 3.96% of the embryophyte GOGs (d) and 27.9 % of the core GOGs (e).

Mitochondrial and plastid proteomes of the Chloroplastida expanded with the origin and diversification of the green lineage

To investigate changes in the proteomes of plastids and mitochondria, we curated 1906 plastid and 825 mitochondrial orthogroups (POGs and MOGs, respectively) based on published proteome data and homology-based protein clustering of 204 eukaryotes, including that of secondarily photosynthetic eukaryotes (Fig. S1B, Table S2A-D). In comparison to rhodophytes and glaucophytes, the green lineage encodes almost twice as many POGs (Fig. 2A, Table S2E). Within the green lineage, from the Zygnematophyceae and embryophytes onwards, plastid proteomes further expanded both in terms of the number of proteins within each POG and the number of unique POGs. The former is likely a consequence of genome duplications, while the latter underscores functional divergence that followed gene duplications. The distribution of MOGs appears qualitatively similar to that of POGs (Fig. 2B, Table S2F). 60% of the POGs could be functionally annotated, predominantly operating in biosynthetic and other metabolic pathways such as photosynthesis (Fig. 2C, Table S2G). Around 75% of the MOGs could be annotated, containing proteins for mitochondrial biogenesis, membrane trafficking and translation (Fig. 2D, Table S2H). Protein biosynthesis-related proteins are abundant in both, POGs and MOGs, underscoring their biosynthetic activity. Proteins for mitochondrial biogenesis also appear in both. For instance, about 60 POGs are annotated as mitochondrial biogenesis and they encompass numerous PPR and mTERF proteins (crucial for RNA editing and metabolism) and proteins involved in various other information processing activities, probable to function in both organelles. Analysis of the N-terminal 20 amino acids show their charge to range from 0 to 2, indicating they might be dually targeted to plastids and mitochondria [42]. Five of the mTERFs are part of a POG and MOG simultaneously (Fig. S3D). Overall, the trends show that in embryophytes the number of protein families associated with an endosymbiotic organelle function increased.

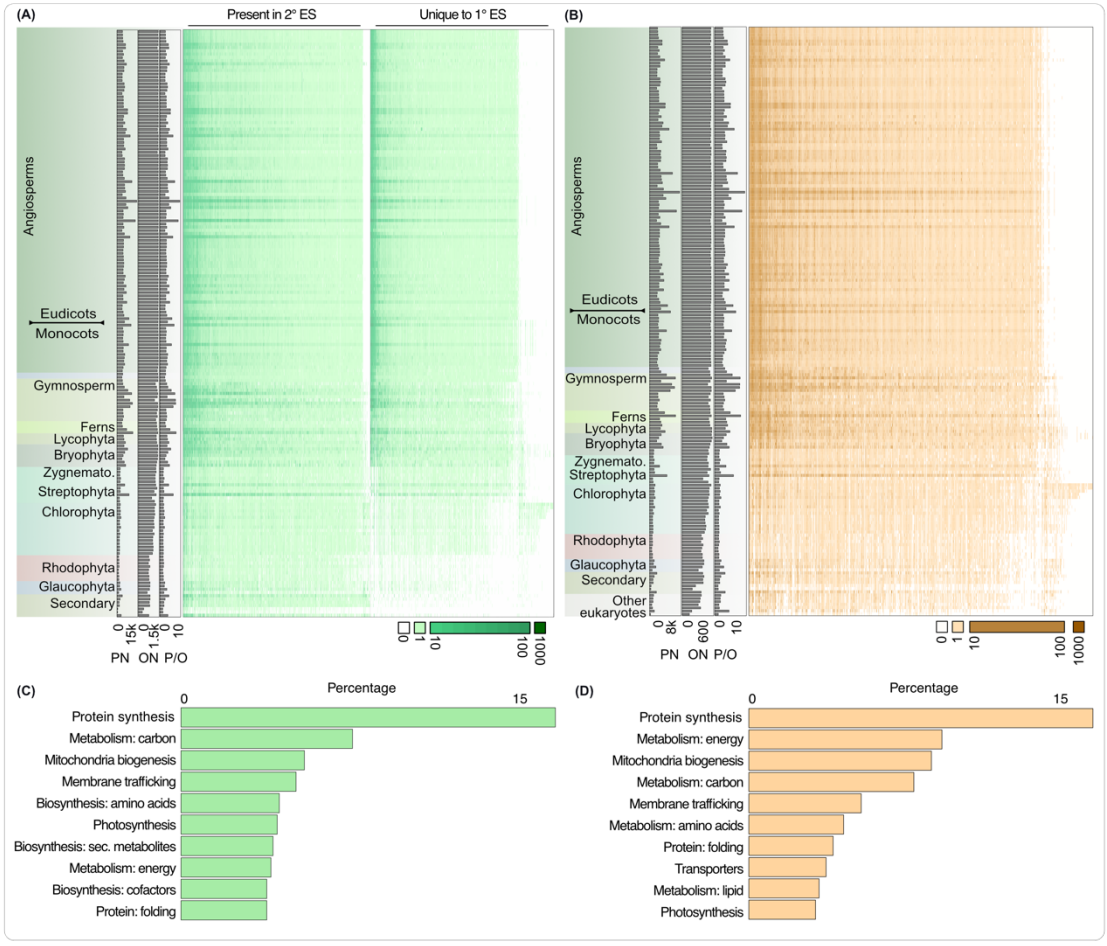

Fig. 2: Mitochondrial and plastid orthogroups across archaeplastidal species. Distribution of plastid (POGs; A) and mitochondrial orthogroups (MOGs; B). The distribution of POGs was determined for plastids of primary (1° ES) and secondary endosymbiotic origin (2° ES). Protein copy numbers within each POG or MOG across species is shown in the heat-map as per the key on the bottom right of the heatmaps. Horizontal bars on the left side of the heatmaps show the total protein numbers (PN) likely localised to organelles, total POG or MOG numbers (ON) and distribution of protein number per OG (P/O) for a given species. Major functional categories of POGs and MOGs in (C) and (D), respectively.

The increased number of POGs and MOGs in the green lineage is explained by a combination of two phenomena: (a) new gains in the green ancestor, and (b) secondary losses at the origin of rhodophytes [43]. We used ancestral state reconstruction (ASR) to resolve between these two possibilities. The branching order of the archaeplastidal lineages remains challenging [44], as sometimes glaucophytes [45] and sometimes rhodophytes come out as the sister to the other remaining archaeplastidal lineages [4,46]. An inferred eukaryotic tree (with 31 non-Archaeplastida eukaryotes as an outgroup to Archaeplastida) placed the rhodophytes and glaucophytes as sister clades (Fig. S2). This tree and ASR pipeline were validated using rbcS control (Fig. S3A), and further undergirded the main results which are also consistent with varying thresholds of probability of presence and absence in a given ancestor on this eukaryotic tree (Fig. S3B) as well as Archaeplastida only phylogeny manually rooted to have glaucophyte and rhodophyte as an outgroup to chloroplastida (Fig. S4-7).

The result suggests that the plastid proteome of the last common ancestor of Archaeplastida united ca. 1000 POGs (Fig. 3A, Fig. S3B, Fig. S6, Table S3A-C). This inferred proteome witnessed significant

gains of protein families at the emergence of the green ancestor (and later speciation). 50% of these newly gained POGs could be functionally annotated (Fig. 3C, Table S4A), showing that at the origin of the green lineage novel photosynthesis- and metabolism-related POGs were recruited, while the transition to land (Z/E and embryophyte ancestors) added metabolism-related, as well as protein synthesis- and ubiquitin-related POGs to the toolkit (Table S4A). Using hidden Markov searches, we verify that more than half of the protein families recruited in embryophyte and Z/E ancestors are absent in non-zygnematophyceae algae (Fig. S3C). The mitochondrial proteome followed a qualitatively similar trend of expansion (Fig. 3B, Fig. S3B, Fig. S7, Table S3D-F). ca. 500 MOGs trace back to the archaeplastidal ancestor, while ca. 700 MOGs were identified at the root of angiosperms (Fig. 3C, Fig. S3B). Around 50% of the newly gained MOGs could be functionally annotated, showing that the chloroplastidal gains contribute to carbon metabolism, protein synthesis and mitochondrial biogenesis. Terrestrialization also witnessed a similar gain of MOGs, most of which function in metabolism as well as mitochondrial biogenesis and membrane trafficking (Fig.3C, Table S4B).

In summary, across plant species, plastid and mitochondrial proteomes are predicted to have gained a significant number of protein families reflecting the dynamic nature of organellar proteomes post-endosymbiosis [47,48]. A closer look at the function of the newly gained organelle proteins shows a wide variety, including lipid and carbon metabolism, information processing, development and division of organelles.

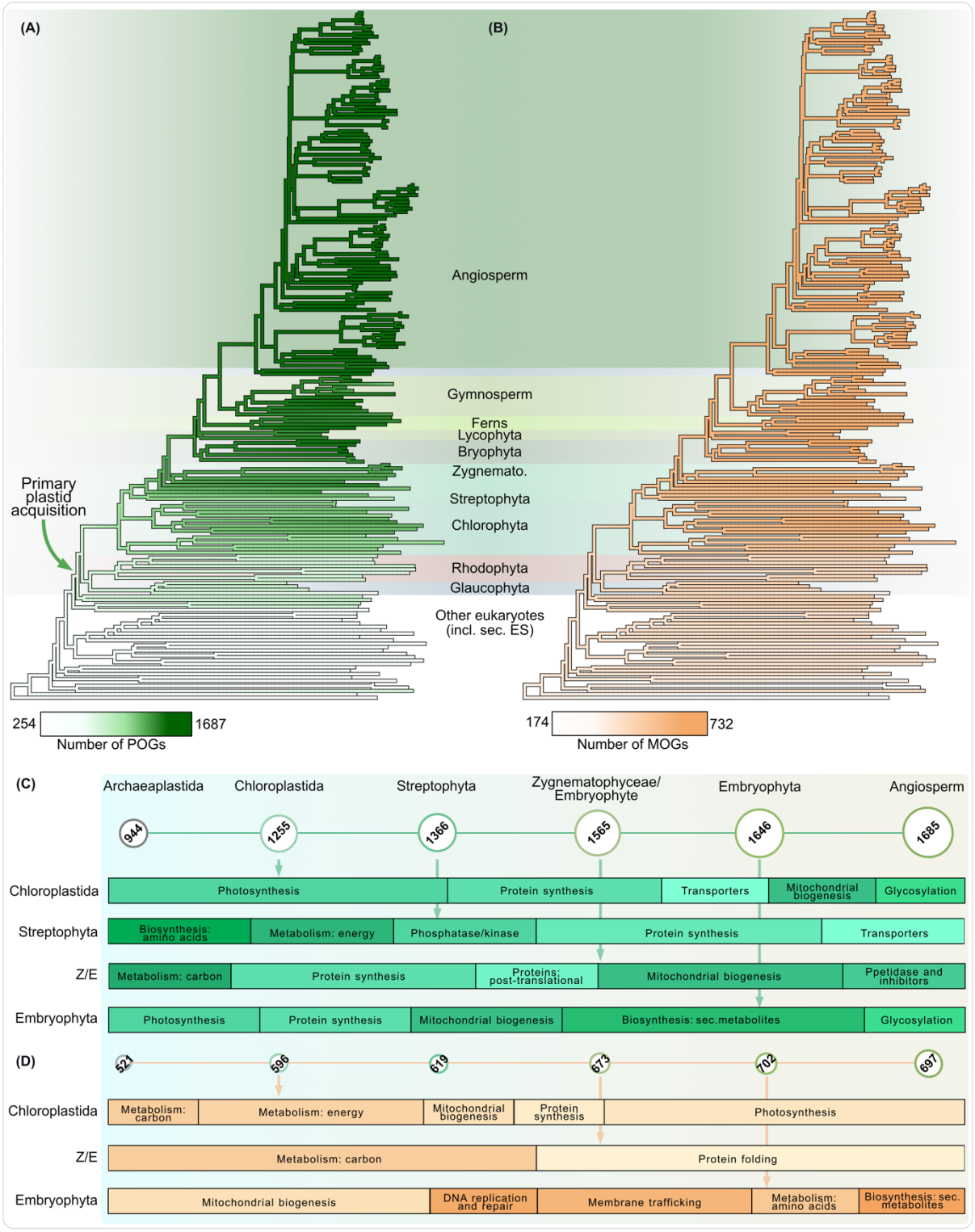

Fig. 3: Evolution of organelle proteomes in Archaeplastida. Gains in plastid (POGs; A) and mitochondrial orthogroups (MOGs; B) across all nodes of archaeplastidal evolution and POGs coinciding with primary and secondary plastid acquisitions. Gains across main nodes of interest in (C), where each circle represents an ancestor, with its predicted number of protein families shown in the circle and whose diameter correlates with the number of OGs. Major gains occurred in the chloroplastidal ancestor, the common ancestor of Zygnematophyceae-embryophytes (Z/E) and that of embryophytes. In (D) the same as in (C), but for mitochondrial OGs. Their functions are shown in the proportionate bar charts below the ancestors.

Increased complexity of RNA metabolism and photosynthetic adaptability

RNA metabolisms such as editing intercepts the linear information flow from mRNA to protein and is crucial for organelles to function [49–51]. Two main domains, the PPR and mTERF domain, are associated with RNA editing and metabolism [52,53]. We first screened for organelle orthogroups containing either of these two domains in at least 60% of all proteins within each respective orthogroup (Fig. S1C). Around 50 POGs and 20 MOGs were found. More than 80% of them were restricted to embryophytes, only few were present in some algae (Fig. 4). A closer look revealed that most of the algal homologues lacked PPR and mTERF domains and they are hence unlikely true orthologues. More generally, this shows that any detailed interpretation regarding an inferred orthogroup's function should be supported by screening for functionally relevant domains.

True PPR or mTERF domain-containing RNA-editing, splicing (and processing at large) proteins increased significantly in number by recruiting new orthogroups, also through adding the two domains to proteins that did not contain these in their algal ancestor. A presence-absence pattern shows that >90% of proteins containing PPR/mTERF domains are exclusive to land plants, except for *Chara braunii* and *Klebsormidium flaccidum* (Fig. 4B). These proteins include, but are not limited to, OTP51 and SOT5 (present in embryophytes and *Chara*) as well as SOT1, SVR7, THA8, PDM4 (present only in embryophytes; Fig. S9). Target transcripts of these RNA metabolism factors point to the synthesis and assembly of photosynthesis-related proteins and to proteins of the thylakoid membrane (Fig. 6B). Likewise, mTERFs, which are crucial for plastid and leaf development, are also uniquely expanded in the terrestrial clade with examples of protein re-targeting across organelles [54]. The dual targeted (plastid and mitochondrion) mTERF6, unique to the land plants (Fig. S9) and the streptophyte alga *Klebsormidium*, takes part in retrograde signalling to the nucleus via ABA and imparts abiotic stress tolerance [55]. Overall, RNA metabolism across plants has undergone major changes and has a significant impact on photosynthesis, improvement of which was key to thriving on land (Fig. 6B).

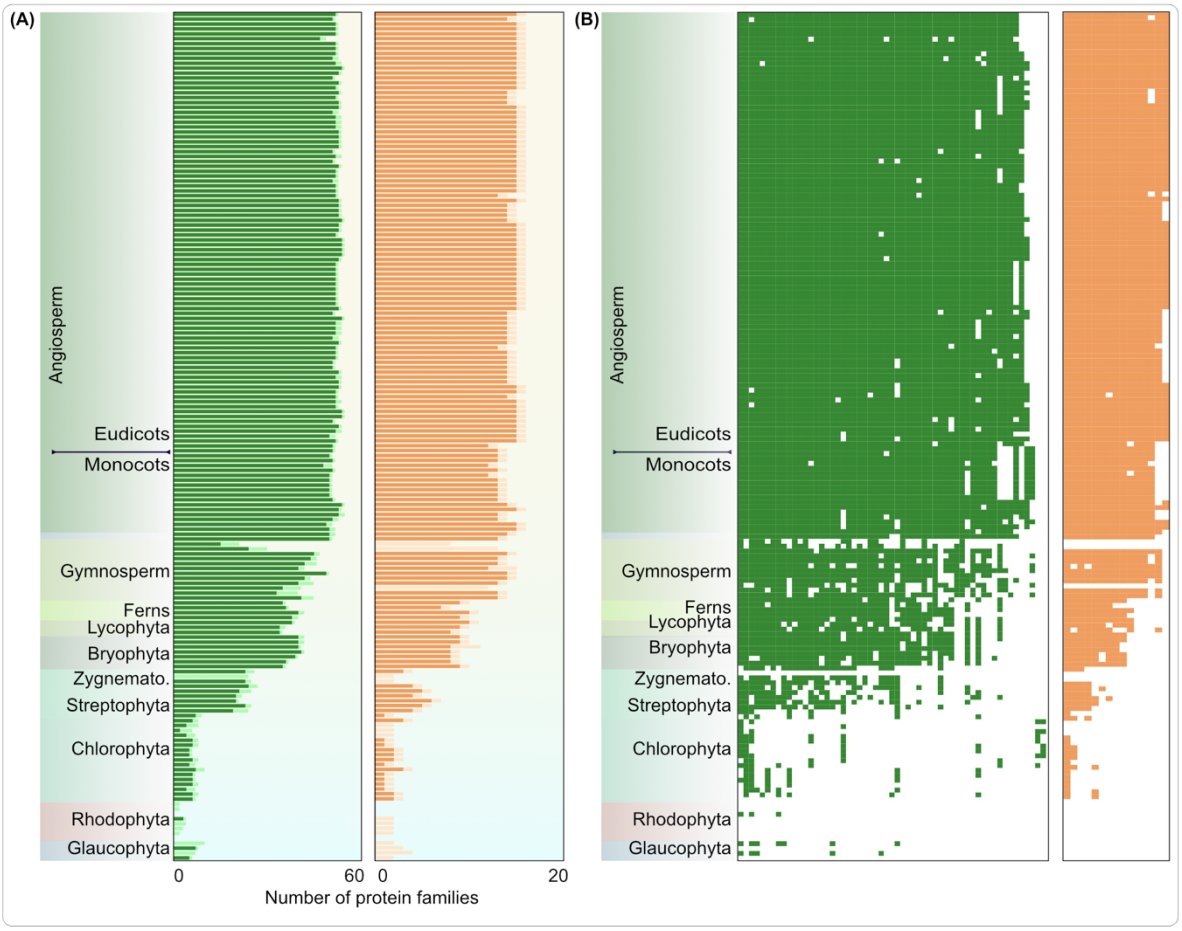

Fig. 4: Recruitment of PPR and mTERF domains in organelle proteins. (A) Number of POGs (left) and MOGs (right) where at least one protein contains a PPR/mTERF domain is shown in bars with dark shades of colors. Total number of orthogroups (regardless of presence or absence of PPR/mTERF domain in that particular species) is shown in lighter shade. It shows the presence of the orthogroups in question in algae, but that they only later obtained PPR/mTERF domains in embryophytes. (B) Each cell represents an orthogroup and a coloured cell indicates the presence of a PPR or mTERF domain in the protein family (column) of a respective species (rows).

Adaptation to the terrestrial habitat and changes in plastid biochemistry

Main terrestrial stresses include draught, high (UV)light and swift temperature changes. Cutin and suberin, two of the most abundant lipid polymers on Earth [56], evolved as one countermeasure [57]. We find that cutin and suberin evolution was enabled by the recruitment of an organelle-specific GPAT (Glycerol-3-phosphate acyltransferases) family in the embryophyte ancestor (Fig. 5), which includes GPAT1 (mitochondrial), GPAT 4,6 and 8 of the endoplasmic reticulum [58,59]. Trafficking of these lipids across organelles was made possible by a dual targeted TGD4 [60] that was recruited in the chloroplastida ancestor (Fig. 5). Acyl carrier thioesterases, responsible for the export of fatty acids from the plastid, acyl carrier protein desaturases (ACP-desaturase) and acyl-carrier proteins co-factors of fatty acid bio-synthesis were uniquely retained and expanded in the green lineage (Fig. S9). Duplication and divergence of ACP desaturases in embryo- and spermatophytes played an important role in regulating lipid composition shifts in response to temperature and drought, the regulation of seed oil content and development [61]. Likewise, acyl-carrier proteins also increased in copy number (Fig. S9) and adapted towards a light-induced expression and regulation of the seed fatty acid content [62,63]. These changes in organelle lipid synthesis and trafficking underpinned embryophyte adaptations to cope

with draught and high temperature stress (wax biosynthesis, deposition on the layer of leaves and cuticle development), as well as seed development and germination in spermatophytes (Fig. 6D).

Changes in starch metabolism mostly pertain to its regulation. ADP-glucose pyrophoshorylase (AGPase), an enzyme responsible for a rate-limiting step in starch metabolism, is uniquely retained in the green lineage and increased in copy number in streptophytes (Fig. S9). AGPases diverged to regulate starch metabolism under osmotic and light stress, as well as the differential regulation of starch synthesis and degradation [64–68]. Another key regulatory enzyme, PGI (phosphoglucose isomerase) evolved a distinct family (PGI1) in Zygnematophyceae (Fig. S9). It likely kickstarted the regulation of starch metabolism at the water-to-land interface and later assumed significant roles in embryophyte fatty acid content regulation and the yield of seeds [69]. PTST3 also emerged around the time of terrestrialization (Fig. S9), which evolved to regulate starch synthesis with significant impact on plastid development [70]. In contrast to the flow of carbon through glycolysis, GSM2 (which originated in streptophytes; Fig. S9), shunts carbon towards the pentose-phosphate pathway and protects plastids from oxidative stress in *Arabidopsis* [71].

Emergence of sophisticated antero- and retrograde communication cascades

Communication across compartments is critical for a concerted response to environmental stimuli. Plastids are key environmental sensors that interconnect cellular metabolism with physiological requirements and stress responses, and terrestrial stressors are key triggers of plastid-to-nucleus retrograde signalling [12,13,22]. We screened for the origin and diversification of EXECUTOR and SVR4, both components of retrograde signalling. We also screened for WHIRLY, a protein family that acts on RNA splicing and ribosome biogenesis, but also relocates between compartments and remains a candidate for retrograde signalling [18,72–76] . EXECUTOR, key to regulating retrograde signalling, oxygen and light stress regulation [77–79], originated in the ancestor of the Chloroplastida and so did WHIRLY (Fig. 5); the latter underwent copy number expansion in embryophytes and was likely lost in some bryophytes (Fig. S9). Divergence of these copies led to a localisation across multiple organelles and today they are crucial for maintaining functional respiration, photosynthesis and the response of mitochondria and plastids to biotic and abiotic stresses [80–82]. These emergence of the Chloroplastida was marked by the two components EXECUTOR and WHIRLY. Additional paralogs evolved, each with a specific function in the main green lineages, and they likely aided in the colonization of the terrestrial habitat by the ancestor of land plants (Fig. 6B).

SVR4, a dual targeted (plastid and nucleus) recruited around terrestrialization (Fig. 5), likely communicates required gene expression changes needed for light-induced plastid development, thylakoid stacking and thermomorphogenesis [83,84]. In combination, this facilitates light-induced photomorphogenesis, a process key for surviving on land. An increase in the complexity of retrograde signaling was a precursor for terrestrialization [12], for instance via innovations associated with the the 3′-phosphoadenosine-5′-phosphate family, which facilitated the emergence of stomatal closing in land plants [85]. The recruitment and diversification of the proteins we highlight were quintessential for responding to two major stressors that are more pronounced and more rapidly changing on land than in water: light and temperature (Fig. 6B).

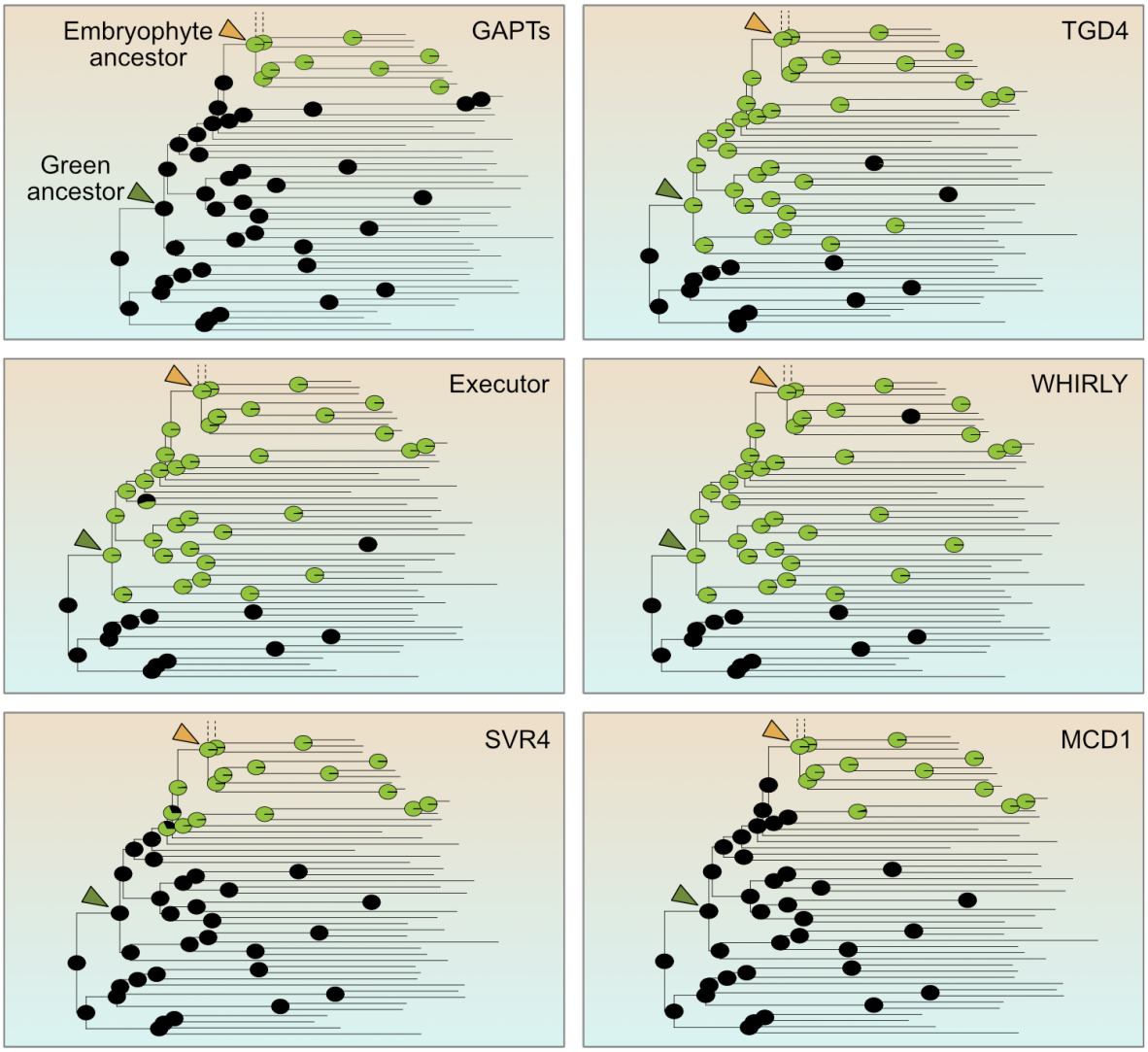

Fig. 5: Origins of key proteins involved in metabolism, communication and development. Ancestor state reconstruction (ASR) for selected lipid metabolism (GAPT and TGD4), retrograde signalling (Executor and Whirly), plastid development (SVR4) and division (MCD1) related proteins. The pie charts at each node represent the probability of presence (green) or absence (black) of a protein family in that node.

Recruitment of new proteins and changes in organelle development

The coordination of tissue and plastid development is linked to ensure an appropriate response to biotic and abiotic factors, especially in morphologically complex plants [86–88]. Polyplastidy is a trait of land plants and many macroscopic algae such as *Bryopsis* or *Chara* [89] and known molecular determinants include MinD, MinE, ARC3 and the FtsZ proteins [16,87]. Our data supports that MULTIPLE CHLOROPLAST DIVISION SITE 1 (MCD1), a core component of the plastid division machinery [90], originated in the ancestral embryophyte (Fig. 5). The cotyledon chloroplast biogenesis factor CYO1 and the transcriptionally active chromosome factor 7 (TAC7) are important components of thylakoid biogenesis and the plastid translation machinery, respectively. Both originated in the streptophyte ancestor (Fig. S9) and, in *Arabidopsis*, play key roles in chloroplast, cotyledon, thylakoid and leaf development [91–93]. Lastly, CRUMPLED LEAF (CRL), a protein residing in the outer plastid membrane, emerged during terrestrialization, too (Fig. S9), likely for regulating plastid division and securing correct plastid inheritance during embryogenesis [94,95].

Crucial for plastid biogenesis, especially in light of an expanding proteome, is the import of proteins. The membrane GTPase TOC159 is essential for chloroplast biogenesis via the selective recognition and import of the photosynthetic proteins [96] and is unique to the green lineage (Fig. S9). The membrane recruitment of this protein requires TOC75, of which a special variant evolved in the green ancestor after the duplication of OEP80 [14,97]. The copy number of TOC159 expanded from the Zygnematophyceae onwards (Fig. S9), hinting at its functional diversification. Unlike in the chlorophyte alga *Chlamydomonas*, land plant TOC159 homologues possess an N-terminal acidic domain that gets phosphorylated to alter substrate specificity [96,98]. Furthermore, TOC159, along with TOC132 and TOC120, play important roles in regulating plastid lipid synthesis and membrane fluidity and in *Arabidopsis* show tissue specific expression (The Arabidopsis Information Resource) [99–101]. Further on the course of evolution, the J-domain-containing protein TOC12 [102] was likely recruited in the ancestral embryophyte for supporting the import machinery at the intermembrane space (Fig. S9). The terrestrial habitat demands a highly efficient and fluid import of proteins, for example upon high light and other abiotic stresses [14,103]. The expansion of the TOC/TIC system in the embryophyte ancestor reflects how the organelle dealt with an ever-increasing diversity of substrates that were required to be processed.

Discussion

The settling of land by a streptophyte alga and the subsequent evolution and spreading of plants (Fig. 6A) was pivotal in the transformation of the terrestrial habitat and it laid the foundation for the concomitant evolution and diversification of animals [1,2]. Throughout the hundreds of millions of years of plant evolution, both organelles of endosymbiotic origin underwent a multitude of molecular adaptations, hereby evolving into the plastid and mitochondrion of modern plants. We identified 31,650 protein families unique to the green lineage, approximately 50% of which are unique to embryophytes. It demonstrates an expansion and divergence of protein families at the time of plant terrestrialization and in line with a recent study that identified around 10,000 duplications at the birth of embryophytes [104].

Expansion of proteins families is evident in both organellar proteomes at the origin of the green lineage itself and at the water-to-land transition. The gain of protein families at the origin of the Chloroplastida needs to be treated with caution due to the documented genetic bottleneck that characterizes rhodophyte origin [105–109] and the sparse availability of glaucophyte genome data. Some of the newly recruited protein families at the origin of the green lineage might rather be explained by a loss in rhodophytes and a retention in the chloroplastidal ancestor instead of a gain. Regardless, this has little bearing on the biological significance of a given protein family with respect to the overall increase in complexity of organelle biology – both concerning the variety as well as the number of proteins targeted to plastids and mitochondria – throughout streptophyte evolution. It affected the organelles metabolic, informational and developmental complexity, and facilitated the evolutionary successful transition from water to land more than 500 million years ago (Fig. 6).

Changes in organelle lipid biochemistry contributed to one of the key adaptations in land plants that is the cuticle. Land plant GPATs (Glycerol-3-phosphate acyltransferases; crucial to lipid synthesis for cutin and suberin) contribute to increased hydrophobicity and water retention in embryophytes [57] and their activity in embryophytes differs from that in algae [110,111]. Our analyses pinpoint the origins of organelle specific GPATs (GPAT 1,4,6, and 8) to the embryophyte ancestor, and of which deleting GPAT4 and GPAT8 distorts cuticles and increases water loss by several fold [58,59]. In parallel, lipid trafficking was mediated by the recruitment or divergence of proteins such as TGD4 and acyl carrier thioesterases, which contributed to wax biosynthesis and deposition on leaves, cuticle development,

thylakoid membrane stacking [60], seed development and germination [61]. As for starch metabolism, the archaeplastidal ancestor likely stored starch in the cytosol [112], but the red and green lineage experienced different fates from there on. Rhodophytes continued to store starch in the cytosol in the form of Floridean starch [113], while in the green lineage, particularly in complex plants, more localized control of starch synthesis and degradation was facilitated by changes in regulatory proteins (eg AGPase). Together, organelle metabolism evolved to serve key roles in the synthesis, regulation and trafficking of lipids involved in wax coating to prevent water loss in the land plant ancestor, as well as synthesis and storage of starch (Fig. 6D).

RNA processing and editing is a crucial component of information processing and overall functionality of plant organelles [49,50]. Changes in RNA metabolism are evident from the origin of the green lineage itself, where RNAse-P (tRNA maturation) was replaced by protein only RNAse P or PROPs [114,115]. Subsequent expansion of PROPs in embryophytes (Fig. S9) led to organelle-localised copies, of which some are essential for maintaining organelle morphology, function and plant viability [116]. Components associated with plastid encoded RNA polymerase (PEP associated proteins, PAPs) also show a gradual recruitment from the green ancestor to embryophyte ancestor (Fig. S8). RNA editing of C to U is not found in algae, however, and editing sites in embryophytes are unlike those of any other eukaryote, suggesting they emerged independently [50]. Of the many RNA-metabolism proteins we find that were gained during terrestrialization, known targets are transcripts involved in photosynthesis and stress tolerance-related transcripts, both key to colonising the land (Fig. 6B). For instance, THA8, PDM4, SVR7 and SOT1 associate with transcripts such as ycf2 and ycf3, and contribute to thylakoid development and biogenesis [117], the generation of photosynthetic complex proteins, grana stacking, and embryo and plastid development [117,119,120]. OTP51 and SOT5 splice transcripts related to chlorophyll synthesis, photosynthesis and thylakoid membranes (ycf3, TRNK and RPL2) [121–123], whereas DOG1 is important for high temperature response and chloroplast development [124]. This elaborate RNA processing in organelles, especially plastids, appears to serve photosynthesis (and thylakoid) related transcripts. It is feasible that by benefitting photosynthesis, organelle RNA editing continued to be positively selected for during terrestrialization and was expanded.

One evolutionary step towards efficient photosynthesis, where RNA editing also plays a key role, are grana stacks [86]. The evolutionary origin of grana remains elusive, along with the underlying developmental pathways involved in regulating its formation and maintenance [86,125,126]. Highly organized grana stacks are observed in embryophytes and some Zygnematophyceae (e.g. the *Cosmarium* genus) [127], but not chlorophytes such as *Chlamydomonas* [128]. We noticed a patchy distribution of grana morphology associated proteins such as CURT1, RIQ1 and RIQ2 (Fig. S9), with both RIQs being present in all streptophytes and some chlorophytes but excluding *Chlamydomonas*. In light of the many key adaptations in Zygnematophyceae discussed here and elsewhere [11,129], we speculate that a sophisticated stacking of grana originated in streptophytes and was beneficial for thriving on land through photosynthesis optimization, in particular with respect to photosystem repair and the separation of the photosystems and the ATP synthase [130,131].

This expansion of an organelle proteome necessitates improving the capacity to import proteins. Changes in some import receptors within the green lineage and in targeting sequences at its origins are known, with phosphorylation likely emerging as a key regulator for sorting the newly expanded proteome differentially to plastid and mitochondria (Fig. 6C) [14,42]. Despite such adaptations, protein sorting is never perfect and some mistargeting might be positively selected for. A regulated distribution of newly recruited proteins (e.g. WHIRLY, TGD4, mTERF6; Fig. 6B) to multiple organelles (with distinct organellar functions) hints at adaptive values of this apparent mis-sorting. How many of newly recruited proteins get ‘mis-sorted’ owing to biological adaptability versus stochasticity remains to be

386 explored together with obtaining a more comprehensive picture of (regulatory) mechanisms associated
387 with sorting in general.

388 Embryophyte cells target proteins not to a single plastid, but many simultaneously. The presence of
389 multiple plastids per cell, polyplastidy, in the green lineage, evolved in an embryophyte ancestor, maybe
390 the common ancestor of embryo– and charophytes, likely through changes in plastid fission and a
391 decoupling of the latter from the cell cycle [15,16]. We find that MCD1, a core regulator of the plastid
392 division proteins FtsZ2 and ARC3, emerged in the embryophyte ancestor, which corroborates the idea
393 of a mono- to polyplastidy switch during the land transition of green algae [16,89,132,133]. A change
394 in the copy number of plastids also requires a mechanism that maintains a functional organelle to cell
395 volume ratio and resource allocation (Fig. 6C). The REDUCED CHLOROPLAST COVERAGE (REC)
396 protein is involved in such a mechanism in *Arabidopsis* [134] and the phylogenetically related protein
397 FRIENDLY regulates the distribution of mitochondria, also in plants and non-photosynthetic organisms
398 [135,136]. REC and FRIENDLY share almost all of their domains. How they exactly function and
399 differentiate between the two organelles remains elusive. From what we can tell, FRIENDLY emerged
400 during eukaryogenesis and the origin of mitochondria. REC we can trace back to the streptophyte
401 ancestor (Fig. S9) and after a likely duplication event of FRIENDLY. We speculate that the origin of
402 REC helped to cement polyplastidy, which itself supports larger body plans and the diversification of
403 different plastid types [15]. Lastly, an increase in organelle copy number also requires an overall
404 increase in the capacity to synthesize proteins. The largest fraction of organelle proteins operate in
405 tRNA, amino acid and ribosomal biosynthesis and undergird the biosynthetic capacity of organelles, an
406 adaptation strategy akin to their bacterial ancestor [137,138].

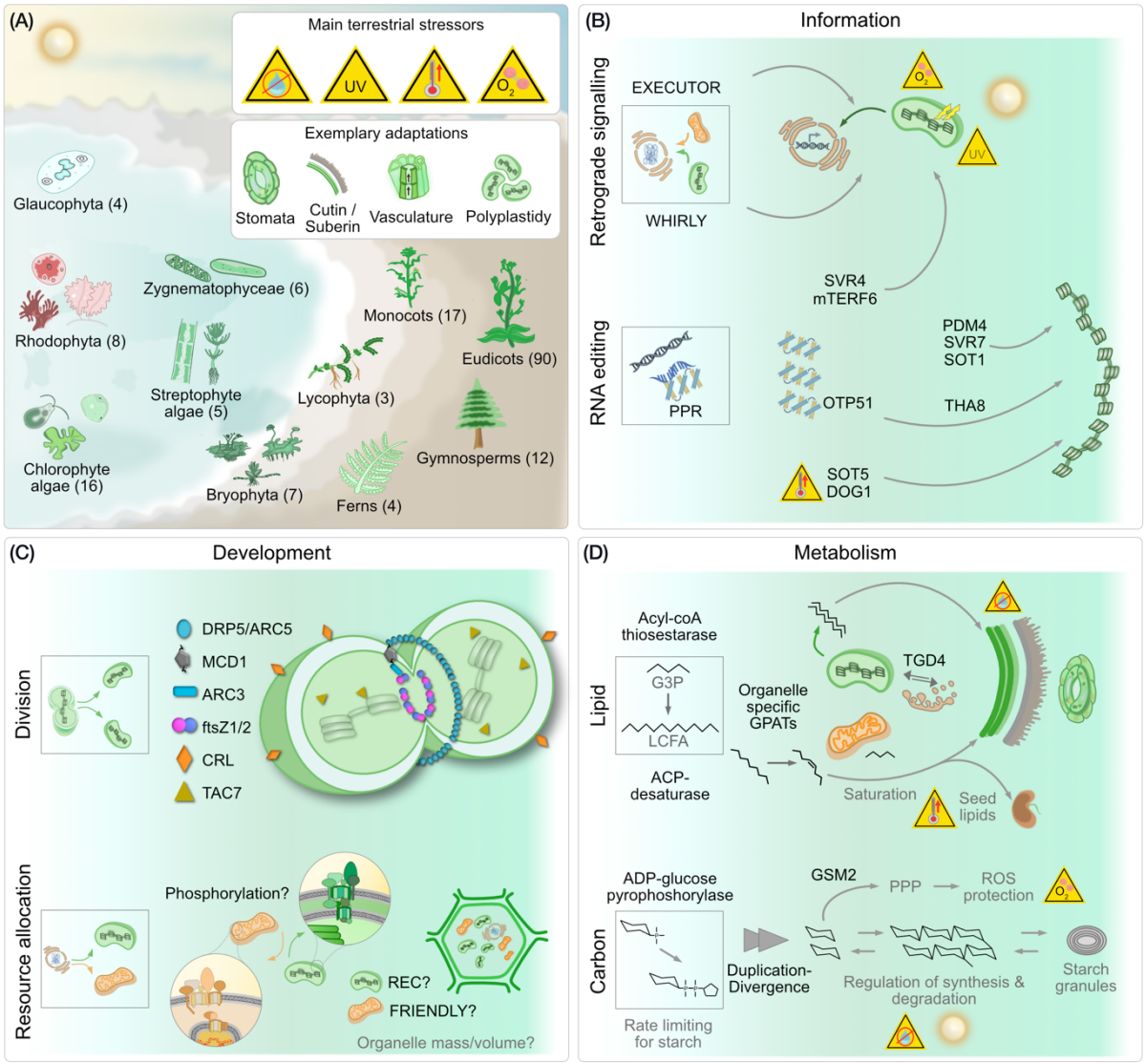

Fig. 6: The global greening and endosymbiotic organelles. (A) After the endosymbiotic origin of the plastid, three aboriginal lineages emerged that form the Archaeplastida: the glaucophytes, rhodophytes and chlorophytes. From the latter, streptophyte algae evolved, including the zygnematophyceae, that represent the algal sister clade to land plants (embryophytes). Abiotic stresses encountered during terrestrialization (water scarcity, high UV, swiftly altering temperatures and higher levels of O_2) selected for adaptive features such as stomata and a cutin layer. The numbers in parenthesis indicate the number of genomes from each major group that was screened. Recruitment of new organelle proteins improved three key aspects of organelle biology in light of terrestrialization: (B) information processing, (C) development and (D) metabolism. Details for each tile are discussed in the main text.

The accommodation of the early mitochondrial endosymbiont is associated with the origin of the endomembrane system and necessitated the emergence of eukaryotic traits including mito- and autophagy [139–141]. Our analyses show that the integration of a subsequent endosymbiont, the plastid, coincided with the emergence of proteins that work for the endomembrane system. Salient are changes in the ubiquitin system during terrestrialization, when polyplastidy in the green lineage also emerged (Table S2G). Ubiquitination is key to proteosome-mediated degradation and is performed chiefly by the E3 ubiquitin ligase family, which are important in land plants also for photomorphogenesis[142]. RING (Really interesting new gene) E3 ligases contribute to growth, development and stress response

via also mediating protein-protein interactions [143–146]. We trace a number of RING finger and related proteins to terrestrialization (Fig. S9) that include, but are not limited to, *DAL1* and *DAL2* (for *Drosophila* DIAP1 like 1 and 2), KEG (Keep on going), and NIP1 and NIP2. *DAL1* and *DAL2* play a key role in regulation of programmed cell death [147], peroxisome and chloroplast biogenesis [148–150]. KEG contributes to stress mitigation [151,152], while NIP1 and NIP2 play a role in plastid development by docking plastid RNA polymerase to the thylakoid membrane [153]. The regulated degradation of plastids and other changes in the endomembrane system are a prerequisite for housing multiple plastids per cell and we find many more recruitments broadly affiliated with the endomembrane system, with poorly characterised functions until now. Exploring the functions of these proteins will add valuable insights into the cell biological changes that endosymbiosis stipulates.

In closing, although experimentally reported plant plastid and mitochondrial proteomes are scarce, we were able to generate a first comprehensive molecular atlas of the changes of plastid and mitochondrial protein families in the evolution of the green lineage. Ancestral state reconstruction (ASR) allows to map the organelle transformations that facilitated the major transitions such as terrestrialization and which will improve with every new proteome that is added. By inferring plastid and mitochondrial proteomes for 173 species, we set testable expectations for new proteomes to come and provide a solid database, where origins and across species orthologues of any known (organelle) protein can be searched (Table S2C-D). Additional proteomes, once available, will likely solidify the general pattern observed and uncover more lineage-specific curiosities. We identify numerous mitochondrial protein recruitments, whose physiological roles and adaptive values help to better understand plant mitochondrial biology. For plastid proteins, we infer their functions and physiological importance based on the extensively studied *Arabidopsis* system. Utilizing an advanced orthology search technique [40], we postulate that orthologues of *Arabidopsis* are likely to exhibit similar functions in other species. Our methodologically robust approach maps various changes in evolution, associated in particular with terrestrialization, that can now be experimentally explored across selected models and with a focus on less-well studied streptophyte algal and bryophyte species [154,155].

Conclusions

Endosymbiotic organelles have a distinct place in the evolutionary tapestry of life. Through the combination of organelle proteome data and phylogeny, we trace the evolution of mitochondria and plastids over a span of a billion years of plant evolution by inferring their proteomes for over a hundred Archaeplastida species. Our comprehensive molecular atlas identifies main changes in their metabolism, communication, information processing and biogenesis. Key adaptations in plant organelles fostered the emergence of wax and cutin (see organelle lipid synthesis and transport), improved the photosynthetic yield (see organelle RNA metabolism and highly structured grana stacks) and the response to abiotic stressors (see inter-organelle communication), and mediated the transition from mono- to polyplastidy (see division and volume control). By connecting the molecular adaptations of mitochondria and plastids to macroevolutionary trends, we show how important changes in organelles of endosymbiotic origin were for the speciation that gave rise to the Chloroplastida and later the origin of land plants from a charophyte algal ancestor.

Material and Methods

Curation of green orthogroups (GOGs). Input protein sequences from 686 proteomes (from KEGG [156]and Phytozome [29], Table S1A) were clustered using Orthofinder version 2.5.4 [40], after all vs all blasts were conducted (E-value cutoff 10e-10) using diamond blast version2.011 [38]. From orthogroups (OGs) recovered, OGs with at least 3 Chloroplastida species green species and less than 3 species other than Chloroplastida were annotated as green orthogroup (GOGs). Schematic in Fig. S1A. (Inhouse python script used for this, and other data processing, are available on Github as repository 'Molecular-Atlas-of-plant-organelle-evolution'.)

Curation of plastid and mitochondria orthogroups (POGs and MOGs). 5,452,977 proteins from 204 eukaryotes (Table S2A) were clustered using Orthofinder as described above. Orthogroups that contained at least one experimentally verified organelle protein from any one of the four experimentally verified organelle proteome of *C. reinhardtii* [157], *P. patens* [158] , *Z. mays* [159], *A. thaliana* [159], were annotated as organelle (plastid and mitochondria) orthogroups. Schematic in Fig. S1B.

Functional annotation of orthogroups. The source of >90% species was Kyoto Encyclopedia of Genes and Genomes (KEGG), which included KEGG orthology identification (KOID) for protein sequences. For all proteins within each GOG, KOIDs were retrieved and the most frequent KOID (i.e. majority rule) was annotated to each GOG (Fig. S1C). From the assigned KOIDs, their KO BRITE functional category was assigned to each GOG. KOIDs for POGs and MOGs were retrieved the same way. For each KOID, the pathway names and BRITE categories at various level of resolutions were used for assigning functional categories manually to each OG. Manual assignment was necessary since BRITE names included a large fraction of categories such as 'enzymes' and 'exosomes'. These were either not very informative or were misleading as many of 'exosome' annotated proteins took part in protein synthesis or folding. Lastly, for OGs or proteins discussed with respect to their physiological relevance, the functions were retrieved from the literature (cited in the text).

Inference of ancestral states. A phylogeny of Archaeplastidal species was inferred based on all genes conserved in all species, using 'Species tree inference from all genes (STAG)' method [160], as a part of orthofinder analysis. STAG infers a species tree by taking greedy consensus of gene trees from each protein family (including that of multigene families). This phylogeny was rooted using minimal ancestral deviation [161] which places Rhodophyta as the sister to all others. Independently, the same unrooted phylogeny was manually rooted using FigTree (v1.4.4) [162] such that Glaucophyta were at the base. Ancestor state of presence and absence of organelle protein families across nodes, were inferred using Phytool [163] package 0.7.80. Based on character state at the tips of the tree, Phytool inferred Bayesian posterior probabilities under a single rate model [164,165] of the character state across nodes of the tree. All OGs that were present in major ancestors of plant groups with probability higher than 0.75 and absent in the preceding ancestor, were considered as newly recruited in that lineage. OGs or proteins discussed with respect to its physiological role in a given clade, their absence outside the group was verified in our copy number database as well as on homologue database available on TAIR.

Searching for potential RNA metabolism POGs and MOGs. Hidden Markov models (HMM) of PPR and mTERF domains were downloaded from pFAM [166] with the IDs: PF01535, PF12854, PF13041, PF13812, PF02536. Each of these HMMS was used as a query to search against the full sequences of all proteins within each POG and MOG. If a given OG had more than 60% of individual proteins containing PPR or mTERF, the OG was annotated as RNA metabolism OG. Origin of such OGs were traced using ASR as described above.

Author contributions

PKR: Conceptualization; Experimental design; Methodology; Investigation, Data curation; Formal analysis; Visualization; Writing - original draft, review and editing. AIM: Methodology, Investigation, Formal analysis; Writing - original draft, review and editing SBG: Conceptualization; Project administration; Funding acquisition; Resources; Supervision; Visualization; Writing - original draft, review and editing.

Funding

We thank the Deutsche Forschungsgemeinschaft (SFB 1208-2672 05415 and SPP2237–440043394) and the Moore and Simons Initiative grant (9743) for financial support.

Acknowledgments

We acknowledge support from the high-performance computing cluster (HILBERT) at the Heinrich Heine University Düsseldorf and thank Michael Knopp (HHU Düsseldorf) for his help. We thank Alice Barkan (University of Oregon) for her useful feedback on our bioXRiv preprint and three anonymous reviewers for their time evaluating and improving our manuscript.

References

1. Schreiber M, Rensing SA, Gould SB. The greening ashore. Trends in Plant Science. 2022. doi:10.1016/j.tplants.2022.05.005

2. Bowles AMC, Williamson CJ, Williams TA, Lenton TM, Donoghue PCJ. The origin and early evolution of plants. Trends in Plant Science. 2023;28: 312–329. doi:10.1016/j.tplants.2022.09.009

3. Li L, Wang S, Wang H, Sahu SK, Marin B, Li H, et al. The genome of Prasinoderma coloniale unveils the existence of a third phylum within green plants. Nature Ecology & Evolution. 2020;4: 1220–1231. doi:10.1038/s41559-020-1221-7

4. Leebens-Mack JH, Barker MS, Carpenter EJ, Deyholos MK, Gitzendanner MA, Graham SW, et al. One thousand plant transcriptomes and the phylogenomics of green plants. Nature. 2019;574: 679–685. doi:10.1038/s41586-019-1693-2

5. Li X, Hou Z, Xu C, Shi X, Yang L, Lewis LA, et al. Large Phylogenomic Data sets Reveal Deep Relationships and Trait Evolution in Chlorophyte Green Algae. Genome Biology and Evolution. 2021;13: evab101–evab101. doi:10.1093/gbe/evab101

6. Puginier C, Keller J, Delaux P-M. Plant–microbe interactions that have impacted plant terrestrializations. Plant Physiology. 2022;190: 72–84. doi:10.1093/plphys/kiac258

7. de Vries S, Fürst-Jansen JMR, Irisarri I, Dhabalia Ashok A, Ischebeck T, Feussner K, et al. The evolution of the phenylpropanoid pathway entailed pronounced radiations and divergences of enzyme families. Plant J. 2021;107: 975–1002. doi:10.1111/tpj.15387

8. Bowman JL. Stomata: Active Portals for Flourishing on Land. Current Biology. 2011;21: R540–R541. doi:https://doi.org/10.1016/j.cub.2011.06.021

9. Kong L, Liu Y, Zhi P, Wang X, Xu B, Gong Z, et al. Origins and Evolution of Cuticle Biosynthetic Machinery in Land Plants. Plant Physiol. 2020;184: 1998–2010. doi:10.1104/pp.20.00913

10. Davies KM, Jibran R, Zhou Y, Albert NW, Brummell DA, Jordan BR, et al. The Evolution of Flavonoid Biosynthesis: A Bryophyte Perspective. Frontiers in Plant Science. 2020;11. doi:10.3389/fpls.2020.00007

11. Cheng S, Xian W, Fu Y, Marin B, Keller J, Wu T, et al. Genomes of Subaerial Zygnematophyceae Provide Insights into Land Plant Evolution. Cell. 2019. doi:10.1016/j.cell.2019.10.019

12. de Vries J, Stanton A, Archibald JM, Gould SB. Streptophyte Terrestrialization in Light of Plastid Evolution. Trends in Plant Science. 2016. doi:10.1016/j.tplants.2016.01.021

13. de Vries J, Archibald JM. Plant evolution: landmarks on the path to terrestrial life. New Phytologist. 2018. doi:10.1111/nph.14975

14. Knopp M, Garg SG, Handrich M, Gould SB. Major Changes in Plastid Protein Import and the Origin of the Chloroplastida. iScience. 2020. doi:10.1016/j.isci.2020.100896

15. de Vries J, Gould SB. The monoplastidic bottleneck in algae and plant evolution. Journal of Cell Science. 2018. doi:10.1242/jcs.203414

16. MacLeod AI, Raval PK, Stockhorst S, Knopp MR, Frangedakis E, Gould SB. Loss of Plastid Developmental Genes Coincides With a Reversion to Monoplastidy in Hornworts. Frontiers in Plant Science. 2022;13. Available: https://www.frontiersin.org/article/10.3389/fpls.2022.863076

17. Shimizu T, Kacprzak SM, Mochizuki N, Nagatani A, Watanabe S, Shimada T, et al. The retrograde signaling protein GUN1 regulates tetrapyrrole biosynthesis. Proceedings of the National Academy of Sciences. 2019;116: 24900–24906. doi:10.1073/pnas.1911251116

18. Foyer CH, Karpinska B, Krupinska K. The functions of WHIRLY1 and REDOX-RESPONSIVE TRANSCRIPTION FACTOR 1 in cross tolerance responses in plants: a hypothesis. Philosophical Transactions of the Royal Society B: Biological Sciences. 2014;369: 20130226. doi:10.1098/rstb.2013.0226

19. Bar-On YM, Phillips R, Milo R. The biomass distribution on Earth. Proceedings of the National Academy of Sciences. 2018;115: 6506–6511. doi:10.1073/pnas.1711842115

20. Pérez-Sancho J, Vanneste S, Lee E, McFarlane HE, Esteban del Valle A, Valpuesta V, et al. The Arabidopsis Synaptotagmin1 Is Enriched in Endoplasmic Reticulum-Plasma Membrane Contact Sites and Confers Cellular Resistance to Mechanical Stresses. Plant Physiology. 2015;168: 132–143. doi:10.1104/pp.15.00260

21. Gao H, Metz J, Teanby NA, Ward AD, Botchway SW, Coles B, et al. In Vivo Quantification of Peroxisome Tethering to Chloroplasts in Tobacco Epidermal Cells Using Optical Tweezers. Plant Physiology. 2016;170: 263–272. doi:10.1104/pp.15.01529

22. Wang Y, Selinski J, Mao C, Zhu Y, Berkowitz O, Whelan J. Linking mitochondrial and chloroplast retrograde signalling in plants. Philosophical Transactions of the Royal Society B: Biological Sciences. 2020;375: 20190410–20190410. doi:10.1098/rstb.2019.0410

595 23. Møller IM, Rasmusson AG, Van Aken O. Plant mitochondria – past, present and future. The
596 Plant Journal. 2021;108: 912–959. doi:10.1111/tpj.15495

597 24. Kanai R, Edwards GE. The Biochemistry of C4 Photosynthesis. C4 Plant Biology. 1999.
598 doi:10.1016/b978-012614440-6/50004-5

599 25. Peers G, Niyogi KK. Pond scum genomics: the genomes of Chlamydomonas and
600 Ostreococcus. Plant Cell. 2008;20: 502–507. doi:10.1105/tpc.107.056556

601 26. Christin PA, Osborne CP. The evolutionary ecology of C4 plants. New Phytologist. 2014.
602 doi:10.1111/nph.13033

603 27. Khan K, Van Aken O. The colonization of land was a likely driving force for the evolution of
604 mitochondrial retrograde signalling in plants. Jones M, editor. Journal of Experimental Botany.
605 2022;73: 7182–7197. doi:10.1093/jxb/erac351

606 28. Heinnickel ML, Grossman AR. The GreenCut: re-evaluation of physiological role of
607 previously studied proteins and potential novel protein functions. Photosynthesis Research.
608 2013;116: 427–436. doi:10.1007/s11120-013-9882-6

609 29. Goodstein DM, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, et al. Phytozome: a
610 comparative platform for green plant genomics. Nucleic Acids Research. 2012;40: D1178–
611 D1186. doi:10.1093/nar/gkr944

612 30. Hori K, Maruyama F, Fujisawa T, Togashi T, Yamamoto N, Seo M, et al. Klebsormidium
613 flaccidum genome reveals primary factors for plant terrestrial adaptation. Nature
614 Communications. 2014. doi:10.1038/ncomms4978

615 31. O'Leary NA, Wright MW, Brister JR, Ciufo S, Haddad D, McVeigh R, et al. Reference
616 sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional
617 annotation. Nucleic Acids Research. 2016;44: D733–D745. doi:10.1093/nar/gkv1189

618 32. Bowman JL, Kohchi T, Yamato KT, Jenkins J, Shu S, Ishizaki K, et al. Insights into Land
619 Plant Evolution Garnered from the Marchantia polymorpha Genome. Cell. 2017.
620 doi:10.1016/j.cell.2017.09.030

621 33. Lang D, Ullrich KK, Murat F, Fuchs J, Jenkins J, Haas FB, et al. The Physcomitrella patens
622 chromosome-scale assembly reveals moss genome structure and evolution. The Plant Journal.
623 2018;93: 515–533. doi:10.1111/tpj.13801

624 34. Nishiyama T, Sakayama H, de Vries J, Buschmann H, Saint-Marcoux D, Ullrich KK, et al.
625 The Chara Genome: Secondary Complexity and Implications for Plant Terrestrialization. Cell.
626 2018. doi:10.1016/j.cell.2018.06.033

627 35. Li F-W, Nishiyama T, Waller M, Frangedakis E, Keller J, Li Z, et al. Anthoceros genomes
628 illuminate the origin of land plants and the unique biology of hornworts. Nature Plants. 2020;6:
629 259–272. doi:10.1038/s41477-020-0618-2

630 36. Wang S, Li L, Li H, Sahu SK, Wang H, Xu Y, et al. Genomes of early-diverging streptophyte
631 algae shed light on plant terrestrialization. Nature Plants. 2020. doi:10.1038/s41477-019-0560-
632 3

633 37. Zhang J, Fu XX, Li RQ, Zhao X, Liu Y, Li MH, et al. The hornwort genome and early land
634 plant evolution. Nature Plants. 2020. doi:10.1038/s41477-019-0588-4

635 38. Buchfink B, Xie C, Huson DH. Fast and sensitive protein alignment using DIAMOND. Nature
636 Methods. 2015;12: 59–60. doi:10.1038/nmeth.3176

637 39. Emms DM, Kelly S. OrthoFinder: solving fundamental biases in whole genome comparisons
638 dramatically improves orthogroup inference accuracy. Genome biology. 2015;16: 157–157.
639 doi:10.1186/s13059-015-0721-2

640 40. Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics.
641 Genome Biology. 2019;20: 238–238. doi:10.1186/s13059-019-1832-y

642 41. Emms DM, Kelly S. SHOOT: phylogenetic gene search and ortholog inference. Genome
643 Biology. 2022;23: 85–85. doi:10.1186/s13059-022-02652-8

644 42. Garg SG, Gould SB. The Role of Charge in Protein Targeting Evolution. Trends in Cell
645 Biology. 2016;26: 894–905. doi:10.1016/j.tcb.2016.07.001

646 43. Janouškovec J, Liu S-L, Martone PT, Carré W, Leblanc C, Collén J, et al. Evolution of Red
647 Algal Plastid Genomes: Ancient Architectures, Introns, Horizontal Gene Transfer, and
648 Taxonomic Utility of Plastid Markers. PLOS ONE. 2013;8: e59001.
649 doi:10.1371/journal.pone.0059001

650 44. Strassert JFH, Irisarri I, Williams TA, Burki F. A molecular timescale for eukaryote evolution
651 with implications for the origin of red algal-derived plastids. Nature Communications.
652 2021;12: 1879. doi:10.1038/s41467-021-22044-z

653 45. Price DC, Goodenough UW, Roth R, Lee J-H, Kariyawasam T, Mutwil M, et al. Analysis of
654 an improved Cyanophora paradoxa genome assembly. DNA Research. 2019;26: 287–299.
655 doi:10.1093/dnares/dsz009

656 46. Wang S, Liang H, Xu Y, Li L, Wang H, Sahu DN, et al. Genome-wide analyses across
657 Viridiplantae reveal the origin and diversification of small RNA pathway-related genes.
658 Communications Biology. 2021;4: 412. doi:10.1038/s42003-021-01933-5

659 47. Keeling PJ. The number, speed, and impact of plastid endosymbioses in eukaryotic evolution.
660 Annual Review of Plant Biology. 2013. doi:10.1146/annurev-arplant-050312-120144

661 48. Ku C, Nelson-Sathi S, Roettger M, Sousa FL, Lockhart PJ, Bryant D, et al. Endosymbiotic
662 origin and differential loss of eukaryotic genes. Nature. 2015;524: 427–432.
663 doi:10.1038/nature14963

664 49. Hao W, Liu G, Wang W, Shen W, Zhao Y, Sun J, et al. RNA Editing and Its Roles in Plant
665 Organelles. Frontiers in Genetics. 2021;12. Available:
666 https://www.frontiersin.org/articles/10.3389/fgene.2021.757109

667 50. Small ID, Schallenberg-Rüdinger M, Takenaka M, Mireau H, Ostersetzer-Biran O. Plant
668 organellar RNA editing: what 30 years of research has revealed. Plant J. 2020;101: 1040–1056.
669 doi:10.1111/tpj.14578

670 51. Barkan A. Expression of Plastid Genes: Organelle-Specific Elaborations on a Prokaryotic
671 Scaffold. Plant Physiology. 2011;155: 1520–1532. doi:10.1104/pp.110.171231

672 52. Barkan A, Small I. Pentatricopeptide Repeat Proteins in Plants. Annu Rev Plant Biol. 2014;65:
673 415–442. doi:10.1146/annurev-arplant-050213-040159

674 53. Wobbe L. The Molecular Function of Plant mTERFs as Key Regulators of Organellar Gene
675 Expression. Plant and Cell Physiology. 2021;61: 2004–2017. doi:10.1093/pcp/pcaa132

676 54. Robles P, Quesada V. Research Progress in the Molecular Functions of Plant mTERF Proteins.
677 Cells. 2021;10. doi:10.3390/cells10020205

678 55. Robles P, Núñez-Delegido E, Ferrández-Ayela A, Sarmiento-Mañús R, Micol JL, Quesada V.
679 Arabidopsis mTERF6 is required for leaf patterning. Plant Science. 2018;266: 117–129.
680 doi:10.1016/j.plantsci.2017.11.003

681 56. Wu L, Zhou Z-Y, Zhang C-G, Chai J, Zhou Q, Wang L, et al. Functional Roles of Three Cutin
682 Biosynthetic Acyltransferases in Cytokinin Responses and Skotomorphogenesis. PLOS ONE.
683 2015;10: e0121943.

684 57. Kong L, Liu Y, Zhi P, Wang X, Xu B, Gong Z, et al. Origins and evolution of cuticle
685 biosynthetic machinery in land plants. Plant Physiology. 2020;184: 1998–2010.
686 doi:10.1104/pp.20.00913

687 58. Chen X, Truksa M, Snyder CL, El-Mezawy A, Shah S, Weselake RJ. Three Homologous
688 Genes Encoding sn-Glycerol-3-Phosphate Acyltransferase 4 Exhibit Different Expression
689 Patterns and Functional Divergence in Brassica napus. Plant Physiology. 2011;155: 851–865.
690 doi:10.1104/pp.110.169482

691 59. Fernández-Santos R, Izquierdo Y, López A, Muñiz L, Martínez M, Cascón T, et al. Protein
692 Profiles of Lipid Droplets during the Hypersensitive Defense Response of Arabidopsis against
693 Pseudomonas Infection. Plant and Cell Physiology. 2020;61: 1144–1157.
694 doi:10.1093/pcp/pcaa041

695 60. Xu C, Fan J, Cornish AJ, Benning C. Lipid Trafficking between the Endoplasmic Reticulum
696 and the Plastid in Arabidopsis Requires the Extraplastidic TGD4 Protein. The Plant Cell.
697 2008;20: 2190–2204. doi:10.1105/tpc.108.061176

698 61. Yonghua Li-Beisson, Basil Shorrosh, Fred Beisson, Mats X. Andersson, Vincent Arondel,
699 Philip D. Bates, et al. Acyl-Lipid Metabolism. The Arabidopsis Book. 2013;2013.
700 doi:10.1199/tab.0161

701 62. Huang J, Xue C, Wang H, Wang L, Schmidt W, Shen R, et al. Genes of ACYL CARRIER
702 PROTEIN Family Show Different Expression Profiles and Overexpression of ACYL
703 CARRIER PROTEIN 5 Modulates Fatty Acid Composition and Enhances Salt Stress
704 Tolerance in Arabidopsis. Front Plant Sci. 2017;8: 987. doi:10.3389/fpls.2017.00987

705 63. Bonaventure G, Ohlrogge JB. Differential regulation of mRNA levels of acyl carrier protein
706 isoforms in Arabidopsis. Plant Physiol. 2002;128: 223–235.

707 64. Figueroa CM, Asencion Diez MD, Ballicora MA, Iglesias AA. Structure, function, and
708 evolution of plant ADP-glucose pyrophosphorylase. Plant Molecular Biology. 2022;108: 307–
709 323. doi:10.1007/s11103-021-01235-8

710 65. Liu K, Zou W, Gao X, Wang X, Yu Q, Ge L. Young seedlings adapt to stress by retaining
711 starch and retarding growth through ABA-Dependent and -independent pathways in
712 Arabidopsis. Biochemical and Biophysical Research Communications. 2019;515: 699–705.
713 doi:https://doi.org/10.1016/j.bbrc.2019.06.023

714 66. Crevillén P, Ventriglia T, Pinto F, Orea A, Mérida Á, Romero JM. Differential Pattern of
715 Expression and Sugar Regulation of Arabidopsis thaliana ADP-glucose Pyrophosphorylase-

716 encoding Genes*. Journal of Biological Chemistry. 2005;280: 8143–8149.
717 doi:https://doi.org/10.1074/jbc.M411713200

718 67. Eliyahu E, Rog I, Inbal D, Danon A. ACHT4-driven oxidation of APS1 attenuates starch
719 synthesis under low light intensity in *Arabidopsis* plants. Proceedings of the National Academy
720 of Sciences. 2015;112: 12876–12881. doi:10.1073/pnas.1515513112

721 68. Pourtau N, Jennings R, Pelzer E, Pallas J, Wingler A. Effect of sugar-induced senescence on
722 gene expression and implications for the regulation of senescence in Arabidopsis. Planta.
723 2006;224: 556–568. doi:10.1007/s00425-006-0243-y

724 69. Bahaji A, Almagro G, Ezquer I, Gámez-Arcas S, Sánchez-López ÁM, Muñoz FJ, et al.
725 Plastidial Phosphoglucose Isomerase Is an Important Determinant of Seed Yield through Its
726 Involvement in Gibberellin-Mediated Reproductive Development and Storage Reserve
727 Biosynthesis in Arabidopsis. The Plant Cell. 2018;30: 2082–2098. doi:10.1105/tpc.18.00312

728 70. Seung D, Boudet J, Monroe J, Schreier TB, David LC, Abt M, et al. Homologs of PROTEIN
729 TARGETING TO STARCH Control Starch Granule Initiation in Arabidopsis Leaves. The
730 Plant Cell. 2017;29: 1657–1677. doi:10.1105/tpc.17.00222

731 71. Zheng M, Zhu C, Yang T, Qian J, Hsu Y-F. GSM2, a transaldolase, contributes to reactive
732 oxygen species homeostasis in Arabidopsis. Plant Mol Biol. 2020;104: 39–53.
733 doi:10.1007/s11103-020-01022-x

734 72. Melonek J, Mulisch M, Schmitz-Linneweber C, Grabowski E, Hensel G, Krupinska K.
735 Whirly1 in chloroplasts associates with intron containing RNAs and rarely co-localizes with
736 nucleoids. Planta. 2010;232: 471–481. doi:10.1007/s00425-010-1183-0

737 73. Lepage E, Zampini E, Brisson N. Plastid Genome Instability Leads to Reactive Oxygen
738 Species Production and Plastid-to-Nucleus Retrograde Signaling in Arabidopsis. PLANT
739 PHYSIOLOGY. 2013;163: 867–881. doi:10.1104/pp.113.223560

740 74. Desveaux D, Subramaniam R, Després C, Mess J-N, Lévesque C, Fobert PR, et al. A “Whirly”
741 Transcription Factor Is Required for Salicylic Acid-Dependent Disease Resistance in
742 Arabidopsis. Developmental Cell. 2004;6: 229–240. doi:10.1016/S1534-5807(04)00028-0

743 75. Comadira G, Rasool B, Kaprinska B, García BM, Morris J, Verrall SR, et al. WHIRLY1
744 Functions in the Control of Responses to Nitrogen Deficiency But Not Aphid Infestation in
745 Barley. Plant Physiol. 2015;168: 1140–1151. doi:10.1104/pp.15.00580

746 76. Ren Y, Li Y, Jiang Y, Wu B, Miao Y. Phosphorylation of WHIRLY1 by CIPK14 Shifts Its
747 Localization and Dual Functions in Arabidopsis. Molecular Plant. 2017;10: 749–763.
748 doi:10.1016/j.molp.2017.03.011

749 77. Lee KP, Kim C, Landgraf F, Apel K. EXECUTER1- and EXECUTER2-dependent transfer of
750 stress-related signals from the plastid to the nucleus of Arabidopsis thaliana. Proceedings of
751 the National Academy of Sciences. 2007;104: 10270–10275. doi:10.1073/pnas.0702061104

752 78. Kim C, Meskauskiene R, Zhang S, Lee KP, Lakshmanan Ashok M, Blajecka K, et al.
753 Chloroplasts of Arabidopsis Are the Source and a Primary Target of a Plant-Specific
754 Programmed Cell Death Signaling Pathway. The Plant Cell. 2012;24: 3026–3039.
755 doi:10.1105/tpc.112.100479

756 79. Uberegui E, Hall M, Lorenzo Ó, Schröder WP, Balsera M. An Arabidopsis soluble chloroplast
757 proteomic analysis reveals the participation of the Executer pathway in response to increased

light conditions. Journal of Experimental Botany. 2015;66: 2067–2077. doi:10.1093/jxb/erv018

80. Maréchal A, Parent J-S, Véronneau-Lafortune F, Joyeux A, Lang BF, Brisson N. Whirly proteins maintain plastid genome stability in Arabidopsis. Proceedings of the National Academy of Sciences. 2009;106: 14693–14698. doi:10.1073/pnas.0901710106

81. Krupinska K, Desel C, Frank S, Hensel G. WHIRLIES Are Multifunctional DNA-Binding Proteins With Impact on Plant Development and Stress Resistance. Frontiers in Plant Science. 2022;13. Available: https://www.frontiersin.org/articles/10.3389/fpls.2022.880423

82. Taylor RE, West CE, Foyer CH. WHIRLY protein functions in plants. Food and Energy Security. 2023;12: e379. doi:10.1002/fes3.379

83. Yu F, Park S-S, Liu X, Foudree A, Fu A, Powikrowska M, et al. SUPPRESSOR OF VARIEGATION4, a New var2 Suppressor Locus, Encodes a Pioneer Protein that Is Required for Chloroplast Biogenesis. Molecular Plant. 2011;4: 229–240. doi:10.1093/mp/ssq074

84. Powikrowska M, Khrouchtchova A, Martens HJ, Zygadlo-Nielsen A, Melonek J, Schulz A, et al. SVR4 (suppressor of variegation 4) and SVR4-like: two proteins with a role in proper organization of the chloroplast genetic machinery. Physiologia Plantarum. 2014;150: 477–492. doi:10.1111/ppl.12108

85. Wang S-W, Li Y, Zhang X-L, Yang H-Q, Han X-F, Liu Z-H, et al. Lacking chloroplasts in guard cells of crumpled leaf attenuates stomatal opening: both guard cell chloroplasts and mesophyll contribute to guard cell ATP levels. Plant, Cell & Environment. 2014;37: 2201–2210. doi:10.1111/pce.12297

86. Jarvis P, López-Juez E. Biogenesis and homeostasis of chloroplasts and other plastids. Nature Reviews Molecular Cell Biology. 2013. doi:10.1038/nrm3702

87. Osteryoung KW, Pyke KA. Division and dynamic morphology of plastids. Annual Review of Plant Biology. 2014. doi:10.1146/annurev-arplant-050213-035748

88. Richardson LGL, Schnell DJ. Origins, function, and regulation of the TOC–TIC general protein import machinery of plastids. Journal of Experimental Botany. 2020;71: 1226–1238. doi:10.1093/jxb/erz517

89. de Vries J, Gould SB. The monoplastidic bottleneck in algae and plant evolution. Journal of Cell Science. 2018. doi:10.1242/jcs.203414

90. Chen L, Sun B, Gao W, Zhang Q, Yuan H, Zhang M. MCD1 Associates with FtsZ Filaments via the Membrane-Tethering Protein ARC6 to Guide Chloroplast Division. The Plant Cell. 2018;30: 1807–1823. doi:10.1105/tpc.18.00189

91. Muranaka A, Watanabe S, Sakamoto A, Shimada H. Arabidopsis cotyledon chloroplast biogenesis factor CYO1 uses glutathione as an electron donor and interacts with PSI (A1 and A2) and PSII (CP43 and CP47) subunits. Journal of Plant Physiology. 2012;169: 1212–1215. doi:10.1016/j.jplph.2012.04.001

92. Tominaga J, Mizutani H, Horikawa D, Nakahara Y, Takami T, Sakamoto W, et al. Rice CYO1, an ortholog of Arabidopsis thaliana cotyledon chloroplast biogenesis factor AtCYO1, is expressed in leaves and involved in photosynthetic performance. Journal of Plant Physiology. 2016;207: 78–83. doi:10.1016/j.jplph.2016.10.005

93. Yu Q-B, Lu Y, Ma Q, Zhao T-T, Huang C, Zhao H-F, et al. TAC7, an essential component of the plastid transcriptionally active chromosome complex, interacts with FLN1, TAC10, TAC12 and TAC14 to regulate chloroplast gene expression in Arabidopsis thaliana. Physiologia Plantarum. 2013;148: 408–421. doi:10.1111/j.1399-3054.2012.01718.x

94. Asano T, Yoshioka Y, Kurei S, Sakamoto W, Machida Y. A mutation of the CRUMPLED LEAF gene that encodes a protein localized in the outer envelope membrane of plastids affects the pattern of cell division, cell differentiation, and plastid division in Arabidopsis. Plant J. 2004;38: 448–459. doi:10.1111/j.1365-313X.2004.02057.x

95. Chen Y, Asano T, Fujiwara MT, Yoshida S, Machida Y, Yoshioka Y. Plant Cells Without Detectable Plastids are Generated in the crumpled leaf Mutant of Arabidopsis thaliana. Plant and Cell Physiology. 2009;50: 956–969. doi:10.1093/pcp/pcp047

96. Smith MD, Rounds CM, Wang F, Chen K, Afitlhile M, Schnell DJ. atToc159 is a selective transit peptide receptor for the import of nucleus-encoded chloroplast proteins. Journal of Cell Biology. 2004;165: 323–334. doi:10.1083/jcb.200311074

97. Day PM, Potter D, Inoue K. Evolution and targeting of Omp85 homologs in the chloroplast outer envelope membrane. Front Plant Sci. 2014;5: 535. doi:10.3389/fpls.2014.00535

98. Agne B, Andrès C, Montandon C, Christ B, Ertan A, Jung F, et al. The Acidic A-Domain of Arabidopsis Toc159 Occurs as a Hyperphosphorylated Protein. Plant Physiology. 2010;153: 1016–1030. doi:10.1104/pp.110.158048

99. Afitlhile M, Fry M, Workman S. The TOC159 mutant of Arabidopsis thaliana accumulates altered levels of saturated and polyunsaturated fatty acids. Plant Physiology and Biochemistry. 2015;87: 61–72. doi:10.1016/j.plaphy.2014.12.018

100. Afitlhile M, Worthington R, Heda G, Brown L. The TOC159 null mutant of Arabidopsis thaliana is impaired in the accumulation of plastid lipids and phosphatidylcholine. Plant Physiology and Biochemistry. 2021;159: 148–159. doi:10.1016/j.plaphy.2020.12.011

101. Afitlhile M, Worthington R, Baldric J. The toc132toc120 heterozygote mutant of Arabidopsis thaliana accumulates decreased levels of the major chloroplast lipids. Phytochemistry. 2021;184: 112652. doi:10.1016/j.phytochem.2020.112652

102. Chiu C-C, Chen L-J, Li H. Pea Chloroplast DnaJ-J8 and Toc12 Are Encoded by the Same Gene and Localized in the Stroma. Plant Physiology. 2010;154: 1172–1182. doi:10.1104/pp.110.161224

103. Ling Q, Jarvis P. Regulation of Chloroplast Protein Import by the Ubiquitin E3 Ligase SP1 Is Important for Stress Tolerance in Plants. Curr Biol. 2015;25: 2527–2534. doi:10.1016/j.cub.2015.08.015

104. Harris BJ, Clark JW, Schrempf D, Szöllősi GJ, Donoghue PCJ, Hetherington AM, et al. Divergent evolutionary trajectories of bryophytes and tracheophytes from a complex common ancestor of land plants. Nature Ecology & Evolution. 2022. doi:10.1038/s41559-022-01885-x

105. Brawley SH, Blouin NA, Ficko-Blean E, Wheeler GL, Lohr M, Goodson HV, et al. Insights into the red algae and eukaryotic evolution from the genome of Porphyra umbilicalis (Bangiophyceae, Rhodophyta). Proceedings of the National Academy of Sciences. 2017;114: E6361–E6370. doi:10.1073/pnas.1703088114

106. Bhattacharya D, Price DC, Chan CX, Qiu H, Rose N, Ball S, et al. Genome of the red alga Porphyridium purpureum. Nature Communications. 2013;4: 1941. doi:10.1038/ncomms2931

107. Collén J, Porcel B, Carré W, Ball SG, Chaparro C, Tonon T, et al. Genome structure and metabolic features in the red seaweed Chondrus crispus shed light on evolution of the Archaeplastida. Proceedings of the National Academy of Sciences. 2013;110: 5247–5252. doi:10.1073/pnas.1221259110

108. Qiu H, Price DC, Yang EC, Yoon HS, Bhattacharya D. Evidence of ancient genome reduction in red algae (Rhodophyta). Journal of Phycology. 2015;51: 624–636. doi:10.1111/jpy.12294

109. Lee J, Yang EC, Graf L, Yang JH, Qiu H, Zelzion U, et al. Analysis of the Draft Genome of the Red Seaweed Gracilariopsis chorda Provides Insights into Genome Size Evolution in Rhodophyta. Molecular Biology and Evolution. 2018;35: 1869–1886. doi:10.1093/molbev/msy081

110. Yang W, Pollard M, Li-Beisson Y, Beisson F, Feig M, Ohlrogge J. A distinct type of glycerol-3-phosphate acyltransferase with sn-2 preference and phosphatase activity producing 2-monoacylglycerol. Proceedings of the National Academy of Sciences of the United States of America. 2010;107: 12040–12045. doi:10.1073/pnas.0914149107

111. Yang W, Simpson JP, Li-Beisson Y, Beisson F, Pollard M, Ohlrogge JB. A land-plant-specific glycerol-3-phosphate acyltransferase family in arabidopsis: Substrate specificity, sn-2 preference, and evolution. Plant Physiology. 2012;160: 638–652. doi:10.1104/pp.112.201996

112. Ball S, Colleoni C, Cenci U, Raj JN, Tirtiaux C. The evolution of glycogen and starch metabolism in eukaryotes gives molecular clues to understand the establishment of plastid endosymbiosis. J Exp Bot. 2011;62: 1775–1801. doi:10.1093/jxb/erq411

113. Viola R, Nyvall P, Pedersén M. The unique features of starch metabolism in red algae. Proc Biol Sci. 2001;268: 1417–1422. doi:10.1098/rspb.2001.1644

114. Lechner M, Rossmanith W, Hartmann RK, Thölken C, Gutmann B, Giegé P, et al. Distribution of ribonucleoprotein and protein-only RNase P in Eukarya. Molecular Biology and Evolution. 2015;32: 3186–3193. doi:10.1093/molbev/msv187

115. Gutmann B, Gobert A, Giegé P. PRORP proteins support RNase P activity in both organelles and the nucleus in Arabidopsis. Genes and Development. 2012;26: 1022–1027. doi:10.1101/gad.189514.112

116. Gobert A, Gutmann B, Taschner A, Gössringer M, Holzmann J, Hartmann RK, et al. A single Arabidopsis organellar protein has RNase P activity. Nature structural & molecular biology. 2010;17: 740–744. doi:10.1038/nsmb.1812

117. Ban T, Ke J, Chen R, Gu X, Tan MHE, Zhou XE, et al. Structure of a PLS-class Pentatricopeptide Repeat Protein Provides Insights into Mechanism of RNA Recognition. Journal of Biological Chemistry. 2013;288: 31540–31548. doi:10.1074/jbc.M113.496828

118. Wang X, Zhao L, Man Y, Li X, Wang L, Xiao J. PDM4, a Pentatricopeptide Repeat Protein, Affects Chloroplast Gene Expression and Chloroplast Development in Arabidopsis thaliana. Frontiers in Plant Science. 2020;11. Available: https://www.frontiersin.org/article/10.3389/fpls.2020.01198

119. Zoschke R, Qu Y, Zubo YO, Börner T, Schmitz-Linneweber C. Mutation of the pentatricopeptide repeat-SMR protein SVR7 impairs accumulation and translation of

chloroplast ATP synthase subunits in Arabidopsis thaliana. Journal of Plant Research. 2013;126: 403–414. doi:10.1007/s10265-012-0527-1

120. Wu W, Liu S, Ruwe H, Zhang D, Melonek J, Zhu Y, et al. SOT1, a pentatricopeptide repeat protein with a small MutS-related domain, is required for correct processing of plastid 23S–4.5S rRNA precursors in Arabidopsis thaliana. The Plant Journal. 2016;85: 607–621. doi:10.1111/tpj.13126

121. De Longevialle AF, Hendrickson L, Taylor NL, Delannoy E, Lurin C, Badger M, et al. The pentatricopeptide repeat gene OTP51 with two LAGLIDADG motifs is required for the cis-splicing of plastid ycf3 intron 2 in Arabidopsis thaliana. The Plant Journal. 2008;56: 157–168. doi:10.1111/j.1365-313X.2008.03581.x

122. Ye J-W, Gong Z-Y, Chen C-G, Mi H-L, Chen G-Y. A Mutation of OSOTP 51 Leads to Impairment of Photosystem I Complex Assembly and Serious Photo-damage in Rice. Journal of Integrative Plant Biology. 2012;54: 87–98. doi:10.1111/j.1744-7909.2012.01094.x

123. Huang W, Zhu Y, Wu W, Li X, Zhang D, Yin P, et al. The Pentatricopeptide Repeat Protein SOT5/EMB2279 Is Required for Plastid rpl2 and trnK Intron Splicing. Plant Physiology. 2018;177: 684–697. doi:10.1104/pp.18.00406

124. Cyrek M, Fedak H, Ciesielski A, Guo Y, Sliwa A, Brzezniak L, et al. Seed Dormancy in Arabidopsis Is Controlled by Alternative Polyadenylation of DOG1. Plant Physiology. 2016;170: 947–955. doi:10.1104/pp.15.01483

125. Mullineaux CW. Function and evolution of grana. Trends in Plant Science. 2005;10: 521–525. doi:10.1016/j.tplants.2005.09.001

126. Xu X, Ouyang M, Lu D, Zheng C, Zhang L. Protein Sorting within Chloroplasts. Trends in Cell Biology. 2021;31: 9–16. doi:10.1016/j.tcb.2020.09.011

127. Stamenković M, Woelken E, Hanelt D. Ultrastructure of Cosmarium strains (Zygnematophyceae, Streptophyta) collected from various geographic locations shows species-specific differences both at optimal and stress temperatures. Protoplasma. 2014;251: 1491–1509. doi:10.1007/s00709-014-0652-x

128. Wietrzynski W, Schaffer M, Tegunov D, Albert S, Kanazawa A, Plitzko JM, et al. Charting the native architecture of chlamydomonas thylakoid membranes with single-molecule precision. eLife. 2020. doi:10.7554/eLife.53740

129. Jiao C, Sørensen I, Sun X, Sun H, Behar H, Alseekh S, et al. The Penium margaritaceum Genome: Hallmarks of the Origins of Land Plants. Cell. 2020. doi:10.1016/j.cell.2020.04.019

130. Khatoon M, Inagawa K, Pospíšil P, Yamashita A, Yoshioka M, Lundin B, et al. Quality Control of Photosystem II: Thylakoid unstacking is necessary to avoid further damage to the D1 protein and to facilitate D1 degradation under light stress in spinach thylakoids. Journal of Biological Chemistry. 2009;284: 25343–25352. doi:https://doi.org/10.1074/jbc.M109.007740

131. Kirchhoff H, Hall C, Wood M, Herbstová M, Tsabari O, Nevo R, et al. Dynamic control of protein diffusion within the granal thylakoid lumen. Proceedings of the National Academy of Sciences. 2011;108: 20248–20253. doi:10.1073/pnas.1104141109

132. Nakanishi H, Suzuki K, Kabeya Y, Miyagishima S. Plant-Specific Protein MCD1 Determines the Site of Chloroplast Division in Concert with Bacteria-Derived MinD. Current Biology. 2009;19: 151–156. doi:10.1016/j.cub.2008.12.018

133. de Vries J, Stanton A, Archibald JM, Gould SB. Streptophyte Terrestrialization in Light of Plastid Evolution. Trends in Plant Science. 2016. doi:10.1016/j.tplants.2016.01.021

134. Larkin RM, Stefano G, Ruckle ME, Stavoe AK, Sinkler CA, Brandizzi F, et al. REDUCED CHLOROPLAST COVERAGE genes from Arabidopsis thaliana help to establish the size of the chloroplast compartment. Proceedings of the National Academy of Sciences. 2016;113: E1116–E1125. doi:10.1073/pnas.1515741113

135. El Zawily AM, Schwarzländer M, Finkemeier I, Johnston IG, Benamar A, Cao Y, et al. FRIENDLY Regulates Mitochondrial Distribution, Fusion, and Quality Control in Arabidopsis. Plant Physiology. 2014;166: 808–828. doi:10.1104/pp.114.243824

136. Zhu Q, Hulen D, Liu T, Clarke M. The cluA- mutant of Dictyostelium identifies a novel class of proteins required for dispersion of mitochondria. Proc Natl Acad Sci U S A. 1997;94: 7308–7313. doi:10.1073/pnas.94.14.7308

137. Raval PK, Ngan WY, Gallie J, Agashe D. The layered costs and benefits of translational redundancy. Pilpel Y, Barkai N, Schirman D, editors. eLife. 2023;12: e81005. doi:10.7554/eLife.81005

138. Roller BRK, Stoddard SF, Schmidt TM. Exploiting rRNA operon copy number to investigate bacterial reproductive strategies. Nature Microbiology. 2016;1: 16160. doi:10.1038/nmicrobiol.2016.160

139. Raval PK, Garg SG, Gould SB. Endosymbiotic selective pressure at the origin of eukaryotic cell biology. Chacinska A, Perry GH, Chacinska A, Perry GH, editors. eLife. 2022;11: e81033. doi:10.7554/eLife.81033

140. Raval PK, Martin WF, Gould SB. Mitochondrial evolution: Gene shuffling, endosymbiosis, and signaling. Science Advances. 2023;9: eadj4493. doi:10.1126/sciadv.adj4493

141. Gould SB, Garg SG, Martin WF. Bacterial Vesicle Secretion and the Evolutionary Origin of the Eukaryotic Endomembrane System. Trends in Microbiology. 2016;24: 525–534. doi:https://doi.org/10.1016/j.tim.2016.03.005

142. Hoecker U. The activities of the E3 ubiquitin ligase COP1/SPA, a key repressor in light signaling. Current Opinion in Plant Biology. 2017;37: 63–69. doi:https://doi.org/10.1016/j.pbi.2017.03.015

143. Borden KLB. RING domains: master builders of molecular scaffolds?11Edited by P. E. Wright. Journal of Molecular Biology. 2000;295: 1103–1112. doi:10.1006/jmbi.1999.3429

144. Stone SL. The role of ubiquitin and the 26S proteasome in plant abiotic stress signaling. Front Plant Sci. 2014;5. doi:10.3389/fpls.2014.00135

145. Jiménez-López D, Muñóz-Belman F, González-Prieto JM, Aguilar-Hernández V, Guzmán P. Repertoire of plant RING E3 ubiquitin ligases revisited: New groups counting gene families and single genes. PLOS ONE. 2018;13: e0203442. doi:10.1371/journal.pone.0203442

146. Thayale Purayil F, Sudalaimuthuasari N, Li L, Aljneibi R, Al Shamsi AM, David N, et al. Transcriptome Profiling and Functional Validation of RING-Type E3 Ligases in Halophyte Sesuvium verrucosum under Salinity Stress. International Journal of Molecular Sciences. 2022;23. doi:10.3390/ijms23052821

147. Basnayake BMVS, Li D, Zhang H, Li G, Virk N, Song F. Arabidopsis DAL1 and DAL2, two RING finger proteins homologous to Drosophila DIAP1, are involved in regulation of programmed cell death. Plant Cell Reports. 2011;30: 37–48. doi:10.1007/s00299-010-0941-6

148. Ling Q, Huang W, Baldwin A, Jarvis P. Chloroplast Biogenesis Is Regulated by Direct Action of the Ubiquitin-Proteasome System. Science. 2012;338: 655–659. doi:10.1126/science.1225053

149. Pan R, Satkovich J, Hu J. E3 ubiquitin ligase SP1 regulates peroxisome biogenesis in Arabidopsis. Proceedings of the National Academy of Sciences. 2016;113: E7307–E7316. doi:10.1073/pnas.1613530113

150. Oikawa K, Matsunaga S, Mano S, Kondo M, Yamada K, Hayashi M, et al. Physical interaction between peroxisomes and chloroplasts elucidated by in situ laser analysis. Nature Plants. 2015;1: 15035. doi:10.1038/nplants.2015.35

151. Liu H, Stone SL. Abscisic Acid Increases Arabidopsis ABI5 Transcription Factor Levels by Promoting KEG E3 Ligase Self-Ubiquitination and Proteasomal Degradation. The Plant Cell. 2010;22: 2630–2641. doi:10.1105/tpc.110.076075

152. Liu H, Stone SL. Cytoplasmic Degradation of the Arabidopsis Transcription Factor ABSCISIC ACID INSENSITIVE 5 Is Mediated by the RING-type E3 Ligase KEEP ON GOING*. Journal of Biological Chemistry. 2013;288: 20267–20279. doi:10.1074/jbc.M113.465369

153. Azevedo J, Courtois F, Hakimi M-A, Demarsy E, Lagrange T, Alcaraz J-P, et al. Intraplastidial trafficking of a phage-type RNA polymerase is mediated by a thylakoid RING-H2 protein. Proceedings of the National Academy of Sciences. 2008;105: 9123–9128. doi:10.1073/pnas.0800909105

154. Naramoto S, Hata Y, Fujita T, Kyozuka J. The bryophytes Physcomitrium patens and Marchantia polymorpha as model systems for studying evolutionary cell and developmental biology in plants. The Plant Cell. 2021;34: 228–246. doi:10.1093/plcell/koab218

155. Szövényi P, Frangedakis E, Ricca M, Quandt D, Wicke S, Langdale JA. Establishment of Anthoceros agrestis as a model species for studying the biology of hornworts. BMC Plant Biology. 2015;15: 98. doi:10.1186/s12870-015-0481-x

156. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Research. 2017;45: D353–D361. doi:10.1093/nar/gkw1092

157. Terashima M, Specht M, Hippler M. The chloroplast proteome: a survey from the Chlamydomonas reinhardtii perspective with a focus on distinctive features. Current Genetics. 2011;57: 151–168. doi:10.1007/s00294-011-0339-1

158. Mueller SJ, Lang D, Hoernstein SNW, Lang EGE, Schuessele C, Schmidt A, et al. Quantitative Analysis of the Mitochondrial and Plastid Proteomes of the Moss Physcomitrella patens Reveals Protein Macrocompartmentation and Microcompartmentation. Plant Physiology. 2014;164: 2081–2095. doi:10.1104/pp.114.235754

159. Sun Q, Zybailov B, Majeran W, Friso G, Olinares PDB, van Wijk KJ. PPDB, the Plant Proteomics Database at Cornell. Nucleic Acids Research. 2009;37: D969–D974. doi:10.1093/nar/gkn654

160. Emms DM, Kelly S. STAG: Species Tree Inference from All Genes. bioRxiv. 2018; 267914–267914. doi:10.1101/267914

161. Tria FDK, Landan G, Dagan T. Phylogenetic rooting using minimal ancestor deviation. Nature Ecology & Evolution. 2017;1: 0193. doi:10.1038/s41559-017-0193

162. Rambaut A, Drummond A. FigTree Version 1.4.4. 2018.

163. Revell LJ. phytools: An R package for phylogenetic comparative biology (and other things). Methods in Ecology and Evolution. 2012. doi:10.1111/j.2041-210X.2011.00169.x

164. Mooers AØ, Schluter D. Reconstructing Ancestor States with Maximum Likelihood: Support for One-and Two-Rate Models. Systematic Biology. 1999;48: 623–633. doi:10.1080/106351599260193

165. Schluter D, Price T, Mooers AØ, Ludwig D. Likelihood of ancestor states in adaptive radiation. Evolution. 1997;51: 1699–1711. doi:10.1111/j.1558-5646.1997.tb05095.x

166. Mistry J, Chuguransky S, Williams L, Qureshi M, Salazar GA, Sonnhammer ELL, et al. Pfam: The protein families database in 2021. Nucleic Acids Res. 2021;49: D412–D419. doi:10.1093/nar/gkaa913