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ABC1K atypical kinases in plants; filling the organellar kinase void

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Abstract

Surprisingly few protein kinases have been demonstrated in chloroplasts or mitochondria. Here we discuss the "activity of bc_1 complex kinase" (ABC1K) protein family which we suggest locate in mitochondria and plastids, thus filling the kinase void. The ABC1Ks are atypical protein kinases and their ancestral function is the regulation of quinone synthesis. ABC1Ks have proliferated from 1–2 members in non-photosynthetic organisms to more than 16 members in algae and higher plants. In this review we reconstruct the evolutionary history of the ABC1K family, provide a functional domain analysis for angiosperms and a nomenclature for ABC1Ks in *Arabidopsis* (*Arabidopsis thaliana*), rice (*Oryza sativa*) and maize (*Zea mays*). Finally, we hypothesize that targets of ABC1Ks include enzymes of prenyl-lipid metabolism as well as components of the organellar gene expression machineries.

The protein kinase-like superfamily

The protein kinase-like (PKL) superfamily encompasses all protein kinases and a subset of small molecule/metabolite kinases (eg phosphatidyl-inositol phosphate kinases) [1, 2]. The PKL superfamily displays an enormous variability in sequence and structure to match the wide array of target substrates. PKLs can be subdivided between the eukaryotic protein kinases (ePKs) prevalent in the eukaryotes, and the atypical protein kinases (aPKs), which predominate in the prokaryotes [2] (Figure 1A). Two-component kinases (ie histidine-aspartate kinases) form a separate family and are important in prokaryotes [3], but have been adapted by eukaryotes [4]. Sequence alignment analyses of numerous, diverse ePKs have established a ~250 amino acid ePK catalytic domain containing twelve subdomains [5, 6], while x-ray crystal structures of ePKs provide functional context for these subdomains (see e.g. [7–11]). The aPKs share little or no homology with ePKs, although crystal structures indicate that most maintain a similar overall protein kinase fold [12]. *In silico* sequence and structural studies of the entire PKL superfamily reveal only ~10 residues conserved across the ePKs and aPKs; and even these residues have been shown to be dispensable in certain PKLs [2, 12]. The ePK-like (ELK) group of aPKs, that share some sequence identity with ePKs (usually < 15%), have emerged as important regulatory kinases of bacteria [13–16] and include the activity of bc_1 complex kinase" (ABC1K) family [17], RIO kinases [18, 19], aminoglycoside kinases [20], and others [2] (Figure 1A). In this article we will explore the evolutionary history of ABC1Ks, and provide support for their significance in plant mitochondria and plastids. We also propose a logical and complete numbering for all

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Arabidopsis, maize and rice ABC1K proteins based on phylogeny. We note that the ABC1K family has no relationship with the ATP-binding cassette (ABC) membrane transporter family.

A protein kinase void in plant plastids and mitochondria

The coordination of complex processes in chloroplasts and mitochondria, such as photosynthesis and respiration, and their need for adaptations to changes in environmental conditions, likely involves an assortment of kinases. So far nearly 200 *Arabidopsis* chloroplast phosphoproteins have been identified in *Arabidopsis* leaves [21, 22]. The true number of kinase targets in chloroplast and non-photosynthetic plastids is likely much larger, considering the technical challenges to identify phosphopeptides, and considering that samples from only a small number of developmental states and (a)biotic conditions have been analyzed [23–25]. Phosphoproteome analysis of yeast mitochondria revealed a kinase network including 48 phosphoproteins involved in critical mitochondrial functions including carbohydrate metabolism, redox regulation, and apoptosis [26]. The phosphoproteome of plant mitochondria is poorly understood and fewer than 20 phosphoproteins have been detected in *Arabidopsis* [27].

So far, identified protein kinases are under-represented in the characterized proteomes of plant plastids and mitochondria [28, 29], likely because most attention has focused on ePKs. However, many kinases in plastids and mitochondria may be bacterial-derived kinases of the poorly annotated aPK family [2]. Indeed, because the ABC1Ks do not have many of the typical ePK features they often are not recognized as kinases (e.g. see [29]). Thus far in *Arabidopsis*, apart from ABC1Ks, 6–7 PKL kinases have been conclusively demonstrated to localize to the plastid or mitochondria, despite significant and systematic efforts [28, 29]. In addition, one chloroplast and one mitochondrial two-component sensor kinase have been identified, which are the chloroplast sensor kinase (CSK) [30] and pyruvate dehydrogenase kinase (PDK) [31], respectively. The experimentally identified ABC1Ks in *Arabidopsis* include seven kinases localized in plastids and one kinase in mitochondria (Table 1).

Characterization of plastid protein kinases has emphasized the role of phosphorylation in plastid gene expression and regulation of the photosynthetic thylakoid electron transport. The state transition kinases, STN7 and STN8 localize to the thylakoid membrane system and phosphorylate subunits of the light-harvesting complex (LHC) and photosystem (PS), respectively, driving rapid alterations in light harvesting and electron transport in response to fluctuating environment [32–35]. Chloroplast stromal casein kinase IIα (cpCK2, also named plastid transcription kinase - PTK) is involved in regulation of plastid gene expression [29, 36], but based on phosphorylation motifs determined from chloroplast phosphoproteome analysis, also phosphorylates a subset of chloroplast metabolic proteins [23]. cpCK2 was shown to interact with CSK providing a link between redox sensing and plastid transcriptional control [37]. The identification of two-component sensor kinases in plastids and mitochondria emphasizes the bacterial ancestry of the organelles and justifies the expectation that many of the organellar kinases are bacterial-derived aPKs.

ABC1Ks in plastids and mitochondria

The ABC1Ks are an evolutionarily-ancient gene family, conserved throughout species of all three primary kingdoms (archaea, bacteria, and eukaryotes), but have greatly expanded in number in photosynthetic organisms. The gene family in *Arabidopsis* contains seventeen members (Table 1, Figure 2). Previously we assigned numbers to the few identified Arabidopsis and maize ABC1K proteins in the Plant Proteome Database (PPDB at http://ppdb.tc.cornell.edu/); here we propose a more logical and complete numbering for all

Arabidopsis, maize and rice ABC1K proteins based on phylogeny (see below). Analyses of purified plastoglobules (PGs) from *Arabidopsis* chloroplasts identified six ABC1K proteins (AtABC1K1, 3–7) that localize predominantly to this plastid location [38–40]. A seventh protein (AtABC1K8/OSA1) was shown to localize to the inner plastid envelope [41]. Plastid localization of AtABC1K1,3,5 was confirmed by YFP fusions [29]. Furthermore, ABC1K13 is expected to be localized in mitochondria, based on the localization of its functional homolog in baker's yeast (*Saccharomyces cerevisiae*) [17, 42]. Several other ABC1K proteins were observed in leaf or pollen samples (Table 1), but their subcellular localization was not determined. Proteome analysis of maize leaf fractions further supported plastid localization of eight ABC1K proteins; ZmABC1K1, ZmABC1K3 (2 homologues), ZmABC1K4, ZmABC1K5, ZmABC1K6, ZmABC1K8 and ZmABC1K9 were identified in maize proplastid and chloroplast fractions [43, 44], with ZmABC1K4 and ZmABC1K8 enriched in plastid nucleoids [45] (Table 1). Furthermore, seven rice ABC1K proteins were identified in chloroplasts (Table 1). Finally, TargetP, an *in silico* predictor of protein localization [46], predicts plastid or mitochondrial localization for most of the maize, rice and *Arabidopsis* ABC1K proteins (Table 1). Therefore, we suggest that most, if not all, ABC1K proteins in higher plants are located in plastids or mitochondria. Based on mass spectrometry analyses of leaf samples, as well as isolated chloroplast and mitochondrial fractions, ABC1Ks assigned to plastids are in general far more abundant than those assigned to mitochondria (see PPDB); this is in agreement with the notion that the plastids contribute much more protein biomass to the leaf than mitochondria.

Identification of the ABC1K gene in yeast

The founding member of the ABC1K protein family, *ABC1/COQ8* in yeast (hereafter called, *ScCOQ8*), is a nuclear-encoded protein required for ubiquinone (UQ) synthesis in the mitochondria. This gene was found to be necessary for redox activity of the mitochondrial bc_1 complex involved in cellular respiration and was thus given the name abc1 (activity of bc_1 complex) [47]. Loss of the *ScCOQ8* gene causes a UQ deficiency and accumulation of the biosynthetic precursor 3-hexaprenyl-4-hydroxybenzoic acid, leading to instability of the bc_1 complex and the lack of bc_1 activity [48]. Missteps in the original analysis of *ScCOQ8* gene function have caused confusion. The *ScCOQ8* gene was initially believed to suppress a deleterious mutation in a cytochrome b translational activator (cbs2-223) leading to the incorrect conclusion that ScCOQ8p functions as a chaperone of cytochrome b [47, 49]. Not until a decade later was it found that *ABC1* is the *ScCOQ8* gene and that suppression of the translational activator mutant (cbs2-223) was due to a neighboring $tRNA^{Trp}$ gene [48, 50]. It is thus currently accepted that ScCOQ8p is required specifically for regulation of UQ synthesis, and is not involved in chaperone activity.

Conservation of COQ8 function in UQ biosynthesis

Analysis of homologs of yeast ScCOQ8p from diverse species has revealed remarkable functional conservation in UQ biosynthesis. Loss of UbiB or aarF, ScCOQ8p homologs in *Escherichia coli* and *Providencia stuartii*, respectively, causes UQ deficiency and concomitant accumulation of 2-octaprenylphenol, indicating a block in the first monoxygenation step in their UQ biosynthetic pathway [51]. The enzyme catalyzing this monoxygenation step has not yet been identified in either *E. coli* or *P. stuartii* and is the postulated target of UbiB/AarF [51]. The functional homolog in *Arabidopsis* was found by complementation of the yeast *ScCOQ8* deletion mutant with an *Arabidopsis* cDNA library; the only complementing cDNA was *AtABC1K13* (At4g01660). This suggests that the *Arabidopsis* genome only encodes a single functional homolog of *ScCOQ8*, which is supported by the phylogenetic analysis of the angiosperm *ABC1K* homologs (see below). Deleterious mutations in the human ABC1K homolog, ADCK3 (for aarF-domain containing

kinase 3), also displays a UQ deficiency [52, 53]. Functional homology of this gene was confirmed by successful complementation of the *ScCOQ8* deletion mutant, when expressed along with a yeast mitochondrial transit peptide [17]. This heterologous expression of HsADCK3 in the *ScCOQ8* mutant restored both UQ biosynthetic complex stability and phosphorylation of several enzymes of the pathway (COQ3, COQ4 and COQ7), suggesting that the kinase activity of this ABC1K protein can target multiple enzymes in the UQ pathway.

Phylogeny of the ABC1K proteins

Homologs of ScCOQ8p among the three branches of archaeal species (crenarchaea, euryarchaea, thaumarchaea) demonstrate strong BLAST hits (E-value < $3e^{-16}$). The presence of ABC1K homologs in all three branches of archaeal species, and throughout the bacterial kingdom, indicates their ancient origin prior to the archaea/bacterial split (Figure 3). Importantly, the archaea and many bacterial species do not synthesize UQ (a benzoquinone), but other types of prenylquinones (napthoquinones), in particular menaquinone [54], suggesting that the ancient function of ABC1Ks is regulation of quinones other than UQ. A striking proliferation of the ABC1K family is found in algae and land plants (11–27 homologs per species), whereas non-photosynthetic prokaryotes and non-photosynthetic eukaryotes consistently contain 1–2 and 3–5 homologs, respectively (Figure 2).

Construction of a phylogenetic tree from the ABC1K proteins of 42 diverse species of archaea, bacteria, and eukaryotes reveals a division into 15 subfamilies, which we name ABC1K1 to ABC1K15 (Figure 3). Immediately apparent is the fact that the archaeal homologs of multiple species all group in a clade evolutionarily distant from the homologs of bacteria and eukaryotes (except for one of two homologs of archaeal *Methanosarcina acetivorans*). This creates a natural outgroup incorporated into the phylogenetic tree, here assigned the archaeal clade (Figure 3). Within each subfamily, the seven angiosperm species (four monocots and three eudicots) all collapse into their own subclade (Figure 3). Immediately sister to each angiosperm clade are homologs from lycopod (*Selaginella moellendorffii*) and moss (*Physcomitrella patens*) with sequences from green and red algae also closely related, indicating that each of the 15 ABC1K families arose with the emergence of photosynthetic eukaryotes. Strikingly, the phylogenetic tree divides into three clear primary clades characterized by evolutionary origins and sub-cellular localization (Figure 3).

The first clade comprises eight subfamilies (1–8) and is specific for photosynthetic organisms. Cyanobacteria harbor three of the eight photosynthetic-specific subfamilies (1, 2, and 7) and it is likely that plastid endosymbiosis resulted in the introduction of these three proteins to a photosynthetic ancestor (see [55]) which subsequently expanded into the current eight members. The presence of algae in six of the eight subfamilies indicates that expansion of the plastid clade occurred very early in the development of the photosynthetic eukaryotic lineage. It is interesting that a majority of the plastid ABC1Ks (ABC1K2 to ABC1K6) of algae and plants appear to be derived from the ancestral ABC1K2 of cyanobacteria, suggesting that they may have closely related or overlapping targets (Figures 3 and 4). Most of the higher plant proteins in this clade were identified in plastid fractions.

The second clade consists of the subfamilies 11–15, which are all likely targeted to the mitochondria based on localization predictions, and because they were observed by proteomics in leaves or pollen (high in mitochondrial content) but not in isolated chloroplasts(Table 1). Furthermore, in the case of subfamily 13 (which corresponds with ScCOQ8p and its functional homologs in *Arabidopsis* and humans) experimental evidence

supports the mitochondrial location assignment, since ScCOQ8p of subfamily 13 localizes to the inner mitochondrial envelope [17] and the UQ pathway localizes within the mitochondria [56]. Except for *Arabidopsis* mitochondrial ABC1K13, experimental localization data is lacking for plant proteins in this clade. However, *in silico* analysis of proteins by TargetP predicts mitochondrial targeting for most of the *Arabidopsis*, maize and rice members, and none of the plant proteins have been observed in plastids (Table 1). Furthermore, homologs of this clade are prevalent in both photosynthetic and non-photosynthetic plant species, as would be expected from genes of a mitochondrial origin derived from endosymbiosis prior to the divergence of the plants [57]. It is not surprising, that homologs of actinobacteria (*Rhodococcus jostii* and *Streptomyces coelicolor*), and spirochaetes (*Leptospira interrogans*) are present in this clade rather than the α-proteobacteria, the presumed mitochondrial ancestors, considering the influence of a fluid evolutionary model of prokaryotic genomes. This model posits that the ancestral mitochondrial donor genome did not contain the current set of α-proteobacterial genes because of mutations, gene loss and horizontal gene transfer over > 1.5 billion years of genome evolution [58].

The third, central clade with subfamilies 9 and 10 contains the majority of the non-photosynthetic bacterial ABC1Ks, and their homologs in cyanobacteria, metazoa, and plants; this clade represents the ancestral group of ABC1Ks, derived not from organelle endosymbiosis, but from the common ancestor of bacteria, archaea, and the nuclear genome of eukaryotes. However, subfamily 9 contains the photosynthetic prokaryotes (cyanobacteria) which are missing in subfamily 10. Conversely, subfamily 10 contains non-photosynthetic eukaryotes, which are missing in subfamily 9.

Cyanobacteria lack UQ, instead using plastoquinone 9 (PQ-9) for both photosynthetic and respiratory electron transport [59]. The ABC1Ks with demonstrated roles in UQ biosynthesis divide between subfamilies 10 and 13, consistent with the absence of cyanobacteria in these subfamilies. Furthermore, these ABC1Ks in non-photosynthetic prokaryotes (aarF in *P. stuartii* and UbiB in *E. coli*) fall into subfamily 10 (ancestral clade), whereas these ABC1Ks in eukaryotes (HsADCK3, ScCOQ8, and AtABC1K13) fall into subfamily 13 (mitochondrial clade). This may be a reflection of differences in biosynthetic pathways and requirements for regulation.

Remarkably, PQ-9 biosynthesis appears to have arisen from the UQ biosynthesis in ancestral proteobacteria [60]; both PQ and UQ are evolutionary younger benzoquinones with higher redox potentials than the more ancient naphtaquinones [59]. This is supported by: (i) the *Synechocystis* genome encodes for homologs of the *E. coli* UQ biosynthetic pathway (Ubi X, D, H, E), (ii) the two pathways both derive their head group from 4-hydroxybenzoate, rather than homogentisate as in eukaryotic PQ-9 synthesis, and (iii) the PQ-9 prenyl-transferase of *Synechocystis* functionally complements the *ubiA* deletion mutant in *E. coli* [60]. It can be expected that the regulatory ABC1K of UQ synthesis was similarly co-opted for PQ-9 synthesis in cyanobacteria. Thus it appears that plants, through endosymbiosis of plastid and mitochondrial ancestors, have inherited multiple pathways for quinone metabolism along with the corresponding regulatory ABC1Ks, resulting in the proliferation of the ABC1K family in photosynthetic eukaryotes.

Defining the ABC1K protein domain

Based on the alignment of 100 full length ABC1K protein sequences from the seven angiosperm species used in the phylogenetic tree (Figure 3), a common ABC1K domain is observed, spanning ~350 residues and containing twelve conserved motifs (Figure 1B). Furthermore, eight of the ten key residues of the PKL superfamily identified in [2] are

present in the ABC1K family and correspond to motifs III, IVa, IVb, VIIb, and VIII, involved in ATP binding and orientation (III, IVa and IVb), catalysis (VIIb), and Mg^{2+} chelation (VIII) [6, 8, 11, 61]. The other seven motifs of the ABC1K domain (I, II, V, VI, VIIa, IX, X) do not have homologous sequences in ePKs, but can be found in a number of proteins outside of the PKL superfamily with diverse enzyme activities. The significance of these ABC1K motifs is unknown and will likely require crystallization studies and mutational analysis.

Motifs conserved with ePKs

The nucleotide-binding pocket (motif III) in the ABC1K family is unusual in that the first two characteristic Gly residues of nucleotide-binding pockets are both replaced with Ala (see also [29]). Between these two alanines is a single residue, either another Ala, or a serine or threonine. An invariant serine and glutamine, flanking two hydrophobic residues also appears in the ABC1K nucleotide-binding pocket. It cannot be concluded from the primary sequence alone which nucleotide (NTP) the ABC1Ks prefer as co-factor. The invariant lysine of PKLs (in motif II) lies in ABC1K motif IVa, fourteen residues downstream of motif III and is immediately downstream of three hydrophobic residues, as in the ePKs. The lysine helps to anchor the NTP by binding its α- and β-phosphates and positions the γ-phosphate for catalysis [6]. Motif IVb in ABC1Ks is characterized by an invariant (acidic) Asp residue, which we suggest is homologous to the invariant (acidic) Glu of ePKs. An acidic residue is necessary for stabilization of the Lys-NTP interaction [6]. The catalytic motif (VIIb) contains a consensus HADPHPGN sequence in the ABC1K family. The Asp is 100% conserved among tested angiosperms and is likely homologous to the conserved Asp of ePK catalytic motifs, responsible for activating the substrate hydroxyl group via nucleophilic attack [6]. Mutation of this residue in ScCOQ8 resulted in the *abc1* mutant phenotype [17]. The histidines in the consensus sequence are conserved in all *Arabidopsis* ABC1Ks, except for ABC1K14 and ABC1K12 where the His positions are replaced with either Asn or Gln, indicating an absolute requirement for an amine-containing side chain at that position. Motif VIII comprises the D[FYHV]G motif which anchors the Mg^{2+} necessary for positioning NTP, using the invariant Asp to chelate this divalent cation [6]. The C-terminal motif of the ABC1K domain (motif X) deserves special mention because the motif in family 13 (PPEExxSLHRKxxG) is homologous to a motif in a number of proteins from diverse species including the RsbU phosphatase 2C of *Bacillus subtilis* [17]. X-ray crystallography studies of the RsbU protein have indicated that the sequence is critical for homodimerization by stabilizing the protein through helix-helix interaction [62]. Point mutations in motif X of the HsABC1K13, (G549S, E551K) and ScCOQ8 (G475D) cause UQ deficiency, indicating a critical function for this motif in ABC1K function [17, 52, 53]. The homodimerization of the RsbU phosphatase facilitates binding with the RsbT serine kinase, creating a complex that mediates stress responses to environmental and nutritional signals in *B. subtilis* [63]. The other ABC1K families show divergent variants of this motif. Thus motif X in the ABC1Ks may similarly mediate protein-protein interactions necessary for mediating stress responses integral to ABC1K function (see below).

Domain architecture

Land plant and red algae ABC1Ks in subfamily 11 (green algae have no observed homolog in this subfamily) contain a C-terminal β-lactamase domain, which in bacteria catalyzes hydrolysis of the β-lactam ring of penicillin. Identification of the intact catalytic motifs suggests that the lactamase domain is active [64]. Several β-lactamase domain proteins have been found in plants with other enzymatic activities such as glyoxylase proteins in rice and *Arabidopsis* [65] and an *Arabidopsis* DNA ligase [66]. The function of β-lactamase domains in plants and other eukaryotic species is unknown, but it has been suggested that the fusion

of the β-lactamase domain to ABC1K confers a unique mechanism of autoregulation of kinase activity [64].

Kinase activity among the ABC1Ks

A direct demonstration of kinase activity in the ABC1K protein family has proven difficult. However, indirect results from point mutants in predicted kinase residues of the yeast and human *ABC1K* genes [17, 52, 53, 67] and an *in-gelo* study of the *Arabidopsis AtABC1K8/ OSA1* gene have reinforced the hypothesized protein kinase activity [41]. ScCOQ8p-dependent isoelectric point shifts of several subunits of the yeast UQ biosynthetic complex (COQ3, COQ5 and COQ7) have been detected in 2D IEF-SDS-PAGE gels, suggesting that ScCOQ8p either directly or in-directly phosphorylates several enzymes of the UQ biosynthesis complex in yeast [17, 67]. Biochemical phenotypes of point mutations in conserved kinase subdomains have further supported the participation of ScCOQ8p in UQ biosynthetic complex phosphorylation. Five of eight point mutants demonstrating the *ABC1K* deletion phenotype were mutated in residues of shared ePK-ABC1K motifs. In particular, mutation of the invariant lysine-216 (motif IVa) to an alanine caused dramatically reduced steady-state levels of the protein. Similarly, several deleterious mutations in the human ADCK3, causing UQ deficiency, are point mutants in conserved kinase subdomains [52, 53].

Experimental studies of plant ABC1Ks and mRNA co-expression analysis

In non-photosynthetic organisms, the ABC1K family has only been studied in relation to UQ biosynthesis. Yet, proliferation of the ABC1K family in photosynthetic eukaryotes implies an expansion of functions and targets.

Leaf proteome analysis showed that the six most abundant ABC1K proteins in *Arabidopsis* were located in thylakoid-associated lipoprotein particles, called plastoglobules (PGs) (Table 1). Based on genome-wide co-expression analysis of these six ABC1Ks [40], we suggested that they have regulatory functions concerning formation, maintenance and optimization of photosynthetic performance through regulation of specific sets of enzymes involved in carotenoid biosynthesis, photoacclimation, senescence, and plastid gene expression. CrABC1K6 (EYE3) in the green algae *Chlamydomonas reinhardtii*, was located in carotenoid-rich lipophilic plastid particles (the pigment granule) in the eyespot of *Chlamydomonas*. Null mutants in *CrABC1K6* failed to develop pigment granules or eyespots. The *Arabidopsis* homolog of CrABC1K6 (AtABC1K6) is located in chloroplast PGs [40] (Table 1), consistent with the belief that PGs serve as the precursors of the eyespot pigment granule [68]. Additionally, AtABC1K6 tightly co-expressed with zeaxanthin epoxidase (ZEP), suggesting a regulatory role in the xanthophyll cycle [40]. Based on various data (Table 1), we believe that higher plant ABC1K4 is located in the nucleoid, where it may help regulate plastid gene expression. The functions of mitochondrial ABC1K proteins include regulation of UQ biosynthesis and are otherwise unclear, but we speculate that it includes regulation of mitochondrial gene expression.

Other experimental studies of plant ABC1K genes have emphasized a role in various types of abiotic stress tolerance, including the heavy metal cadmium. *AtABC1K8 (OSA1)*, encoding for a chloroplast envelope protein, was transcriptionally upregulated in response to cadmium, and loss of *AtABC1K8* expression rendered plants more susceptible to cadmium toxicity, high-light, and H_2O_2 [41]. Even under optimal growth conditions, these *AtABC1K8* mutants displayed elevated biochemical markers of oxidative stress (e.g. SOD activity). Likewise, a homolog of the PG-localized AtABC1K7 from the heavy-metal over-accumulator species *Brassica juncea* was also up-regulated in response to 24 hours of treatment with cadmium [69]. The maize homolog ZmABC1K8 expressed primarily in

green tissue, with highest expression levels in fully mature leaves, and its expression was enhanced in response to cadmium [70]. Conversely, *ZmABC1K8* mRNA accumulation was down-regulated by treatment with ABA, $H_2O_2$and darkness, and did not respond to cold-treatment. It was suggested that heterologous expression of wheat ABC1K13 in *Arabidopsis* conferred enhanced tolerance against a wide variety of stresses, but no evidence was presented for accumulation of the transgenic protein [71]. mRNA analysis of rice *ABC1K* genes suggested highest expression in leaf tissue and varying responses to abiotic stresses [72].

Conclusions and future directions

Regulation of quinone synthesis is the ancient (archaeal) function of the ABC1K family. Plants, through endosymbiosis of plastid and mitochondrial ancestors, have inherited pathways for quinone metabolism along with the corresponding regulatory ABC1Ks. The requirement for additional quinolic and other prenyl-lipids likely further drove the expansion of the ABC1K family in algae and higher plants. These ABC1Ks localize in plastids and mitochondria in which they represent the majority of known kinases. The direct targets of ABC1Ks are not known, but likely include enzymes of prenyl-lipid metabolism (eg carotenoids) and components of the organellar gene expression machineries. Systematic analysis of ABC1K targets will be critical in defining the functional significance of the ABC1K family in photosynthetic organisms.

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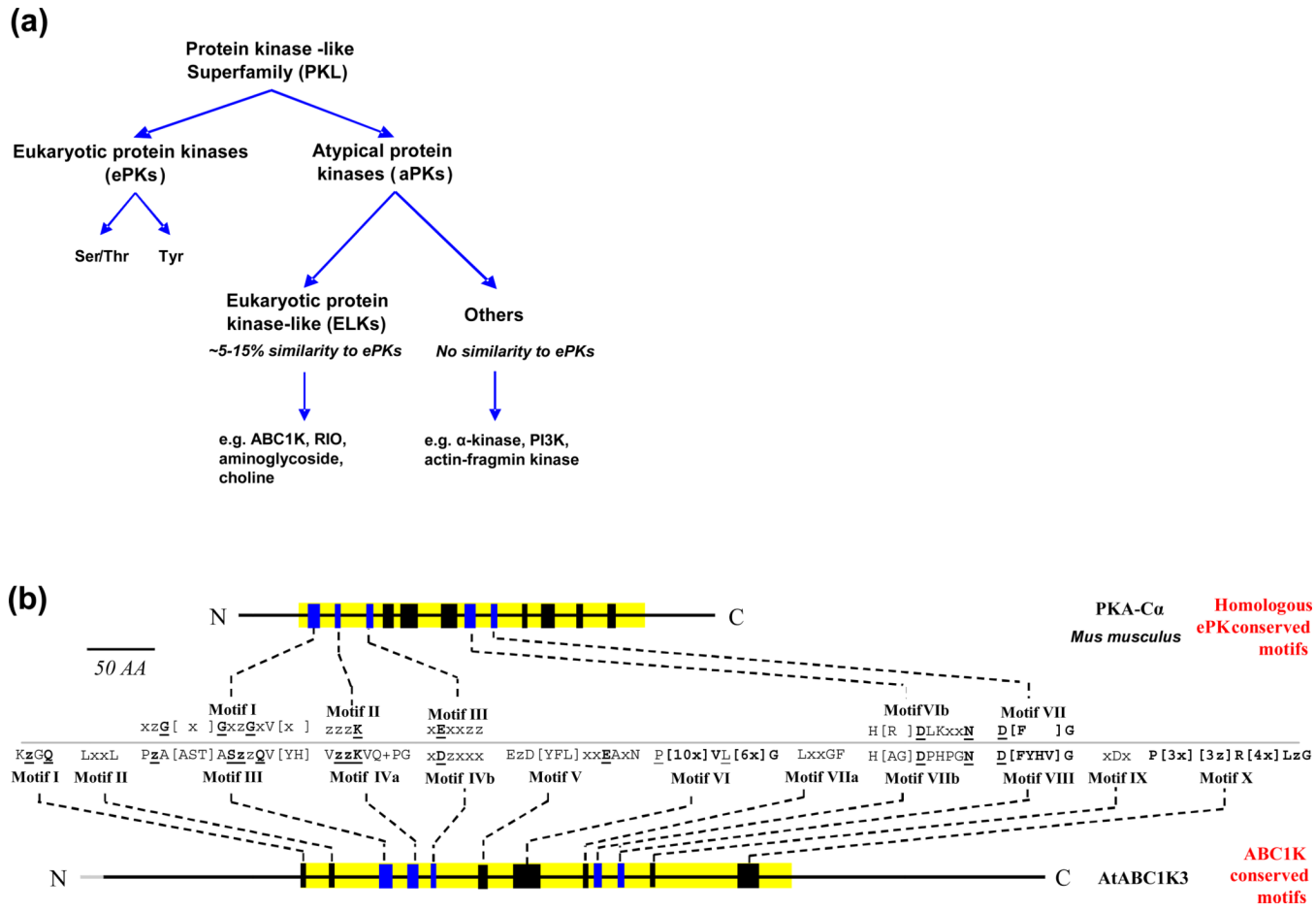
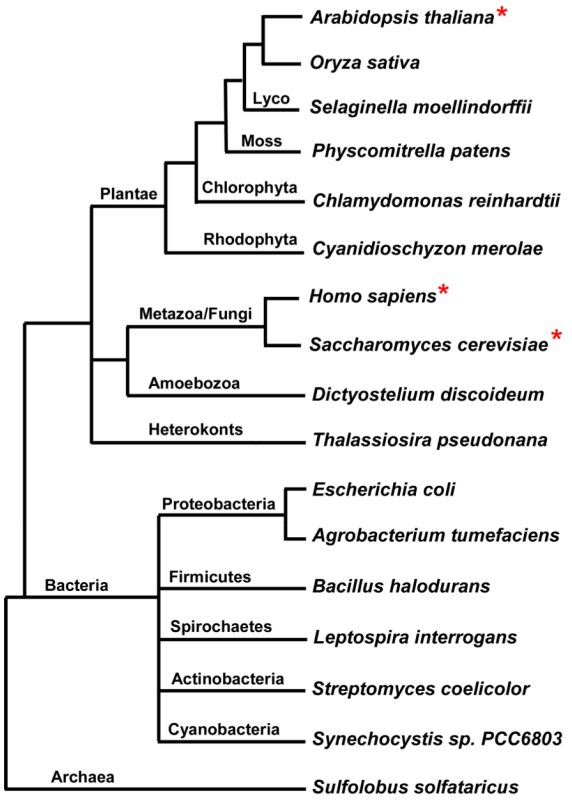


Figure 1.

Categorical relationships and conserved motifs of the ABC1K protein domain, compared with the ePK family of kinases. **(a)** The diagram illustrates the categorization of the protein kinase-like superfamily. **(b)** The twelve conserved motifs found in the ABC1K domain from an alignment of 100 sequences of three eudicot species (*Arabidopsis, M. truncatula*, and *P. trichocarpa*) and four monocot species (*Z. mays, O. sativa, B. distachyon, S. bicolor*), are illustrated using the prototypical ePK of *Mus musculus*, protein kinase C alpha (PKA-Cα), and AtABC1K3. Five motifs are shared between ABC1Ks and ePKs and are colored in blue in the cartoon and are aligned to each other for comparison. Conserved motifs are described using the single letter code of amino acid residues and residues conserved in >75% of sequences are shown. Bold, underlined residues are conserved in 100% of the aligned sequences, z indicates a hydrophobic residue,×indicates any residue, + indicates a positive-charged residue. ABC1K motifs shared with the ePK motif: **Motif III** A part of the NTP-binding pocket, this motif is responsible for anchoring the α- and β-phosphate groups and positioning the γ-phosphate for catalysis. The sequence in ABC1Ks is divergent from the "GxGxxG" sequence seen in ePKs and other NTP-binding pockets. However, the glycine-rich loop of ePKs forms a turn that is easily substituted with the small side-chains of Ala and Ser, as seen in the ABC1K family [60]. **Motif IVa** Contains the invariant lysine helping to anchor the NTP by binding the α- and β-phosphates and also forms a salt bridge with the carboxyl group of the Asp in motif IVb. As in ePKs, this invariant lysine lies 14 residues downstream of the NTP-binding pocket (12–21 residues in ePKs [60]). **Motif IVb** The aspartic acid residue is homologous to the conserved glutamic acid residue of ePK motif III, necessary for stabilization of the interaction between Lys of motif III and the α- and β-

phosphate of NTP. As in casein kinase 2 and a number of other ePKs, the conserved acidic residue lies exactly 13 residues downstream of the invariant lysine [6], however the pair of hydrophobic residues downstream of glutamic acid (+3 from Glu), required in ePKs, is not maintained in ABC1Ks, but they rather conserve a hydrophobic residue at the next position (+1 from Asp), suggesting a different local protein fold. **Motif VIIb** Referred to as the catalytic loop in ePKs, the conserved Asp serves as the catalytic base, activating the substrate hydroxyl group. **Motif VIII** Anchors the Mg^{2+} necessary for positioning the α- and β-phosphate of the NTP. The Asp chelates the divalent cation through the assistance of a hydrophobic bond with the conserved Gly.

Cladogram of diverse species containing ABC1Ks



ABC1K gene family size in 42 representative species

Phylogenetic group	Species	# of loci
Dicots	Arabidopsis thaliana	17
	Medicago truncatula	17
	Populus trichocarpa	23
Monocots	Brachypodium distachyon	16
	Oryza sativa	17
	Sorghum bicolor	16
	Zea mays	20
Lycopod	Selaginella moellindorffii	20
Moss	Physcomitrella patens	27
Red algae	Cyanidioschyzon merolae D10	11
Green algae	Chlamydomonas reinhardtii	17
Cyanobacteria	Synechocystis sp. PCC6803	5
	Anabaena variabilis ATCC29413	4
	Prochlorococcus marinus subsp. Marinus str. CCMP	3
	Nostoc sp. PCC7120	4
	Acaryochloris marina str. MBIC11017	4
Metazoa	Drosophila melanogaster	3
	Homo sapiens	5
	Mus musculus	5
Fungi	Neurospora crassa	4
	Saccharomyces cerevisiae	3
Amoeba	Dictyostelium discoideum str. AX4	4
Oomycete	Phytophthora infestans	4
α-proteobacteria	Agrobacterium tumefaciens str. C58	1
	Brucella melitensis M28	1
β-proteobacteria	Ralstonia solancearum str. GMI1000	1
	Nitrosomonas europaea str. ATCC19718	2
γ-proteobacteria	Escherichia coli K12	1
	Yersinia pestis	1
	Providencia stuartii	1
ε-proteobacteria	Sulfuricurvum kujiense str. DSM 16994	1
Actinobacteria	Streptomyces coelicolor A3(2)	2
	Rhodococcus jostii st. RHA1	2
Firmicutes	Bacillus halodurans	1
Spirochaetes	Leptospira interrogans serovar Lai str. 56601	2
Crenarchaea	Sulfolobus solfataricus	1
	Metallosphaera sedula DSM 5348	1
Euryarchaea	Natrinema pellirubrum DSM 15624	2
	Haladaptatus paucihalophilus DX253	1
	Methanosarcina acetivorans	2
Thaumarchaea	Cenarchaeum symbiosum A	1
	Nitrosopumilus maritimus	1

Figure 2.

A phylogenetic tree and table illustrating the diversity of species containing ABC1K proteins and their evolutionary relationships. A proliferation of homologs in photosynthetic species, especially eukaryotic species, is seen in the table at right. The red asterisk indicates the three species with experimentally demonstrated functional homologs involved in UQ synthesis. Lengths in the phylogenetic tree are not meant to indicate evolutionary distance.

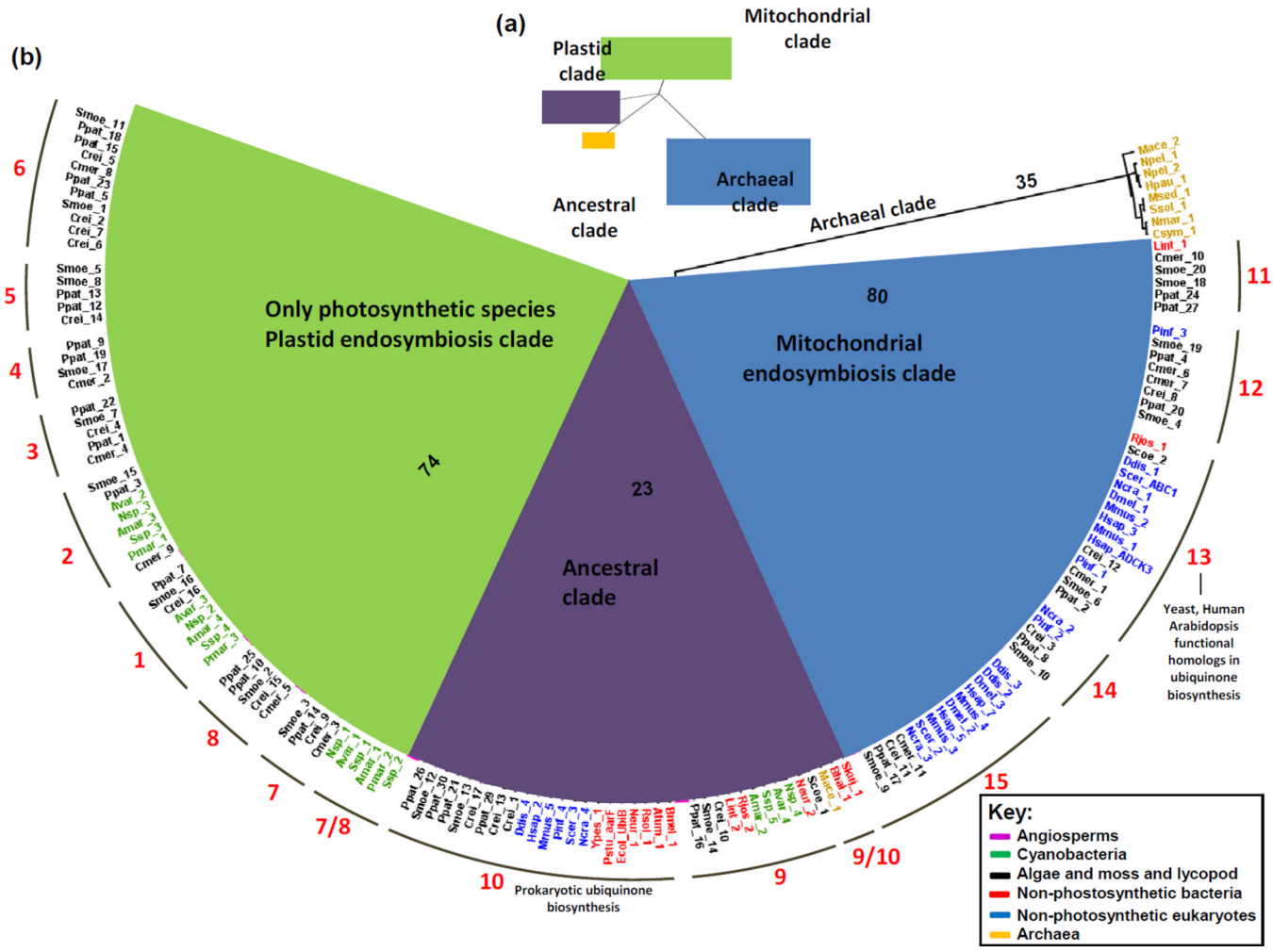


Figure 3.

Phylogenetic tree of the ABC1 kinase family among archaea, bacteria and eukaryotes. The full complement of 274 ABC1K proteins of 42 species, from diverse archaea, prokaryotes and eukaryotes were aligned using MUSCLE 3.5.1 (http://toolkit.tuebingen.mpg.de/muscle) and manually corrected in case of truncated protein sequences. The tree was constructed using the RAxML software tool at CIPRES (http://www.phylo.org/sub_sections/portal/) using the General Time Reversal model with 1000 bootstrap iterations and has been illustrated as an unrooted tree **(a)** and a proportional polar tree layout **(b)** using FigTree v1.3.1 (http://tree.bio.ed.ac.uk/software/figtree/). (a), unrooted tree illustrating the three primary clades, in addition to an archaeal group forming a natural outgroup to the tree. The three clades can be categorized by their presumptive localizations and origins indicated by color; plastid endosymbiosis (green), mitochondrial endosymbiosis (blue), ancestral (purple), and outgroup (archaea specific clade) (orange). (b), Expansion of the tree shown in (a) illustrating the distribution of species. Bootstrap values are indicated for the four primary branches. Species names are colored to distinguish archaea, photosynthetic and non-photosynthetic bacteria and photosynthetic and non-photosynthetic eukaryotes. Angiosperm clades have been collapsed for legibility and are colored magenta. The 15 subfamilies are labeled around the perimeter of the wheel. Species are named as follows: Smoe, *Selaginella moellindorffii*; Ppat, *Physcomitrella patens*; Crei, *Chlamydomonas reinhardtii*; Cmer, *Cyanidioschyzon merolae*; Avar, *Anabaena variabilis*; Nsp, *Nostoc* sp.; Amar, *Acarychloris*

marina; Ssp, *Synechocystis* sp.; Pmar, *Prochlorococcus marinus*; Ddis, *Dictyostelium discoideum*; Pinf, *Phytophthora infestans*; Hsap, *Homo sapiens*; Scer, *Saccharomyces cerevisiae*; Ncra, *Neurospora crassa*; Pstu, *Providencia stuartii*; Ypes, *Yersinia pestis*; Ecol, *Escherichia coli*; Neur, *Nitrosomonas europaea*; Rsol, *Ralstonia solancearum*; Bmel, *Brucella melitensis*; Atum, *Agrobacterium tumefaciens*; Lint, *Leptospira interrogans*; Scoe, *Streptomyces coelicolor*; Mace, *Methanosarcina acetivorans*; Bhal, *Bacillus halodurans*; Skuj, *Sulfuricurvum kujiense*; Mmus, *Mus musculus*; Dmel, *Drosophila melanogaster*; Rjos, *Rhodococcus jostii*; Nmar, *Nitrosopumilus maritimus*; Csym, *Cenarchaeum symbiosum*; Msed, *Metallosphaera sedula*; Ssol, *Sulfolobus solfataricus*; Hpau, *Haladaptatus paucihalophilus*; Npel, *Natrinema pellirubrum*.

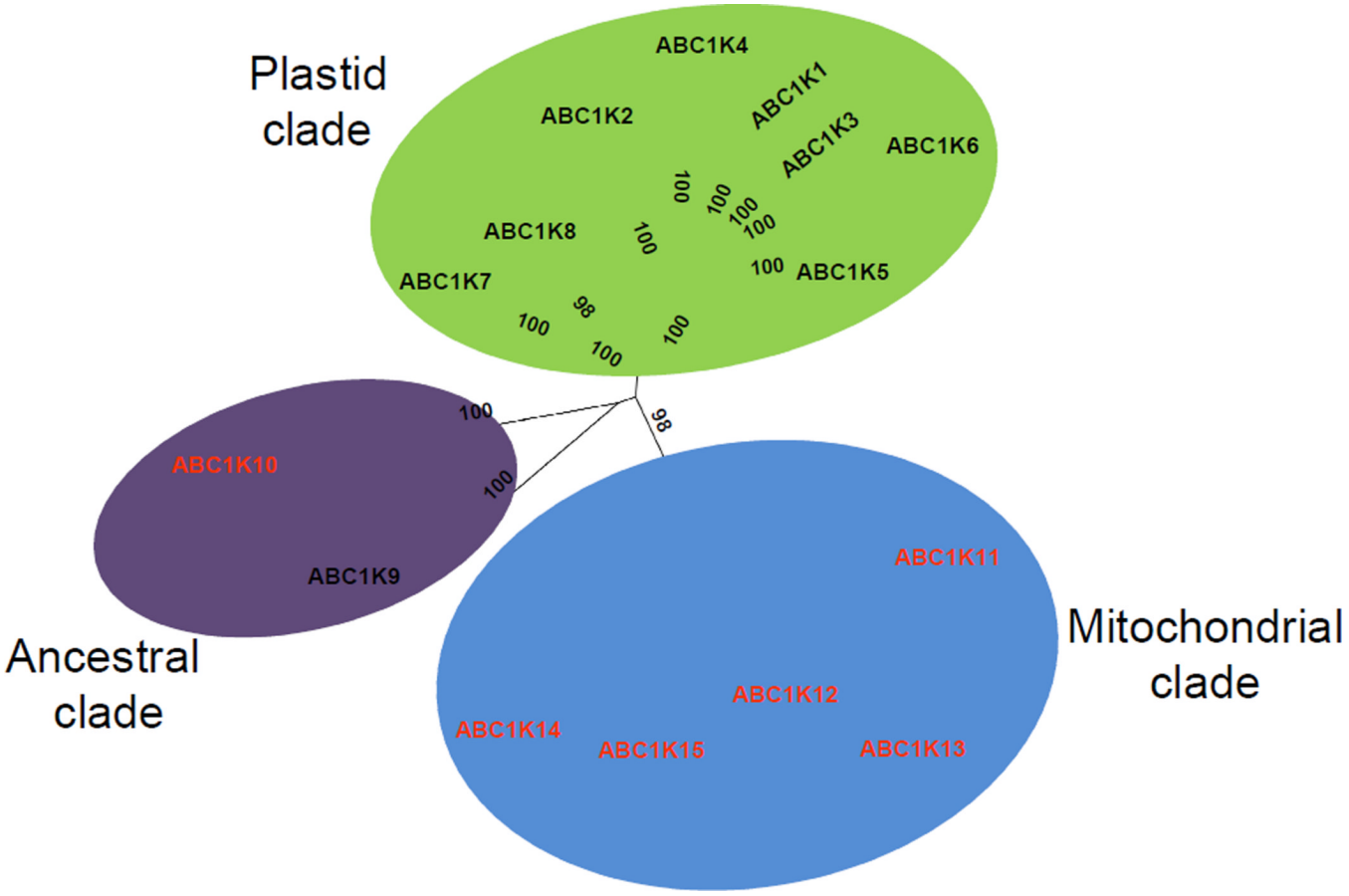


Figure 4.

Phylogenetic tree of the angiosperm ABC1K proteins. The unrooted tree was based on the amino acid sequence alignment of the 126 ABC1K proteins from three eudicot species (*Arabidopsis*, *M. truncatula*, and *P. trichocarpa*) and four monocot species (*Z. mays*, *O. sativa*, *B. distachyon*, *S. bicolor*) which was manually corrected in the case of the truncated protein products. The tree was generated as outlined in figure 3. The three clades identified and illustrated in figure 3 are indicated here using the same color code and each of the 15 subfamilies are labeled. Subcellular localization of each subfamily (black – plastids, red – mitochondria) has been determined by experimental evidence or, in the absence of experimental evidence, by TargetP prediction. Bootstrap values are indicated.

Table 1

The nomenclature and subcellular localization of the ABC1K protein family in *Arabidopsis*, rice and maize

Sub-family[a]	Name[b]	Location[c]	*Arabidopsis* homolog(s)[d]	Tar-getP[e]	Exp. loc.[f]	Rice homolog(s)[g]	Tar-getP[e]	Exp. local.[h]	Maize homolog(s)[g]	Tar-getP[e]	Exp. loc.[j]
Photosynthetic-specific clade[a]											
1	ABC1K1	Plastid (PG)	AT4G31390[k]	P	PG	Os11g11000	P	Plastid	GRMZM2G377115	P	PG
2	ABC1K2	Plastid	AT5G24970	M	Leaf	Os07g27480	M	N.d.	GRMZM5G817551[n]	M	N.d.
3	ABC1K3	Plastid (PG)	AT1G79600[k]	P	PG	Os05g25840	P	Plastid	GRMZM2G315125	P	PG
									GRMZM5G878070[o]	P	PG
4	ABC1K4	Plastid (Nuc)	AT2G39190	P	Leaf	Os02g56200	P	N.d	GRMZM5G855200[p]	C	Nuc
5	ABC1K5	Plastid (PG)	AT1G71810[k]	P	PG	Os04g54790	P	Plastid	GRMZM2G305007	M	PG
6	ABC1K6	Plastid (PG)	AT3G24190	P	PG	Os02g57160	P	Plastid	GRMZM2G157369	P	PG
7	ABC1K7	Plastid (PG)	AT3G07700	C	PG	Os09g07660	P	Plastid	GRMZM5G845129	P	N.d.
8	ABC1K8	Plastid (IE or Nuc)	AT5G64940[l]	P	IE, Nuc[j]	Os02g36570	P	Plastid	GRMZM2G040511	P	Nuc
Ancestral clade[a]											
9	ABC1K9	Plastid (PG)	AT5G05200	M	PG	Os07g12530	P	Plastid	GRMZM2G031780	M	PG
10	ABC1K10	Mitoch.	AT1G11390	M	Leaf	Os07g37180	M	N.d.	GRMZM2G067520	M	N.d.
			AT1G61640	M	N.d.	Os04g56510	M	N.d	GRMZM2G087201	M	N.d.
									GRMZM2G368486	S	N.d.
Mitochondrial clade[a]											
11	ABC1K11	Mitoch.	AT5G24810	M	Leaf	Os06g48770	M	Leaf	GRMZM2G140917	M	N.d.
12	ABC1K12	Mitoch.	AT4G24810	C	N.d	Os01g67720	C	N.d.	GRMZM2G113264	M	N.d.
			AT5G50330	C	N.d				GRMZM5G884972[q]	M	N.d.
13	ABC1K13	Mitoch. (IM)	AT4G01660[m]	C	Pollen	Os01g21610	S	N.d.	GRMZM2G003417	C	N.d.
14	ABC1K14	Mitoch.	AT1G65950	M	Leaf	Os11g34750	M	N.d.	GRMZM2G124553	M	N.d.
						Os11g34830	M	N.d.	GRMZM2G040720	M	N.d.
15	ABC1K15	Mitoch.	AT2G40090	M	Leaf	Os03g49140	C	N.d.	GRMZM2G026180	C	N.d.

Abbreviations: C, cytosol; Exp. loc.; experimental localization; IE, inner envelope; IM, inner membrane; Mitoch., Mitochondria; N.d., not detected; Nuc, nucleoid; P, plastid; PG, plastoglobule; S, secreted.

[a] Subfamilies determined by phylogenetic analysis, as illustrated in figures 3 and 4.

[b] Assigned name based on subfamilies.

[c] Most likely subcellular location in *Arabidopsis*, rice or maize based on experimental data combined with predictions (see also PPDB: http://ppdb.tc.cornell.edu).

[d] Gene/protein accession numbers are from *Arabidopsis* genome assembly TAIR10 (http://www.arabidopsis.org/).

[e] The predicted subcellular location by TargetP based on most likely gene model for each locus.

[f] Subcellular localization reported in the literature (PG [39], IE [40]). Leaf detected in *Arabidopsis* leaf samples. Pollen, this protein was reported in the pollen proteome [73].

[g] Gene/protein accession numbers are from rice genome assembly v6 (http://rice.plantbiology.msu.edu/).

[h] Experimental location based on mass spectrometry analysis of rice chloroplasts and leaves. (N.d. indicates that the protein was not detected in rice lea chloroplasts (Huang, Friso and van Wijk, unpublished)).

[i] Gene/protein accession numbers are from maize genome assembly 5b.60 (http://www.maizesequence.org/).

[j] Experimental location based on mass spectrometry analysis of maize subfractions and leaves by the van Wijk lab.

[k] Null mutant results in conditional stress phenotype (Lundquist, Giacomelli and van Wijk, unpublished).

[l] Mutant results in impaired cadmium tolerance [41]

[m] Functionally complements the ScCOQ8 mutant [17,42]

[n] Gene/protein number in genome assembly 4a53 is GRMZM2G045183

[o] Gene/protein number in genome assembly 4a53 is GRMZM2G020627

[p] Gene/protein number in genome assembly 4a53 is GRMZM2G008643

[q] Gene/protein number in genome assembly 4a53 is GRMZM2G091267