**INTRODUCTION**

Oxygentic photosynthesis is a biophysicochemical process that converts carbon dioxide into organic compounds using sunlight as a source of energy. It occurs in the chloroplasts of land plants and algae, and also in the cytoplasm of cyanobacteria, and uses water as a source of electrons, releasing oxygen as a waste product (for a recent review, see Hohmann-Marriott and Blankenship, 2011). The chloroplast in algae and plants has evolved from a cyanobacterial ancestor via endosymbiosis with a primitive eukaryotic host. It is a highly compartmentalized organelle, with three membrane systems (outer envelope, inner envelope, and thylakoid) and three soluble spaces (intermembrane space, stroma, and thylakoid lumen). A wide variety of solute and metabolite transporters reside within the different types of chloroplast membranes and mediate communication between the cytosol, stroma, and lumen. Several excellent reviews on the identification and functional characterization of these transporters have recently become available (Speetea and Schoefs, 2010; Breuers et al., 2011; Flügge et al., 2011; Weber and Linka, 2011).

Regarding their evolution, the majority of the inner envelope metabolite transporters have been shown to have a host origin, driven by the requirement to establish communication between the host cytosol and the cyanobiont (Facchinelli and Weber, 2011). There are so far no evolutionary studies dedicated to solute transporters from the outer envelope or thylakoid membranes, but it is believed that they originate from proteins in the ancestral cyanobacterial outer and thylakoid membranes, respectively.

Mitochondria and chloroplasts are the two organelles able to synthesize ATP, which is the universal energy currency of cellular metabolism in all living organisms. The difference between these organelles in this respect is that in all eukaryotes mitochondria produce ATP via oxidative phosphorylation on the inner membrane, to be used during cell metabolism. Chloroplasts and also cyanobacteria use sunlight as a source of energy to produce ATP (photophosphorylation) on the thylakoid membrane, which is consumed during CO2 fixation in the stroma. In addition, ATP is used for energy-dependent reactions on the envelope and thylakoid membrane or inside the thylakoid lumen (Speetea and Thuswaldner, 2008).

ATP is the largest and most highly charged solute transported across organelar membranes. Two structurally and phylogenetically different types of ATP transporters are represented in chloroplasts, namely ATP/ADP antiporters (AAA, TC #2.1.12, according to Saier et al., 2009) and mitochondrial ADP/ATP carriers (AAC,
There are two ATP/ADP antiporters (AATPs) in the inner envelope membrane of photosynthetic and heterotrophic plastids, supplying cytosolic ATP to the stroma (for recent reviews, see Haferkamp et al., 2011; Traba et al., 2011). The transport is electroneutral since the counter-ions for ATP4− are ADP3− together with H2PO−4 (Trentmann et al., 2008). AATPs possess 12 putative transmembrane helices, and share a common origin with the ATP/ADP antiporters found in the parasite bacteria Rickettsia prowazekii and Chlamydia psittaci (Haferkamp et al., 2011).

The mitochondrial ADP/ATP carriers are the first and most studied members of the mitochondrial carrier (MC) family and are present only in eukaryotic cells (Haferkamp et al., 2011; Traba et al., 2011). Like the members of the AAA family, AACS are exchangers, but the transport is electrogenic (ATP4−/ADP3−) and proceeds in the opposite direction, since they transport matrix ATP through the intermembrane space out into the cytosol. Yet another functional difference from AAA is that AACS are sensitive to specific inhibitors, such as bongkrekic acid and carboxyatractylsoside (Klingenberg, 2008). The 3D structure of the bovine AAC has been resolved at 2.2 Å resolution in a conformation stabilized with carboxyatractylsoside (Pebay-Peyroula et al., 2003). The structure revealed six transmembrane helices and a selectivity filter for adenine nucleotides, whose sequence could be used to predict other AACS and even other MCs (Nury et al., 2010). From a total of 58 MC in Arabidopsis thaliana, three classical mitochondrial AACS have been characterized, with at least four more paralogous sequences awaiting validation (Palmieri et al., 2011). In addition to the mitochondrion, AAC members have also been found in peroxisomes, endoplasmic reticulum, amyloplasts, and chloroplasts (Haferkamp et al., 2011; Traba et al., 2011).

Using western blotting and activity inhibition with an antibody against the bovine AAC, the activity of an AAC was reported in the spinach thylakoid membrane (Spetea et al., 2004). BLAST searches with the bovine AAC against the Arabidopsis protein database combined with prediction of chloroplast transit peptides revealed one putative chloroplast AAC, encoded by the At3g1500 gene. The corresponding protein was localized to the thylakoid membrane of Arabidopsis (Thuswaldner et al., 2007; Zybailov et al., 2008), and was annotated as the thylakoid ATP/ADP carrier (AtTAAC). Subfractionation as well as immunocytochemical experiments indicated the non-appressed regions as the precise location of TAAC within the thylakoids (Thuswaldner et al., 2007). Another chloroplast AAC is encoded by the At3g51870 gene and was initially localized to the envelope using western blotting (Spetea, C., unpublished data) and mass-spectrometry based proteomics (Ferro et al., 2010).

The AtTAAC sequence contains 415 amino acids that include a predicted transit peptide of 60 amino acids. The sequence of the processed form is 80 residues longer than that of bovine AAC, explaining the observed difference between the two proteins in the reported size in SDS gels (Spetea et al., 2004). The extra 80 residues are distributed as 50 in the N-terminus and 30 in the C-terminus—regions containing many charged residues and a five-glycine repeat that could play a role in the regulation of TAAC activity. TAAC shares about 30% identity with bovine AAC, which is concentrated in the six putative transmembrane helices and to a lesser degree in the connecting loops (Thuswaldner et al., 2007). The selectivity filter for adenine nucleotides, represented by residues K-130, R-186, Y-282, and K-369, is fully conserved, indicating adenine nucleotides as the most likely substrates for the transport activity of TAAC.

Arabidopsis TAAC was characterized in E. coli as an ATP importer in exchange for cytosolic ADP (Thuswaldner et al., 2007), and its activity was found sensitive to bongkrekic acid (Thuswaldner, S., and Spetea, C., unpublished data). Pi is not a substrate for transport by TAAC (Thuswaldner et al., 2007), implying the requirement for a separate thylakoid Pi transporter. Indeed, such a protein has been identified in Arabidopsis, and functionally characterized in yeast and E. coli (Guo et al., 2008; Pavón et al., 2008). When assessed in thylakoid membranes, TAAC transports stromal ATP into the thylakoid lumen in exchange for ADP. The direction of TAAC-mediated transport determined in both E. coli and thylakoids is opposite to the direction of transport by mitochondrial AACS (Thuswaldner et al., 2007). Therefore, to distinguish it from the mitochondrial ADP/ATP carrier, the thylakoid protein has been named ATP/ADP carrier. Through adenine nucleotide exchange, TAAC was proposed to supply ATP for nucleotide-dependent reactions in the thylakoid lumen (Spetea et al., 2004). An extensive review on the structure, function, and evolution of the MC family has become recently available and provides insights into their roles in plants (Palmieri et al., 2011). This review focuses on the evolutionary origin of the TAAC subfamily of the mitochondrial AACS, which aids in elucidating its function in the thylakoid membrane.

**WHEN AND WHERE IN THE TREE OF LIFE DID TAAC ORIGINEATE?**

We assembled a set of protein sequences with which to place TAAC in an evolutionary context. We extracted protein sequences from the curated gene families at ARAMEMNON (Schwacke et al., 2003)1 and from search results to specific clades and genomes at NCBI2 and PHYTOZOME (Goodstein et al., 2011)3, respectively. We added the best BlastP matches to AtTAAC from each major eukaryotic division of life with an E-value <10−55. We also added all 17 members of the Arabidopsis MC family (including AtTAAC) that were listed as protein sequences related to TAAC at ARAMEMNON. We also added the best BlastP hits to proteins from several green plant clades, including Bryophyta, Chlorophyta, Lycopodiophyta, and Pinophyta. An alignment of these sequences was made using MUSCLE (Edgar, 2004, implemented at http://www.ebi.ac.uk). From the alignment (not shown) we could see that only two copies in Arabidopsis (encoded by the At5g01500 and At3g51870 genes) and all other non-Arabidopsis land plant proteins in this sample possessed a partly conserved N-terminal motif, consisting of 19 amino acid residues directly upstream from the first transmembrane helix delimited by Thuswaldner et al. (2007).

For the first phylogenetic analysis we included amino acid positions 121–369 in TAAC (spanning the six transmembrane helices...
domains), which resulted in 340 aligned positions. The analysis was carried out using a Bayesian approach. We used four parallel chains of a reverse model jump protein Bayesian analysis in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001). Each chain was run for two million generations and sampled every 1,000 generations. The tree in Figure 1 shows a well-supported [posterior probability (PP) = 1.0] N-terminal containing clade (the “TAAC clade”) that includes land plants and some copies from Chlorophyta, but no proteins from other taxa. Sister to this clade with strong support (PP = 1.0) is a clade (also PP = 1.0) containing proteins from Chlorophyta that lack the N-terminal motif, including proteins from Chlamydomonas, Chlorella, and Volvox.

![Figure 1: Protein phylogeny of 340 Muscle aligned positions of selected members of the MC family.](image-url)

The analysis involved 51 protein sequences, including 17 from Arabidopsis. The root position shown is arbitrary. The mid-point root position is marked, as is the clade containing all TAAC-like sequences (boxed in red). The clade of Green Plants is marked in green. All plant sequences outside this clade are indicated by green arrows. Accession numbers follow the gene annotation (where available) and names of genera within which the proteins are found. The position of Arabidopsis TAAC is highlighted in red. The scale bar indicates substitutions per site. Posterior probabilities ≥0.90 of clades summarizing two million Markov chain Monte Carlo generations (of a reversible model jump protein Bayesian analysis in MrBayes 3.1.2) are shown above branches. The results discussed are robust to an alternative alignment (MAFFT, not shown).
The best BlastP matches to proteins from the following taxa did not contain the N-terminal motif, although they each possessed the conserved six transmembrane domain structure: Amoebozoa, Animalia, Euglenozoa, Fungi, Opisthokonta, Rhodophyta, and Stramenopiles – the latter includes brown algae and diatoms (nomenclature based on NCBI’s taxonomy). All other eukaryote taxa at one hierarchical level below Eukaryota in NCBI’s taxonomy were searched, but did not contain BlastP matches to AtTAAC with E-values \( < 10^{-55} \). Apart from TAAC, only one other protein from Arabidopsis is also a member of the TAAC clade and contains this motif, namely the one encoded by the At3g51870 gene. The amino acid sequences of these two proteins are 67% identical.

The TAAC clade contains relatively short branches in relation to the remainder of the phylogeny, making it unlikely that the root resides within the clade. This is also consistent with mid-point rooting (marked in Figure 1) as well as the phylogenetic pattern expected among the plants that carry these proteins. Together, this indicates that the TAAC clade contains several proteins orthologous to AtTAAC. This clade also contains duplicated copies (paralogs) from several plant taxa (more are seen in our expanded sample in Figure 2, details below), but these are inferred to have arisen only after green plants diverged from other eukaryotes and are expected due to the action of recent gene and/or genome duplication (e.g., in Arabidopsis, Bowers et al., 2003; legumes, Pfeil et al., 2005; Physcomitrella, Rensing et al., 2007).

The position of the remaining 15 Arabidopsis MC proteins (Figure 1) indicates that the MC protein family was present very early in eukaryote evolution. Scattered across the tree are, for example, homologs from Metazoa (Nematostella), Fungi (Tuber), Amoebozoa (Dictyostelium), Stramenopiles (Aureococcus), Euglenozoa (Leishmania), and Rhodophyta (Chondrus). However, none of these homologs are part of the TAAC clade, which contains only proteins from Chlorophyta and Streptophyta. No matches within the BlastP cut-off used were found to other photosynthetic organisms, such as Bacillariophyta (diatoms), Cyanophyta (cyanobacteria), Glaucoctystophyceae, Phaeophyceae (brown algae), or Rhodobrya (red algae).

Therefore, we conclude that TAAC-like sequences arose only a single time, after the clad including both Chlorophyta and Streptophyta (the latter including land plants) diverged from the other taxa in our sample, but earlier than the divergence of Chlorophyta and Streptophyta. The phylogenetic results are robust to an alternative alignment based on MAFFT (Katoh et al., 2002), although this is not shown here.

**HOW DID THE TAAC COPIES IN ARABIDOPSIS AND OTHER EUDICOTS EVOLVE?**

We increased the sample of TAAC-like proteins in a more focused phylogenetic inference in order to place the two Arabidopsis members of the TAAC subfamily into context. For this purpose, we sampled all copies from the species listed in Table 1 that contain the N- and C-terminal motifs similar to TAAC and that were also part of the TAAC clade. We focused on eudicots, because preliminary analyses indicated that the two Arabidopsis TAAC subfamily members were placed in a clade containing proteins from eudicot species only.
Table 1 | Copy number of TAAC-like proteins found in various eudicot plants.

| Taxon                           | Group (NCBI taxonomy) | TAAC-like copies |
|---------------------------------|-----------------------|------------------|
| Arabidopsis thaliana (L.) Heynh | Malvids               | 2                |
| Thellungiella halophila O. E. Schulz | Malvids     | 2                |
| Eucalyptus grandis W. Hill      | Malvids               | 1                |
| Carica papaya L.                | Malvids               | 1                |
| Citrus sinensis Osbeck          | Malvids               | 1                |
| Populus trichocarpa Torr and A. Gray | Fabids     | 2                |
| Medicago truncatula Gaertn      | Fabids               | 2                |
| Glycine max (L.) Merr           | Fabids               | 5                |
| Vitis vinifera L.               | Rosids incertae sedis | 1             |
| Mmulus guttatus DC              | Asterids             | 1                |
| Aquilegia caerulea E. James    | Stem eudicots        | 2                |

We expanded our search for land plant sequences containing the N-terminal motif by using BlastP with AtTAAC as the query to specific genomes in Phytozome, as well as to Thellungiella (Brassicaceae), because in earlier BlastP searches a Thellungiella sequence closely related to TAAC was detected. We excluded possible allelic variants that differed by only a single amino acid from our enumeration. The alignment of this set of sequences was performed as before. From this alignment (Figure S1 in Supplementary Material), we used amino acids 107–482 in TAAC (corresponding to 375 aligned positions and including the N-terminal and C-terminal motifs) in our second phylogenetic analysis and also added the sister group of the TAAC clade (from Figure 1) as an outgroup. The phylogenetic analysis was performed as described for the data shown in Figure 1, with sample information summarized in Table 2 and the results presented in Figure 2.

The copy number of TAAC-like proteins in the eudicots examined here appears to be rather conservative, ranging from one to five copies (Table 1). Arabidopsis, Medicago, and Populus each have only two copies, but these are perhaps the best-known eudicot genomes. Therefore, the copy number for the other species may be underestimated. The lack of support for some key nodes in the phylogeny (Figure 2) makes it difficult to pinpoint the timing of divergence of the two copies found in Arabidopsis. They almost certainly diverged sometime before this genus diverged from Thellungiella (perhaps 40–50 million years ago; Amtmann, 2009; Beilstein et al., 2010), but it is difficult to say how much earlier this divergence might have been.

Unlike the Arabidopsis copies, one of the copies found in Aquilegia differs from its closest relative by a long branch (Figure 2). In contrast, the rather recently diverged (in comparison to Arabidopsis) multiple copies found in Selaginella and Phycomitrella do not show this pattern. The long branch observed in Aquilegia may indicate functional shifts and positive selection may be involved, as has been shown in e.g., monkey pancreatic ribonucleases (Zhang et al., 2002). These possibilities could be examined further and may lead to novel research questions. However, in the case of one Glycine sequence, poor assembly is a more likely explanation for the long branch observed in that taxon (NB: alignment positions 308–389 in Figure S1 in Supplementary Material), so caution needs to be taken in specific cases.

**HOW DOES THE N-TERMINAL MOTIF VARY WITHIN THE TAAC SUBFAMILY?**

The N-terminal motif may be helpful to identify potential members of the TAAC subfamily. Furthermore, this clade shows complete conservation of the ring of four positively charged residues (Figure S1 in Supplementary Material) proposed to act as a selectivity filter for adenine nucleotides (Thuswaldner et al., 2007), confirming that these aligned proteins are all AACS.

Potentially important is that significant variation in the N-terminal sequences (beyond just the 19 residue motif described earlier) occurs among the proteins sampled here. For example, several proteins contain many charged residues, including paired charged ones, and a five-glycine repeat (Figure S1 in Supplementary Material). These may be implicated in ligand (e.g., calcium) binding and large conformational changes that could regulate their activity, as in the case of Ca2+-dependent members of the MC family (Weber et al., 1997). However, some of these proteins do not contain either of these features. For example, the Selaginella sequences have only 13 residues upstream of the more conserved N-terminal motif, few charged residues, and lack the five-glycine repeat. One Micromonas protein appears to lack the N-terminal motif altogether (Figure S1 in Supplementary Material). Variation in the N-terminal motif among the land plant TAAC-like proteins is smaller (Table 2), but so is the phylogenetic distance. It is tempting to speculate as to what the precise function of the N-terminal residues might be, moreover, interesting research questions can be formulated around these sequences. However, the sequences (especially those apparently lacking the N-terminal motif) should of course be verified before pursuing these lines of investigation, as technical problems (e.g., with the assembly), deposition of incomplete sequences, etc., need to be ruled out first.

**WHAT ARE THE POSSIBLE FUNCTIONS OF TAAC IN LAND PLANTS AND GREEN ALGAE?**

The phylogenetic analyses performed in this work indicate that TAAC is absent in cyanobacteria and that the most recent common ancestor of green plants (Streptophyta + Chlorophyta) was the earliest photosynthetic organism that we can identify to have carried a TAAC-like protein. Although TAAC is a thylakoid protein and the thylakoid membrane originated with the thylakoid membrane of an ancestral cyanobacterium, TAAC appears to be eukaryote-specific, as are all mitochondrial AACS (Palmieri et al., 2011). Nevertheless, in contrast to mitochondrial AACSs, which are found in both photosynthetic and heterotrophic eukaryotes (Palmieri et al., 2011), only some photosynthetic eukaryotes, more specifically, Chlorophyta and Streptophyta, carry TAAC-like copies. Other photosynthetic eukaryotes (red algae, brown algae, and diatoms) and, interestingly, even some Chlorophyta (Chlamydomonas and Volvox) do not appear to have such a protein, i.e., not one with a similar N-terminus nor placed in the TAAC clade, whereas other Chlorophyta do (e.g., Chlorella; Table 2).

A potential answer to the question “why do not all photosynthetic organisms carry TAAC-like proteins?” could be that TAAC may fulfill specialized functions in the thylakoid membrane of...
Table 2 | The presence (green) or absence (red) of TAAC-like proteins within the surveyed genomes is shown.

| Clade          | Species                      | Accessions used          | Annotation<sup>1</sup>                                 | N-terminal<sup>2</sup> % AA identity to TAAC | Accession source |
|----------------|------------------------------|--------------------------|----------------------------------------------------------|-----------------------------------------------|------------------|
| Eudicots       | Aquilegia caerulea           | AcoGoldSmith_v1.006656m  | MC<sup>2</sup> protein                                    | 68                                            | Phyt ozone       |
|                |                              | AcoGoldSmith_v1.006033m  | MC protein                                                | 58                                            | Phyt ozone       |
|                | Arabidopsis thaliana         | At5g01500                | Thylakoid ATP/ADP carrier (TAAC)                          | 100                                           | Phyt ozone       |
|                | Cana papaya                  | At3g51870                | MC protein                                                | 68                                            | Phyt ozone       |
|                | Citrus sinensis              | evm.TU.supercontig_11.156| MC protein                                                | 58                                            | Phyt ozone       |
|                | Eucalyptus grandis           | Glyma03g10900            | MC protein                                                | 53                                            | Phyt ozone       |
|                | Glycine max                  | Glyma03g08120            | Not available                                            |                                               |                  |
|                |                               | Glyma01g28890            | MC protein                                                | 58                                            | Phyt ozone       |
|                |                               | Glyma07g18140            | Not available                                            |                                               |                  |
|                |                               | Glyma18g42950            | MC protein                                                | 58                                            | Phyt ozone       |
|                |                               | Medtr3g166530            | Not available                                            |                                               |                  |
|                |                               | Medtr3g025560            | MC protein                                                | 58                                            | Phyt ozone       |
|                |                               | mgv1a008249m             | MC protein                                                | 47                                            | Phyt ozone       |
|                |                               | Pt0006s10070             | MC protein                                                | 68                                            | Aramemnon        |
|                |                               | Pt0016s12280             | MC protein                                                | 63                                            | Aramemnon        |
|                |                               | BAJ33944                 | MC protein                                                | 68                                            | NCBI             |
|                |                               | BAJ34636                 | MC protein                                                | 68                                            | NCBI             |
| Monocots       | Oryza sativa                 | LOC_Os01g16040           | MC protein                                                | 26                                            | Phyt ozone       |
|                | Brachypodium distachyon       | Bradi2g09790             | MC protein                                                | 37                                            | Phyt ozone       |
|                | Zea mays                      | GRMZM2G178460            | MC protein                                                | 53                                            | Phyt ozone       |
| Pinophyta      | Picea sitchens               | ABR16962                 | MC protein (NCBI)                                         | 42                                            | NCBI             |
|                | Selaginella moellendorfii     | XP_0029777864            | Putative MC protein (NCBI)                                | 42                                            | NCBI             |
|                |                               | XP_002979461             | Putative MC protein (NCBI)                                | 42                                            | NCBI             |
|                |                               | XP_001762055             | Putative MC protein (NCBI)                                | 37                                            | NCBI             |
|                |                               | XP_001767235             | Putative MC protein (NCBI)                                | 42                                            | NCBI             |
|                |                               | XP_002506340             | Putative MC protein (NCBI)                                | 15                                            | NCBI             |
|                |                               | XP_003063489             | Amyloplast brittle-1 (BT1) protein homolog (NCBI)         | Not available                                | NCBI             |
| Bryophyta      | Physcomitrella patens        | EFN55833                 | As above                                                 | 26                                            | NCBI             |
|                |                               | EFN55777                 | ADP/ATP transporter on adenylate translocase; provisional (NCBI) | Not available | NCBI             |
| Chlorophyta    | Micromonas sp. RCC229        | XP_002945883             | ADP/ATP transporter on adenylate translocase; provisional (NCBI) | Not present | NCBI             |
|                | Micromonas pusilla           | XP_001696228             | ADP/ATP transporter on adenylate translocase; provisional (NCBI) | Not present | NCBI             |
|                | Ostreococcus lucimarinus     | XP_001421607             | ADP/ATP transporter on adenylate translocase; provisional (NCBI) | No hits < 10<sup>-55</sup> | No hits < 10<sup>-55</sup> |
|                | Chlorella variabilis         | EFN55833                 | Not present                                              |                                               | NCBI             |
|                |                               | EFN55777                 | Not present                                              |                                               | NCBI             |
| Alveolata      | Volvox carteri               | XP_002945883             | ADP/ATP transporter on adenylate translocase; provisional (NCBI) | No hits < 10<sup>-55</sup> | No hits < 10<sup>-55</sup> |
|                | Chlamydomonas reinhardtii    | XP_001696228             | ADP/ATP transporter on adenylate translocase; provisional (NCBI) | No hits < 10<sup>-55</sup> | No hits < 10<sup>-55</sup> |
|                | Dictyostelium purpureum      | XP_003287896             | Putative MC protein (NCBI)                                | Not present                                  | NCBI             |

(Continued)
### Table 2 | Continued

| Clade          | Species                                           | Accessions used (Figure 2 only) | Annotation\(^1\)                                                                 | N-terminal\(^2\) % AA identity to TAAC | Accession source |
|---------------|---------------------------------------------------|---------------------------------|----------------------------------------------------------------------------------|----------------------------------------|-----------------|
| **Euglenozoa**| *Leishmania mexicana*                              | CBZ25891                        | Putative MC protein (NCBI)                                                       | No hits < 10\(^{-5}\)                  | Not present     |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  | NCBI            |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
| **Fornicata** |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
|               |                                                   |                                 |                                                                                  | No hits < 10\(^{-5}\)                  |                 |
| **Glaucocystophyceae** | *Capsaspora owczarzaki*                          | EFW43855                        | MC protein (NCBI)                                                                | Not present                           | NCBI            |
| **Haptophyceae** |                                                   |                                 |                                                                                  | Not present                           | NCBI            |
| **Jakobida**  |                                                   |                                 |                                                                                  | Not present                           | NCBI            |
| **Katablepharidophyta** | *Trichoplax adhaerens*                          | XP_001626526                    | Putative MC protein (NCBI)                                                       | Not present                           | NCBI            |
| **Malawimonadidae** |                                                 |                                 |                                                                                  | Putative MC protein (NCBI)             | Not present     |                 |
| **Opisthokonta** | *Nematostella vectensis*                          | XP_002114403                    | Putative MC protein (NCBI)                                                       | Not present                           | NCBI            |
|               | *Tubulostoma adhaerens*                           | XP_002837709                    | Putative MC protein (NCBI)                                                       | Not present                           | NCBI            |
|               | *Xenopus tropicalis*                              | NP_001072712                    | MC protein (NCBI)                                                                | Not present                           | NCBI            |
|               | *Metathrixium acidum*                             | EFY93016                        | Putative MC protein (NCBI)                                                       | Not present                           | NCBI            |
|               |                                                   |                                 |                                                                                  | Not present                           | NCBI            |
|               |                                                   |                                 |                                                                                  | Not present                           |                 |
|               |                                                   |                                 |                                                                                  | Not present                           |                 |
| **Oxymonadida** | *Chondrus crispus*                                | ABV25602                        | Putative MC protein (NCBI)                                                       | Not present                           | NCBI            |
| **Parabasalia** |                                                   |                                 |                                                                                  | Putative MC protein (NCBI)             | Not present     |                 |
| **Rhizaria**  | *Gracilaria gracilis*                             | AAD17310                        | Putative MC protein (NCBI)                                                       | Not present                           | NCBI            |
| **Rhodophyta**|                                                   |                                 |                                                                                  | Putative MC protein (NCBI)             | Not present     |                 |
| **Stramenopiles** | *Phaeophytae infestans*                          | XP_002906079                    | Putative MC protein (NCBI)                                                       | Not present                           | NCBI            |
|               |                                                   |                                 |                                                                                  | Putative MC protein (NCBI)             | Not present     |                 |
|               |                                                   |                                 |                                                                                  | Putative MC protein (NCBI)             | Not present     |                 |
| **Bacteria (including cyanobacteria)** |                                                 |                                 |                                                                                  | Putative MC protein (NCBI)             | Not present     |                 |

The taxon, species of origin, accession numbers, annotation, and sequence identity of the N-terminus relative to the Arabidopsis TAAC are indicated. The information about Arabidopsis TAAC is highlighted in bold. The source of these data is given in the last column: Phytozome, ARAMEMNON, or NCBI. Not available, incomplete gene model (no start codon); not present; the sequence appears to be complete, but lacks anything resembling the 19 amino acid N-terminal motif common to most taxa with genes in the TAAC clade. Green = taxa with genes in the TAAC clade. Red = taxa without genes in the TAAC clade. E-value for BlastP (http://blast.ncbi.nlm.nih.gov) against Arabidopsis thaliana TAAC. For clades where TAAC type sequences are not found, the best hit (September 2011) is recorded instead, down to a threshold of 10\(^{-5}\). We assume that matches with E-value < 10\(^{-5}\) will not be closely related to the TAAC clade, given that many matches better than this are also not part of this clade. “Putative” annotations include “hypothetical” annotations and annotations based on conceptual translations. 1Annotations come from Phytozome unless stated otherwise in the table. 2N-terminal refers to the 19 residue N-terminal motif discussed in the text. 3MC, mitochondrial carrier.

The differences in thylakoid membrane organization among cyanobacteria, green algae, and land plants may have implications for biogenesis and turnover of photosynthetic complexes, such as photosystem II (PSII). The biosynthesis of complexes in cyanobacteria occurs at contact sites with the plasma membrane, whereas PSII repair takes place within thylakoid membranes (Zak et al., 2001). In green algae, the site of PSII assembly during de novo D1 synthesis is around a so-called pyrenoid, a specialized sub-compartment within the chloroplast for CO\(_2\) fixation that is different from the site of PSII repair—the stroma-regions of the thylakoid membrane (Uinta and Zerges, 2007). In land plants, the location of thylakoid membrane complex biosynthesis is uncertain. A widely accepted model is that PSII subunits assemble in the non-appressed region of the thylakoid membrane (Baena-González and Aré, 2002). The mechanism of PSII repair in plants has been studied in detail and shown to have important
FIGURE 3 | Evolution of thylakoid membrane organization and composition. Cyanobacterial thylakoid membranes (shown in blue) are located directly in the cytoplasm, are arranged in multiple (three to eight) layers, and make contacts with the plasma membrane. They have attached phycobilisomes for harvesting light, which protrude toward the cytoplasm, thus preventing membrane stacking (Liberton and Pakrasi, 2008). Chloroplasts have evolved via primary symbiosis from a cyanobacterial relative. Thus, similar arrangement of the thylakoid membranes is observed in red algae (shown in red), which also have attached phycobilisomes (Csekos et al., 1996). Thylakoid membranes in diatoms and brown algae (shown in brown) are arranged in groups of three loosely appressed vesicles and contain fucoxanthin–chlorophyll–protein complexes for harvesting light (Bertrand, 2010). Note the four-layer envelopes surrounding the chloroplast as compared to the types found in other algae and plants, as a result of secondary symbiosis. Thylakoid membranes in green algae and higher plants (shown in green) are located inside the chloroplast, are organized in grana stacks (5–20 vesicles) interconnected by stroma-exposed lamellae, and contain chlorophyll–protein complexes for harvesting light (Mustardy and Garab, 2003). Various 3D models of the organization of these thylakoid membranes have been proposed and debated (Daum and Kühlbrandt, 2011). According to the most accepted model, based on electron tomography (Daum et al., 2010; Austin and Staehelin, 2011), grana should be seen as stacks of straight, exactly parallel pairs of thylakoid membranes, each pairs of grana disks merging with stroma lamellae by staggered membranes protrusions. Importantly, but not shown in this figure, the plane of the stromal thylakoids is tilted by 10–25˚ relatively to the plane of the grana stack so that the stromal thylakoids display a helix around the granum (Mustardy et al., 2008). The photosynthetic apparatus is composed of four multisubunit complexes, namely the water-oxidizing photosystem II (PSII), cytochrome b6/f (cytbf), photosystem I (PSI), and the H+-translocating ATP synthase (CF0F1; Nelson and Ben-Shem, 2004). These complexes are laterally distributed in green algae and plants, whereas in other algae and diatoms, they display a more uniform distribution.

differences from that in cyanobacteria, such as the shuttling of PSII subcomplexes between the grana and stroma membranes, which is accompanied by changes in the oligomeric structure of the complex (Mulo et al., 2011).

The question arising is whether TAAC plays a role in the biogenesis and turnover of plant photosynthetic complexes. Its tissue expression pattern has been studied in detail, and supports this possibility, as described below. AtTAAC was found highly expressed in young photosynthetic organs, such as developing leaves, flower buds, and green siliques. Furthermore, it was found expressed in etiolated seedlings, similar to the thylakoid HCF136 protein, which was proven to be required for biogenesis of thylakoids and PSII assembly (Meurer et al., 1998; Plücken et al., 2002). TAAC expression is strongly upregulated in leaves...
undergoing senescence or exposed to wounding, light stress, oxidative stress, salt stress, and desiccation, pointing to an additional role in supplying ATP for energy-dependent processes (e.g., proteolysis, folding) during turnover of photosynthetic complexes.

Thus far, no dedicated studies have been undertaken on the role of TAAC activity during thylakoid biogenesis. Nevertheless, its role during PSII repair has been studied in more detail by taking advantage of taac mutants. Remarkably, the mutants grew slower and were more sensitive to high light stress due to an inability to degrade the reaction center D1 protein (Yin et al., 2010). A model for its function during PSII repair cycle in plants under high light stress is presented in Figure 4. Briefly, ATP translocated by TAAC into the lumen is converted to GTP, which is then bound to the PsbO luminal extrinsic subunit of the PSII dimeric complex (Spetea et al., 2004; Lundin et al., 2007a). The GTPase activity of this protein regulates the monomerization and partial disassembly of PSII, pre-requisite steps for the proteolytic degradation of the D1 protein (Lundin et al., 2007b). Replacement of the damaged D1 with a new copy requires coordination between its degradation, synthesis, insertion, and assembly of the new copy in the PSII complex. We propose that the novel mechanism of ATP transport and GTP signaling across thylakoid membranes, discussed above, may be a plant-specific feature for the following reasons: the D1 degradation is GTP dependent in plants but not in cyanobacterial thylakoids (Spetea et al., 1999); GTP binding to PsbO may be a plant-specific feature, since putative GTP binding sequence domains are not found conserved in cyanobacteria or the green alga Chlamydomonas PsbOs (Lundin et al., 2007a); TAAC belongs to the MC family, which is eukaryotic specific (Palmieri et al., 2011). Thus, we speculate that TAAC is associated with the need for highly controlled regulation of PSII repair in a highly stacked thylakoid membrane system requiring shuttling of complexes between the appressed and non-appressed regions.

CONCLUSION

Study of the adenine nucleotide carrier TAAC in chloroplast thylakoids is interesting because it has revealed similarities but also differences from classical mitochondrial AACs. Most MC (including AACs) are present in both phototrophic and heterotrophic eukaryotes, suggesting functions required for basic eukaryotic processes. On the other hand, TAAC-like proteins have been found only in green plants, indicating at the earliest an appearance after the endosymbiosis of a phototrophic prokaryote with the most recent common ancestor of land plants and green algae. Essentially, TAAC is an AAC with additional N (and C) motifs, apart from the chloroplast targeting sequence. The pathway by which TAAC arrived in the chloroplast and the precise function of the N-motif in TAAC activity remain to be investigated. Nevertheless, based on the phylogenetic study presented here, we propose that TAAC may fulfill functions that may be required in the context of the more complex organization of thylakoid membranes in green algae and land plants, such as biogenesis and repair of photosynthetic complexes. Some of the luminal enzymes participating in the biogenesis/turnover processes may require ATP or other nucleotides resulting from inter-conversion, and therefore ATP must be translocated. This would not be unexpected, taking into consideration the increasing evidence for a complex role of the thylakoid lumen in photosynthetic regulation and plant cell signaling, expanding its function beyond an energetic perspective.

**FIGURE 4 | Proposed model for role of the thylakoid ATP/ADP carrier (TAAC) during photosystem II (PSII) repair cycle in plants.**

The plant thylakoid membrane is organized in grana stacks (appressed region) and stroma-exposed (non-appressed region) lamellae. The composition of the thylakoid membrane is heterogenous, although its luminal space is continuous. TAAC is located in the stroma lamellae and exchanges stromal ATP for luminal ADP. ATP is inter-converted to GTP by the luminal nucleoside diphosphate kinase (NDPK). The active PSII dimer is located in the grana regions and contains luminal extrinsic PsbO proteins. During illumination with excess light, the reaction center D1 protein may be oxidatively damaged and needs to be replaced. The PsbO subunit of the damaged monomer binds and hydrolyzes GTP, leading to its dissociation and partial disassembly of the monomeric complex on the way to the stroma-exposed regions. Here the D1 protein is degraded and replaced with a new copy synthesized by the chloroplast ribosomes and co-translationally inserted in the membrane. Monomers assemble into the dimers and migrate to the grana regions.
(for a recent review, see Spetea, 2011). Thus, learning about TAAC evolution can be very important to understand its functional importance in green plants.

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SUPPLEMENTARY MATERIAL
The Supplementary Material for this article can be found online at http://www.frontiersin.org/Plant_Physiology/10.3389/fpls.2011.00110/abstract

Figure S1 | MUSCLE alignment of TAAC subfamily of full-length amino acid sequences.

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