Short Communication

Evolution of Chloroplast Vesicle Transport

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Vesicle traffic plays a central role in eukaryotic transport. The presence of a vesicle transport system inside chloroplasts of spermatophytes raises the question of its phylogenetic origin. To elucidate the evolution of this transport system we analyzed organisms belonging to different lineages that arose from the first photosynthetic eukaryote, i.e. glaucocystophytes, chlorophytes, rhodophytes, and charophytes/embryophytes. Intriguingly, vesicle transport is not apparent in any group other than embryophytes. The transfer of this eukaryotic-type vesicle transport system from the cytosol into the chloroplast thus seems a late evolutionary development that was acquired by land plants in order to adapt to new environmental challenges.

Keywords: Chloroplast evolution — Thylakoid biogenesis — Vesicle transport.

Abbreviation: PP1, protein phosphatase 1.

Vesicle traffic is a common phenomenon in transport systems of eukaryotic cells but it does not exist in prokaryotic organisms. Nevertheless vesicle-like structures have been observed since early electron microscopic studies in the stroma of higher plant chloroplasts, an organelle that derived from a prokaryotic ancestor (von Wettstein 1958, Mühlethaler and Frey-Wyssling 1959). The exact role of this transport system remains to be clarified but one possible function is the maintenance of the thylakoid membrane system in mature chloroplasts where a connection between the thylakoids and the inner envelope does not exist (Douce and Joyard 1996, Morre et al. 1991, Kroll et al. 2001, Westphal et al. 2001). Using established inhibitors of eukaryotic vesicle traffic it was shown that the chloroplast vesicle transport system utilizes a fusion machinery similar to homotypic membrane fusion in yeast (Morre et al. 1991, Kroll et al. 2001, Westphal et al. 2001) which comprises calmodulin and a protein phosphatase 1 (PP1) (Peters and Mayer 1998, Peters et al. 1999). That vesicle transport is not described for cyanobacteria, the phylogenetic ancestors of chloroplasts, implies that the system is of eukaryotic origin, e.g. cytosolic, and was transferred to the organelle sometime during the evolution from the first photosynthetic eukaryote to the spermatophytes. Thus the function of chloroplast vesicle transport might be closely related to a phylogenetic development of the chloroplasts after the creation of this organelle. All chloroplasts originated from a singular endosymbiotic event that created the first chloroplast-harboring eukaryotic cell. From this cell all other photosynthetic eukaryotes arose (Margulis 1970, Palmer 2000). During the evolution of photosynthetic eukaryotes the thylakoid membrane system underwent a development of its structure from simple membranes to an intertwined membrane network of grana stacks and stroma lamellae. The aim of this study was to determine the onset of chloroplast vesicle transport in evolution and to find a connection between the state of chloroplast development and the acquisition of vesicle transport. We thus analyzed chloroplasts of a number of organisms, representing different steps in the evolution of photosynthetic eukaryotes (Fig. 1), for their thylakoid structure and the presence of chloroplast vesicle transport.

Vesicles can be observed occasionally in ultrathin sections of Arabidopsis thaliana and Pisum sativum chloroplasts even without the application of fusion inhibitors (Morre et al. 1991, Kroll et al. 2001, Westphal et al. 2001). Treatment of leaf discs or isolated chloroplasts with microcystin, an inhibitor of protein phosphatase 1 and 2a, or ophiobolin, a calmodulin inhibitor, results in a considerable accumulation of vesicles (Westphal et al. 2001). We thus included in our screening a treatment of all specimens with inhibitors in order to look for potential vesicle accumulation after membrane fusion inhibition. To be thorough, we also included cyanobacteria, the evolutionary ancestor of the chloroplasts. Fig. 2a shows an ultrathin section of Synechocystis sp. as a representative of the cyanobacteria. All cyanobacteria contain very simply arranged thylakoids and there is no visible differentiation in the thylakoid membrane structure. Instead, all thylakoids appear in a concentric or parallel order. We could not observe vesicle-like structures either in Synechocystis or in Chroococcidiopsis thermalis (Fig. 2a and data not shown), in accordance with the fact that no vesicle transport has been described for these prokaryotic organisms. Cyanobacteria do contain protein phosphatases, including a homologue to PP1. Yet this class of proteins is involved in many different cellular processes. Also, the cyanobacterial PP1 is not inhibited by microcystin (Shi et al. 1999). A phylogenetic analysis of the PP1 proteins in the genome of
Fig. 1  Schematic outline of the evolution of photosynthetic eukaryotes. The first chloroplast-harboring eukaryote gave rise to three different lineages, two of which further created organisms with complex plastids by secondary endosymbiosis. Land plants developed from a single lineage. Organisms from all three lineages as well as cyanobacteria were analyzed for the presence of vesicle transport. The gray box indicates groups where chloroplast vesicle transport was found.
A. thaliana indicated that no true homologue to the microcystin-resistant PP1 from Synechocystis exists in this plant. Instead the Arabidopsis PP1 proteins are closely related to the eukaryotic PP1s. This leads to the conclusion that the membrane fusion machinery inside of chloroplasts is of eukaryotic origin. The genomes of Synechocystis sp. 6803 and Anabaena sp. PCC7120, both of which have been sequenced, furthermore do not contain any genes coding for proteins typically involved in eukaryotic vesicle traffic, i.e. N-ethylmaleimide sensitive factors (NSF), soluble NSF attachment proteins, soluble NSF attachment protein receptors, or calmodulins (Bock et al. 2001). Due to the absence of both, visible vesicle-like structures and appropriate genes, we conclude that the chloroplast vesicle transport system was not inherited from its prokaryotic ancestor. Instead it must have developed after the endosymbiotic event inside the newly arising cell organelle.

We continued our analysis with two representatives of the glaucocystophytes lineage, Glaucocystis nostochinearum and Cyanophora paradoxa (Fig. 2b and data not shown). Glaucocystophytes are primitive eukaryotic algae whose chloroplasts are still surrounded by a peptidoglycan layer between their envelope membranes. They are believed to be the closest relative to the first photosynthetic eukaryote (Palmer 2000). Their thylakoid membranes very much resemble the structures found in cyanobacteria and we found no indication for vesicle-like structures in the chloroplasts. Simply arranged thylakoids are also a common feature of all rhodophytes, like Antithamnion plumula (Fig. 2c), and are conserved in the organisms containing complex plastids that derived by secondary symbiosis from the red algae lineage (Fig. 1, 2d, e). In neither of these organisms could we observe any membrane vesicles even after a treatment with microcystin or ophiobolin.

In most organisms of the chlorophytes lineage, including euglenoids and chloroarachneophytes, the thylakoid structure remains quite simple (Fig. 1, 2f). In some green algae, e.g. Chlamydomonas reinhardtii and Actinastrum gracillium, the thylakoid membranes appear closely spaced, a feature that is considered as an early form of grana stacking (Stefansson et al. 1997). While in green algae we have the onset of the complex thylakoid structure known from higher plant chloroplasts, we could not detect vesicle transport in this lineage either. From the results presented so far we deduce that the acquisition of chloroplast vesicle transport with a eukaryotic type fusion machinery must have taken place somewhere within the third lineage, the streptophytes.

This lineage gave rise to a group of algae, the charophytes (Fig. 1). On a cellular level the charophytes are highly organised multicellular organisms. With regard to their chloroplasts, the distinction between stroma lamellae and grana stacks has become more pronounced (Fig. 2g). However, the thylakoids are still mostly present as long parallel membranes. We examined three species from three different orders within the charophytes, including Chara sp. and Coleochaete scutata, but we found no indication for vesicle-like structures, with or without an inhibitor treatment of the specimen (Fig. 2g).

The streptophytes lineage also gave rise to all land plants,
the embryophytes (Fig. 1). The embryophytes are diverged into the bryophytes, the pteridophytes, and the spermatophytes (Qui and Palmer 1999). Bryophytes are believed to represent the closest extant relatives of the first land plants. However, whether liverworts, mosses, or hornworts represent the earliest form of land plants remains unresolved. Recent phylogenetic analyses strongly favour the liverworts as the oldest of these groups (Qui and Palmer 1999). Somewhere on the way from a common ancestor of today’s charophytes and bryophytes the transition from submerged living organisms to land dwellers occurred but we know little about how this transition took place. From the bryophytes onwards the thylakoid membrane system inside the chloroplasts changes into the intertwined network of grana stacks connected by stroma lamellae that is characteristic for the chloroplasts of higher plants (Fig. 3a–e).

When investigating Marchantia polymorpha, supposedly the most ancient representative of the bryophytes, we instantly could observe sporadic vesicles even without the application of inhibitors. More vesicles accumulated when leaves were treated with microcystin or ophiobolin indicating that a vesicle transport system similar to that in the chloroplasts of spermatophytes is present in this organism (Fig. 3a). The same phenomenon was observed in the fern Sphagnum sp. (Fig. 3b) and in several other pteridophytes, such as Psilotum triquetrum (Fig. 3c), Lycopodium hippocus, and Ceratopteris sp. We also observed vesicles and inhibitor-induced vesicle accumulation in the gymnosperms Ginkgo biloba, Gnetum sp., Cycas revoluta, and Araucaria heterophylla (Fig. 3d) as well as in several angiosperms. The latter included monocotyledons such as Vallisneria spiralis as well as the dicotyledons A. thaliana (Fig. 3e), Utricularia sp., and P. sativum. Vesicle accumulation after membrane fusion inhibition by low temperatures, a phenomenon described for certain types of eukaryotic vesicle transport (Morre et al. 1989), had been observed previously in P. sativum, Nicotiana tabacum, Glycine max, and Spinacia oleracea (Morre et al. 1991). Not a single exception was found in this study. We thus propose that chloroplast vesicle transport with a membrane fusion machinery resembling homotypic membrane fusion in yeast does exist in all organisms that belong to the embryophytes (Fig. 1 grey box).

In our study we could find no indication for vesicles in mature chloroplasts of any algae including members of the charophytes. Our results strongly indicate that a membrane fusion machinery utilising calmodulin and protein phosphatase was transferred to the chloroplasts after the division of the charophytes and the embryophytes and prior to the evolution of the first extant bryophytes. The evolutionary development of the bryophytes also represents the transition from life submerged under water to life in a non-aqueous environment. To clarify whether this drastic environmental change has an influence on the vesicle transport in chloroplasts, we investigated different species belonging to the bryophytes, the pteridophytes, and the spermatophytes that have secondarily returned into the water. Chloroplasts of Riccia fluitans, Salvinia natans as well as Utiri-
cularia sp., respectively, displayed a thylakoid structure that resembles their land plant relatives. Furthermore, we observed inhibitor-induced vesicle accumulation in all three species (data not shown). Thus chloroplast vesicle transport seems to represent a specific evolutionary development.

The question remains as to why the chloroplasts had to acquire a vesicle transport system at exactly this point in evolution. We cannot rule out entirely the possibility that vesicle transport is also present in certain algae. Vesicles have been described in the greening etioplasts of C. reinhardtii (Hooper et al. 1991) but whether they represent part of a vesicles transport system remains to be elucidated. In this study we could observe inhibitor-induced vesicle accumulation only in mature chloroplasts, even in the embryophytes, and not at all in any algae. It is noteworthy to keep in mind that the evolution of the embryophytes represents the transition to life in a non-aqueous environment. At the same time a complex tissue organization was developed. This coincided with the manifestation of a complex thylakoid structure. The photosynthetic apparatus had to be adapted to novel environmental conditions, which could have led to the strong lateral heterogeneity of appressed grana and non-appressed stroma thylakoids as present in the chloroplasts of all embryophytes. The maintenance of this complex and highly organized membrane network might have required the establishment of a vesicle transport system. Vesicle transport is not the only feature that the chloroplast acquired at this stage of plant evolution. For example plastidal RNA editing was originally thought to have arisen after the split of the bryophytes but was later shown to exist in this group and it has been proposed that it was a direct effect of the landing of plants as a means to adapt to various environmental circumstances (Yoshinaga et al. 1996). Chloroplast vesicle transport could be just another way to adapt to environmental challenges of a life outside the water.

The following organisms were included in this study: Synecocystis sp. PCC 6803 and Chroococcidiopsis thermals (Cyanobacteria), Cyanophora paradoxa and Glaucoctystis nostochinearum (Glaucoctystophytes), Antithamnion plumula and Audouinella hermani (Rhodophytes), Cryptomonas sp. (Cryptomonadids), Coccolithophorales sp. (Haptophytes), Nitzschia frustulum, Ochromonas danica, Fucus sp. and Ectocarpus siliculosus (Heterokonts), Actinoprasrum gracilis, Ankistrodesmus gracilis, Chlamydomonas reinhardtii, Chlorella kessleri and Halochlorococcum operculum (Chlorophytes), Chlorarachniophytes, Asista longa and Euglena gracilis (Euglenoids), Closterium ehrenbergii, Coleochaete scutata and Chaura sp. (Charophytes), Spirogyra sp. (Mosses, Bryophytes), Riccia fluitans and Marchantia polymorpha (Liverworts, Bryophytes), Lycopodium huppirus and Selaginella canescens (Lycopods, Pteridophytes), Psilotum triquetrum (Psilotaceae, Pteridophytes), Adiantum tennen, Nephrolepis exaltata and Salvinia natans (Ferns, Pteridophytes), Ginkgo biloba, Gnetum sp., Cycas revoluta and Araucaria heterophylla (Gymnosperms, Spermatophytes), Vallisneria spiralis and Hordeum vulgare (Monocotyledons, Angiosperms, Spermatophytes), Utricularia sp., Pism sativum and Arabidopsis thaliana (Dicotyledons, Angiosperms, Spermatophytes).

The organisms used in this study were either obtained from the ‘Culture Collection of Algae at the University of Göttingen’ or from the Botanical Garden in Kiel.

For transmission electron microscopic studies all unicellular organisms were used directly while plant tissue was cut into pieces of 1 mm² prior to use. The samples were incubated for 30 min at 4°C and then fixed at 4°C overnight in 0.1 M phosphate buffer (pH 7.2), containing 4% glutaraldehyde, followed by post-fixation in 2% OsO₄ overnight. The samples were dehydrated in a graded acetone series and embedded in Epon 812 according to standard procedures. Ultrathin sections were cut with a diamond knife and put on single-hole grids covered with a Formvar film. They were stained by incubation in 2% aqueous uranyl acetate for 10 min, followed by a 5-min incubation in lead citrate. The sections were viewed under a Philips CM10 electron microscope. For the analysis of membrane fusion inhibition the samples were incubated with 100 μM microcystin LR and 100 μM ophiobolin A for 10 min at 4°C. Incubation was continued at room temperature for 30 min before the samples were fixed in glutaraldehyde as described above.

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References


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