The vesicle inducing protein in plastids (VIPP1) is an essential protein for the biogenesis of thylakoids in modern cyanobacteria, algae, and plants. Although its exact function is still not clear, recent work has provided important hints to its mode of action. We believe that these data are consistent with a structural role of VIPP1 within thylakoid centers, which are considered as sites from which thylakoid membranes emerge and at which the biogenesis at least of photosystem II is thought to occur. Here we present a model that may serve as starting point for future research.

The structure and function of the major photosynthetic complexes in thylakoid membranes are well characterized. However, the biogenesis of thylakoid membranes and of most of the major complexes it harbors is barely understood. An important role in the biogenesis of thylakoid membranes, but not of the protein complexes it harbors, was suggested for the vesicle inducing proteins in plastids (VIPP1). However, this assignment was recently challenged by several reports. Based on current knowledge, we will in the following construct a model for a role of VIPP1 as a dynamic, structural component of thylakoid centers.

In cyanobacteria, the biogenesis at least of photosystem (PS) II appears to take place at distinct sites referred to as thylakoid centers. Thylakoid centers are located close to the cytoplasmic membrane and are situated at the origin of multiple thylakoid membrane layers (Fig. 1A). They contain rod-shaped structures of 50- to 1000-nm length and 30- to 50-nm width that consist of ring-like structures with ~14-fold symmetry (Figs. 1B and 1C). Similar structures, referred to as microtubule-like structures (MTLs), have been observed in early electron microscopy studies in plastids of diverse algal and plant species (Fig. 2A). MTLs have diameters of 15 to 40 nm, reach lengths of up to 5 µm, and appear to bridge inner envelope and thylakoid membranes.

VIPP1 is conserved from cyanobacteria to higher plant chloroplasts. However, it is absent in some Prochlorococcus marinus strains that do contain thylakoid membranes. VIPP1 was found to form large ring-like structures of up to 2 MDa that have diameters of 25- to 37-nm and 12- to 17-fold symmetries. VIPP1 rings can further organize into rods, which may have lengths of up to 1.4 µm (Fig. 2B). Hence, VIPP1 rings/rods strongly resemble MTLs and the structures found within the cyanobacterial thylakoid centers. We hypothesize that MTLs might correspond to plastidic thylakoid centers and that these have VIPP1 as a central structural component (Fig. 3). This idea is supported by the following observations:

Already in the first publication on VIPP1 (then termed M30), this protein, VIPP1 by immunogold analysis was detected in clusters at thylakoids and at the inner envelope of pea chloroplasts. GFP-tagged VIPP1 in Arabidopsis was found to organize into rod-shaped structures forming along the inner envelope. Chloroplasts in Arabidopsis vip1 knock-down lines had empty stromal spaces, as if the connection between the thylakoid network and the envelope was lost. Using immunofluorescence, VIPP1...
within the chloroplast of *Chlamydomonas* cells was found to localize to distinct spots sometimes extending into rods. In chloroplasts of *VIPPI*-amiRNA/RNAi cells, harboring only ~25% of wild-type VIPP levels, aberrant prolamellar body-like structures (PBLs) were consistently observed at the origin of multiple thylakoid membrane layers, which appear to coincide with the immunofluorescent VIPP1 spots. PBLs were also found in dark-grown *Chlamydomonas* yellow-in-the-dark mutant cells that are deficient in the light-independent protochlorophyllide reductase. These data suggested that a reduction in VIPP1 levels impairs thylakoid center formation and function, thereby leading to the aggregation of photosystem assembly intermediates in the PBLs. Accordingly, levels of both photosystems were reduced by ~20% in *Chlamydomonas* VIPP1-RNAi strains, while thylakoid membranes in low light-grown cells appeared unaffected in their ultrastructure and lipid composition. Similarly, depletion of VIPP1 in *Synechocystis* first led to a loss of the photosystems from thylakoid membranes before thylakoid membranes themselves were affected. Further support for a role of VIPP1 within thylakoid centers in photosystem biogenesis comes from the observation that VIPP1 interacts with the membrane integrase ALB3.2, whose depletion by RNAi also led to a reduced accumulation of both photosystems.

VIPP1 rods and rings are dynamically (dis)assembled by the chloroplast HSP70B-CDJ2-CGE1 chaperone system (Fig. 3). VIPP1 also interacts with chloroplast HSP90C and co-suppression of the *Arabidopsis* HSP90C homolog resulted in a reduced accumulation of thylakoids and photosynthetic complexes and increased levels of VIPP1 oligomers. These results indicate that the dynamic assembly and disassembly of VIPP1 by the chaperones is required for its function. Accordingly, GFP-tagged VIPP1 in osmotically stressed chloroplasts was found to be highly mobile in the stromal region, such that VIPP1 supercomplexes were formed at the expense of preexisting ones. An interplay between VIPP1/HSP70B/CDJ2 and the membrane integrase ALB3.2 was also suggested by the finding that downregulation of ALB3.2 led to the upregulation of VIPP1 and the chaperones.

Finally, there is also evidence for a role of VIPP1 in supplying structural lipids, like phosphatidyl glycerol (PG), to PSII: 1) thylakoid membranes in *Chlamydomonas* VIPP1-RNAi cells grown on ammonium and exposed to high light intensities swell, suggesting a structural defect in these thylakoid membranes. This may be caused by a reduced ability of PSII to arrange in semicrystalline PSII-LHCII arrays caused by a reduced content in structural lipids in PSII. This scenario is also suggested by a reduced cooperativity of PSII centers in VIPP1-RNAi cells. 2) The integrity of the QA site of PSII is impaired in VIPP1-RNAi cells; in cyanobacterial PSII, PG depletion affected the integrity of the QB site. 3) PSII in VIPP1-RNAi cells is more thermosensitive, supporting the notion of a structural defect in this photosystem.
We speculate that VIPP1 might directly transport lipids to thylakoid centers during photosystem biogenesis. Alternatively, in analogy to eisosomes in the plasma membrane, VIPP1 might create microdomains enriched in specific lipid species, like PG (Fig. 3). Eisosomes are furrow-like invaginations of the plasma membrane enriched in sterols that in yeast are formed by the Pil1 and Lsp1 proteins. Both proteins also form large rods resembling VIPP1 rods that are able to bind and tubulate liposomes in vitro. The specific lipid environment created in microdomains formed by eisosomes...
attracts specific integral membrane proteins, e.g., the arginine transporter Can1, whose efficient function depends on the specific lipid environment present in these microdomains. The enrichment of specific lipids like PG in a membrane microdomain putatively formed by VIPP1 may facilitate their incorporation as structural lipids during photosystem biogenesis. Moreover, translocases and integrases like ALB3, Sec, and Tat that are required for photosystem biogenesis may be enriched and their effective function dependent on the lipid environment in such microdomains (Fig. 3). In support for such a scenario, VIPP1 and its bacterial ancestor PsPa improved bacterial Tat – and Sec-mediated protein secretion. The activity of the bacterial Sec translocase was shown to strictly require the presence of anionic phospholipids and is stimulated by non-bilayer lipids. In this regard it is interesting that PsPa was shown to bind PG and phosphatidylserine and to interact with TatA. Finally, the addition of recombinant VIPP1 to thylakoid membranes was shown to increase Tat-mediated protein import in vitro, although VIPP1 did not directly interact with import substrate or Tat components.

VIPP1 is strongly induced by high light, and the repair of PSI after photoinhibition was retarded in VIPP1-RNAi strains. An attractive hypothesis is that VIPP1 newly synthesized at high light intensities would form additional thylakoid centers/microdomains to form sites for PSI repair. That plastidic HSP70B may catalyze this process is indicated by its strong inducibility by high light and the finding that HSP70B levels correlate with the ability of cells to recover from photoinhibition.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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