

# Lipid trafficking between the endoplasmic reticulum and the chloroplast

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## Abstract

The photosynthetic (thylakoid) membrane of plants is one of the most extensive biological cell membrane systems found in nature. It harbors the photosynthetic apparatus which is essential to life on earth as carbon dioxide is fixed and atmospheric oxygen released by photosynthesis. Lipid biosynthetic enzymes of different subcellular compartments participate in the biogenesis of the thylakoid membrane system. This process requires the extensive exchange of lipid precursors between the chloroplast and the endoplasmic reticulum (ER). The underlying lipid trafficking phenomena are not yet understood at the mechanistic level, but genetic mutants of the model plant *Arabidopsis thaliana* with disruptions in lipid trafficking between the ER and the chloroplast have recently become available. Their study has led to the identification of components of the lipid transfer machinery at the inner chloroplast envelope.

## Introduction

Compared to animal cells, plant cells contain an additional subcellular compartment, the chloroplast, with its extensive photosynthetic membrane system (thylakoids). The extent of this membrane system is so great that plants cannot afford to rely exclusively on phospholipids as components of their membranes. Phosphorus in the form of phosphate is limiting to plant growth and more than 50% of total polar lipids found in a green leaf are represented by non-phosphorous galactoglycerolipids (Dörmann et al. 2002). Despite the presence of these large amounts of galactoglycerolipids in plants, more than 35% of the organic phosphorus is bound in the phospholipids of the plant cell membranes (Poirier *et al.* 1991). Although plant-type galactoglycerolipids are generally absent from animal cells, they share the diacylglycerol (DAG) backbone with phosphoglycerolipids prevalent in non-photosynthetic bacteria and eukaryotes. Moreover, a central metabolite in the biosynthesis of glycerolipids in all organisms is phosphatidic acid (PA). This phospholipid is not only a biosynthetic intermediate, but it is also known to play a regulatory role in intracellular signaling processes (English 1996; Munnik 2001). As will be discussed below, PA might be the substrate of a transporter at the inner chloroplast envelope, which is crucial for the trafficking of lipids from the ER to the thylakoids.

The glycosyltransferases, MGD1 and DGD1, involved in the biosynthesis of the bulk of the two major galactoglycerolipids in plants, monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) respectively, have been identified (Shimojima et al. 1997; Dörmann et al. 1999) and much is known about their biochemistry (Benning et al. 2005). In *Arabidopsis*, the MGDG synthase MGD1 is associated with the outside of the inner chloroplast envelope (Awai et al. 2001; Xu et al. 2005) and the DGDG synthase DGD1 with the outside of the outer chloroplast envelope (Froehlich *et al.* 2001) as shown in Figure 1. This arrangement of the two major enzymes of galactoglycerolipid biosynthesis raises the question, how lipid precursors move

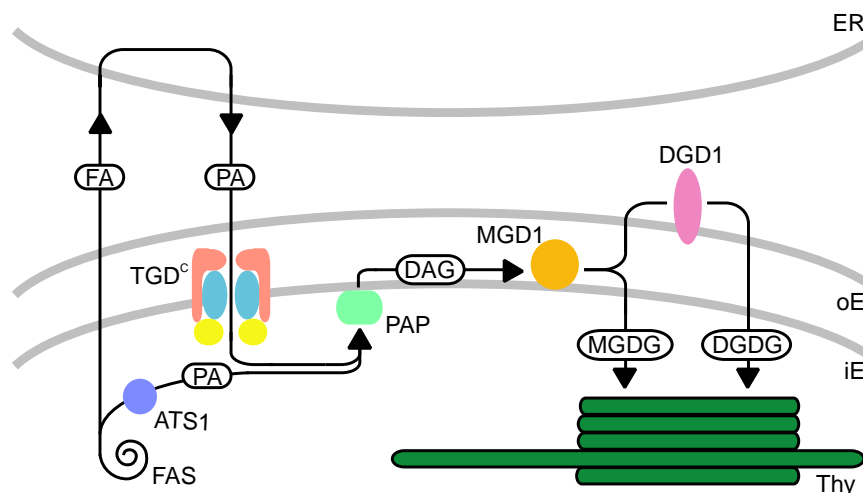
between the two envelopes, and from the envelopes to the thylakoids inside the chloroplast. A further complication arises from the fact that galactoglycerolipids can be assembled either *de novo* in the chloroplast, or from imported precursors assembled at the ER. It is this involvement of multiple membrane systems in the biosynthesis of galactoglycerolipids that necessitates a rich repertoire of lipid transfer phenomena in the biogenesis of chloroplasts. Furthermore, evidence is mounting that a second galactoglycerolipid biosynthetic pathway is induced following phosphate deprivation (Härtel *et al.* 2000) initiating the transfer of galactoglycerolipids from the chloroplast to the plasma membrane (Andersson *et al.* 2003) or even the mitochondrion (Jouhet *et al.* 2004). Thus galactoglycerolipid biosynthesis in plants encompasses not only the import of lipid precursors into the chloroplast, it also extends to the export of galactoglycerolipids from the chloroplast to other organelles under conditions of phosphate limitation. None of the underlying lipid trafficking phenomena are mechanistically understood but a number of transport processes are proposed (Kelly *et al.* 2004), including the direct contact between ER and chloroplast membranes at specialized ER-domains that presumably give rise to plastid associated microsomes (PLAMs) (Kjellberg *et al.* 2000). Below a new set of Arabidopsis lipid trafficking mutants will be discussed. These *tgd* (*trigalactosyldiacylglycerol*) mutants are deficient in the biosynthesis of galactolipids from precursors assembled at the ER, and experience a complex redirection of their lipid metabolism into novel compounds.

### **Disruption of the ER pathway in the *tgd1* mutants of Arabidopsis**

The *tgd* mutants of Arabidopsis were isolated during a suppressor screen in the *dgd1* mutant background (Xu *et al.* 2003). The *dgd1* mutant is deficient in DGDG biosynthesis by the MGD1/DGD1 pathway (Dörmann *et al.* 1995; Dörmann *et al.* 1999) and the screen was intended to discover regulatory components necessary for the induction of an DGD1-independent pathway of galactoglycerolipid biosynthesis activated under phosphate-limiting growth conditions (Härtel *et al.* 2000). As it turns out, *tgd* mutants can just as easily be isolated in the wild-type background based on their diagnostic accumulation of oligogalactolipids, in particular trigalactosyldiacylglycerol, the phenotype giving rise to the name of the mutant class and the respective loci. Oligogalactolipids had been previously observed in isolated chloroplast preparations (van Besow *et al.* 1978; Heemskerk *et al.* 1990; Heemskerk *et al.* 1991) and are synthesized due to the activation of a processive galactolipid:galactolipid galactosyltransferase associated with the outer chloroplast envelope (Benning *et al.* 2005). However, the primary defect in these mutants causes other disturbances in lipid metabolism leading to a complex phenotype. The mutants, exemplified by the published *tgd1* mutant, not only accumulate oligogalactolipids, but also triacylglycerols in their cytoplasm visible as oil droplets (Xu *et al.* 2005). Molecular species analysis of the oligogalactolipids is consistent with their origin from the prokaryotic pathway, whilst triacylglycerols seem to be exclusively derived from the eukaryotic pathway. Moreover, the ER-derived pool of galactoglycerolipids is diminished and results of pulse chase labeling experiments are consistent with a disruption of the eukaryotic pathway of thylakoid lipid biosynthesis in the *tgd* mutants. In other words, in many aspects the *tgd* mutants and the *ats1(act1)* mutant described above show opposite phenotypes. In fact, the *ats1-1(act1)*, *tgd1-1*

double mutant is embryo-lethal consistent with a disruption of both pathways of thylakoid biosynthesis (Xu *et al.* 2005).

The *TGD1* locus has been identified by map-based cloning of the mutant allele and encodes a predicted six-membrane-spanning-domain protein resembling the permease component of bacterial ABC transporters (Xu *et al.* 2003). The protein is integral to the inner envelope of the chloroplast (Xu *et al.* 2005). Additional TGD proteins recently identified in the lab include TGD2 that is predicted to contain a single membrane-spanning domain and appears similar to bacterial substrate-binding proteins associated with ABC transporters, and TGD3 that is predicted to encode an ATP-binding protein (Xu, Awai, Lu, and Benning, unpublished). The identical phenotypes of the respective non-allelic mutants suggest that these three proteins are components of a multipartite bacterial-type ABC transporter system involved in the eukaryotic pathway of thylakoid lipid biosynthesis. While it is not clear what the substrate of the transporter is, first clues can be derived from the fact that PA levels are 5-fold elevated in the *tgdl-1* mutant and that the incorporation of labeled PA supplied to isolated *tgdl-1* mutant chloroplasts into galactoglycerolipids is reduced (Xu *et al.* 2005). Moreover, the TGD2 protein was found to specifically bind PA (Awai, Xu and Benning, unpublished).



**Figure 1.** Lipid trafficking during galactoglycerolipid biosynthesis in Arabidopsis.

Proteins are shown as filled shapes; metabolites are designated with their abbreviations in open shapes. Bilayer membranes are indicated with grey lines. Arrows suggest metabolic flux of intermediates. ATS1, plastid glycerol-3-phosphate acyltransferase; DAG, diacylglycerol; DGD1, digalactosyldiacylglycerol synthase 1; DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; iE, inner chloroplast envelope; oE, outer chloroplast envelope; FA, fatty acid; FAS, fatty acid synthase; MGD1, MGDG synthase 1; MGDG, monogalactosyldiacylglycerol; PA, phosphatidic acid; PAP, plastid phosphatidic acid phosphatase; TGD<sup>c</sup>, TGD-Complex; Thy, thylakoids.

Based on the current analysis of the *tgdl* mutants and the TGD proteins, a plausible model (Fig. 1) for the function of these proteins emerges. It is hypothesized that the TGD1-3 proteins constitute components of an ABC transporter at the inner chloroplast envelope that transfers PA possibly from the outer envelope through the inner envelope. The role of TGD2 might be to facilitate PA transfer between the two envelopes. The substrate transported would be PA synthesized by the eukaryotic pathway and could be

derived from the ER by direct transfer in contact sites. Indeed, a TGD1-GFP fusion protein was associated in a punctate pattern with the chloroplast indicative of the presence of discrete membrane domains (Xu *et al.* 2005). As the substrate for MGD1 is DAG not PA and MGD1 is localized on the outside of the inner envelope (Xu *et al.* 2005), the question arises why PA needs to be transported to the inside of the inner envelope rather than being metabolized in the outer envelope or the intermembrane space. The answer to this question appears to be that PA phosphatase is exclusively associated with the inside of the inner envelope. According to this model a disruption of the TGD complex would lead to lack of ER-derived molecular species of galactoglycerolipids as observed in the mutants. It could explain the observed elevation of PA levels in the *tgdl-1* mutant. Because PA is a signaling compound and a central metabolite of lipid metabolism as mentioned above, its levels are presumably strictly controlled. The accumulation of triacylglycerols might be a mechanism to prevent the excessive accumulation of PA which can be converted in two steps into triacylglycerols. Explaining the accumulation of oligogalactolipids in the *tgdl* mutants is more difficult, as the function of the responsible processive galactosyltransferase in the wild-type is not known. It seems possible that the activity of this enzyme is affected by the local lipid environment which most likely is altered in mutant.

### **Perspectives**

While the TGD proteins provide a new outlook on the components and possible mechanisms involved in ER-chloroplast lipid trafficking as part of the eukaryotic pathway of thylakoid lipid biosynthesis, many questions remain to be answered in order to corroborate the model described above. For example, it needs to be experimentally shown, possibly by *in vitro* reconstitution, that the three TGD1-3 proteins are components of a PA transporter at the inner chloroplast envelope. The identity of the PA phosphatase as well as its localization or possible association with the TGD complex has to be determined. The processive galactosyltransferase at the chloroplast outer envelope that is responsible for the accumulation of oligogalactolipids in the mutants needs to be identified before the mechanism of its activation and its possible role in maintaining lipid homeostasis can be studied. The mechanism of transfer of DAG from the PA phosphatase on the inside of the inner chloroplast envelope to MGD1 on the outside of inner envelope needs to be identified. Likewise, the quest for the nature of the lipid species transferred from the ER to the outer chloroplast envelope and the mechanisms by which this happens remain a daunting challenge. Nevertheless, the availability of the TGD proteins and the *tgdl* mutants of *Arabidopsis* provide many new experimental leads that promise acceleration in the discovery process towards a more complete understanding of ER-chloroplast lipid trafficking in plants.

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