Chloroplast biogenesis: the lipid supply

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Abstract: Chloroplast biogenesis with the emphasis of transport and metabolism of lipids, was studied. With isolated pea fractions, lipids were transported from endoplasmic reticulum, containing radiolabelled lipids, to isolated chloroplasts. Phosphatidyl choline (PC) and phosphatidyl inositol (PI), both constituents of chloroplast membranes, were transported to a greater extent than the non-chloroplast lipid, phosphatidyl ethanolamine (PE). Isolated chloroplasts also possessed the capability of incorporating the fatty acid group derived from acyl-CoA into PC, a reaction highly stimulated by added lysoPC. Following a pulse of radiolabelled lysoPC and acyl-CoA to label PC, UDP-galactose and >10 kDa cytosol stimulated the transfer of radiolabel from PC to monogalactosyl diacylglycerol (MGDG). These results were interpreted as an effect of phospholipase C or phospholipase D and phosphatidic acid phosphatase on PC in the chloroplast outer envelope, producing diacylglycerol (DAG) for MGDG synthesis. I suggest that the apparent transfer of PC from the endoplasmic reticulum to the chloroplast occurs as lysoPC, which is acylated to PC by acyl-CoA in the inner chloroplast envelope. The envelope localised PC is hydrolysed to DAG by cytosolic phospholipases and phosphatidic acid phosphatases and is then used in the synthesis of MGDG.

Transport of lipids from the chloroplast envelope to the thylakoid was investigated in intact chloroplasts. Newly synthesised MGDG and digalactosyl diacylglycerol were transported to the thylakoid in a temperature and time dependent manner. The initial step in intra-plastidial lipid transport, the release of lipids from the chloroplast envelope was studied. Stroma proteins, ATP, GTP and acyl-CoA stimulated the release of lipids from radiolabelled immobilised chloroplast envelope. GTPγS, a non-hydrolysable GTP-analogue, was essentially non-stimulative, or when chloroplast envelope from wheat was used, inhibited lipid release. Several GTP-binding proteins were detected in envelope isolated from spinach chloroplasts. A protein of 39 kDa bound GTP more strongly upon addition of mas7, a mastoparan analogue that activates heterotrimeric GTP-binding proteins. GTP phosphorylated proteins and lipids in the chloroplast envelope and proteins in the stroma and thylakoid. In addition, GTPγS dependent thiophosphorylation of proteins and lipids was also detected. I propose that GTP-binding proteins and/or GTP-dependent phosphorylation of proteins or lipids in the chloroplast envelope or stroma partake in regulating lipid release from the chloroplast envelope, possibly as vesicles.

Keywords: chloroplast biogenesis, chloroplast envelope, lipid transport, lipid metabolism, membrane biogenesis, acyl-CoA, signalling, GTP, MGDG synthesis

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