Metabolic Pathways Involved in Storage Lipid Accumulation

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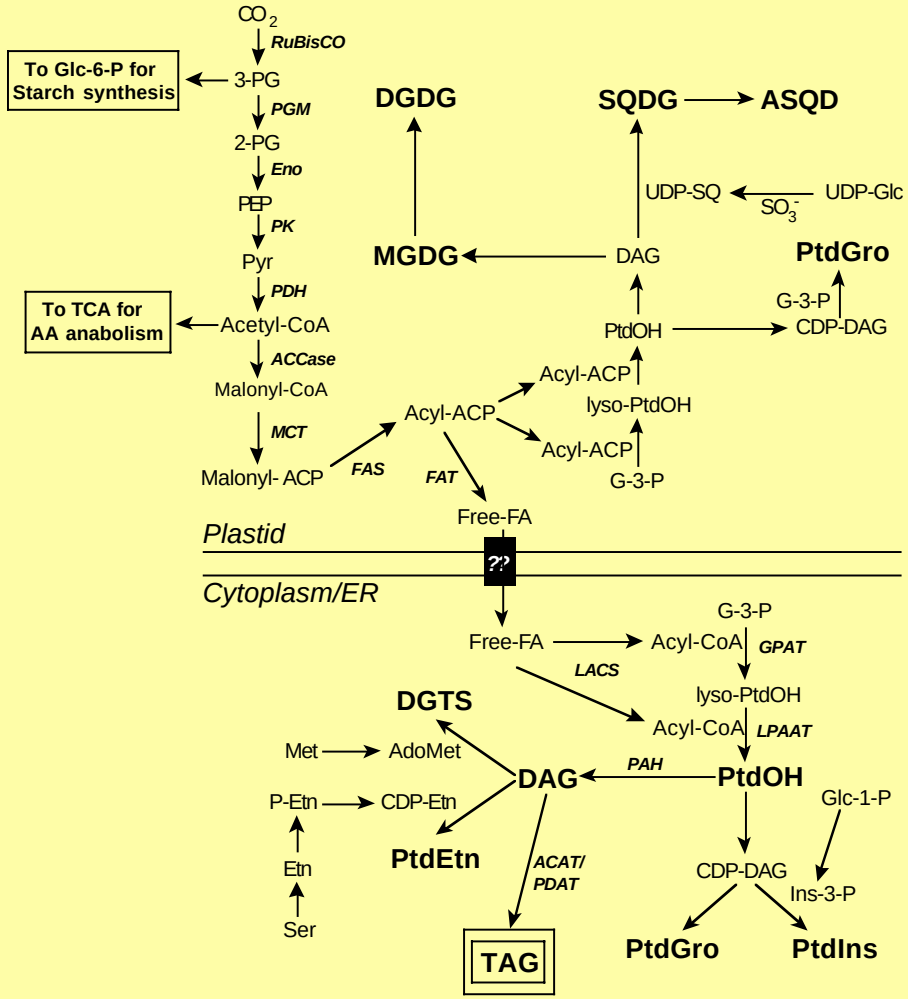
Outline

- Overview of lipid gene annotation of Chlamydomonas genome.
- Pathway from CO_2 to triacylglycerol in algae.
- Carbon partitioning (lipid, starch, and protein) and lessons from other organisms.
- Major unresolved questions and future prospects.

Overview of Lipid Metabolism in *Chlamydomonas*



From Riekhof, Sears, and Benning, Euk. Cell, 2005

Based on annotation of genome version 3.

<u>Major conclusions:</u>

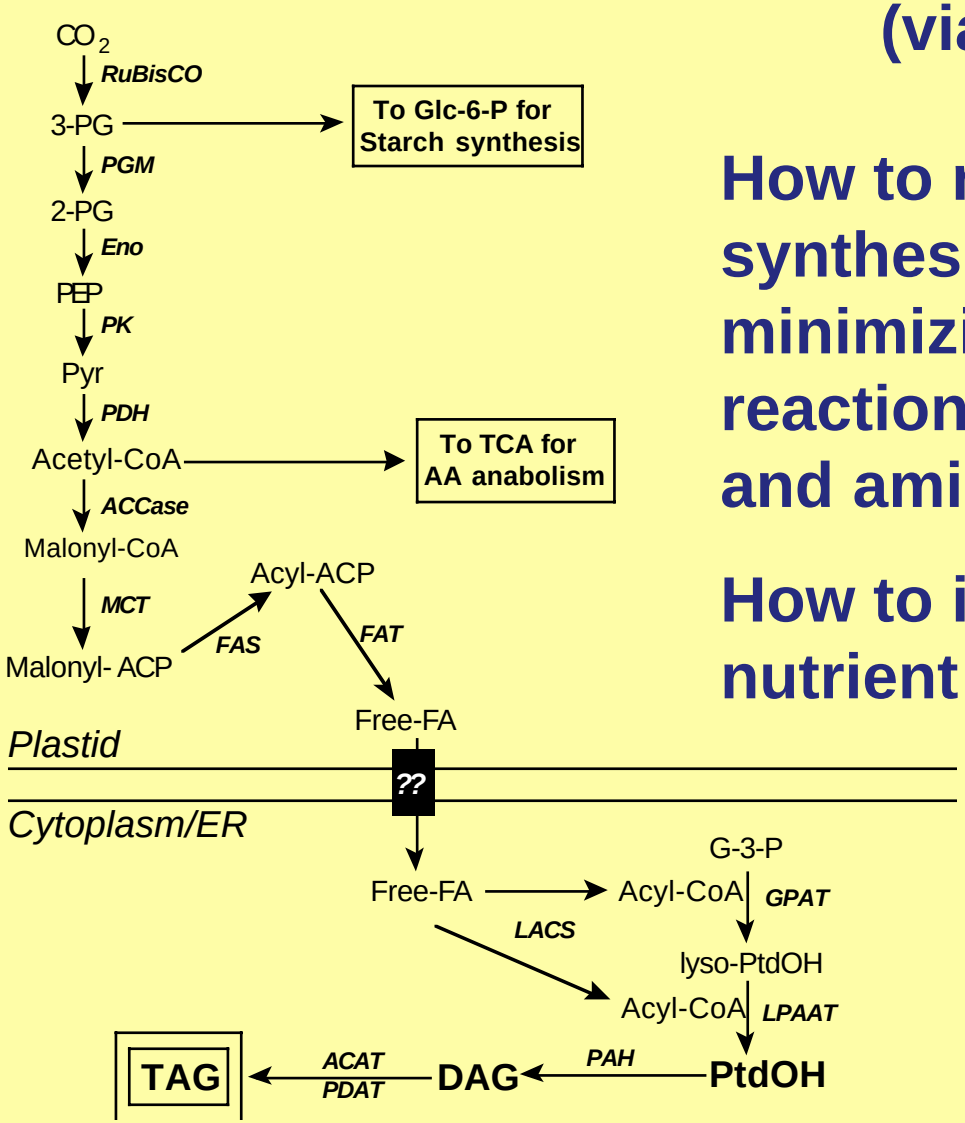
Lipid metabolism is simpler than in seed plants.

Isozyme numbers are much smaller than Arabidopsis.

Many potential dual-targeted proteins.

From CO_2 to Triacylglycerol

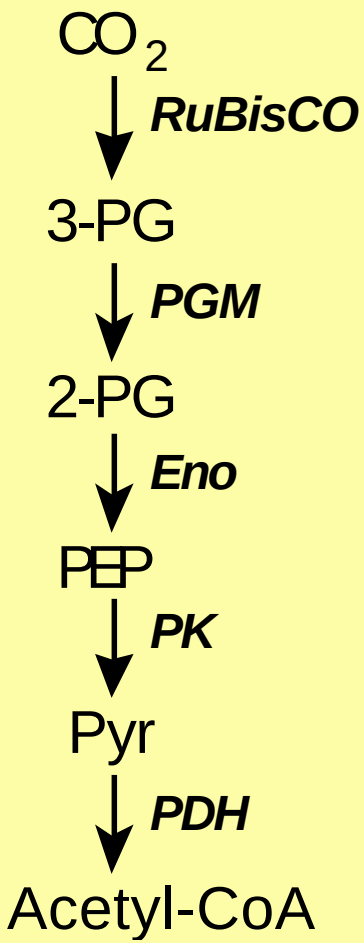
(via the most direct route)



How to maximize the synthesis of TAG while minimizing the "side reactions" of carbohydrate and amino acid metabolism?

How to induce this pathway in nutrient replete cells?

1. Production of Acetyl-CoA



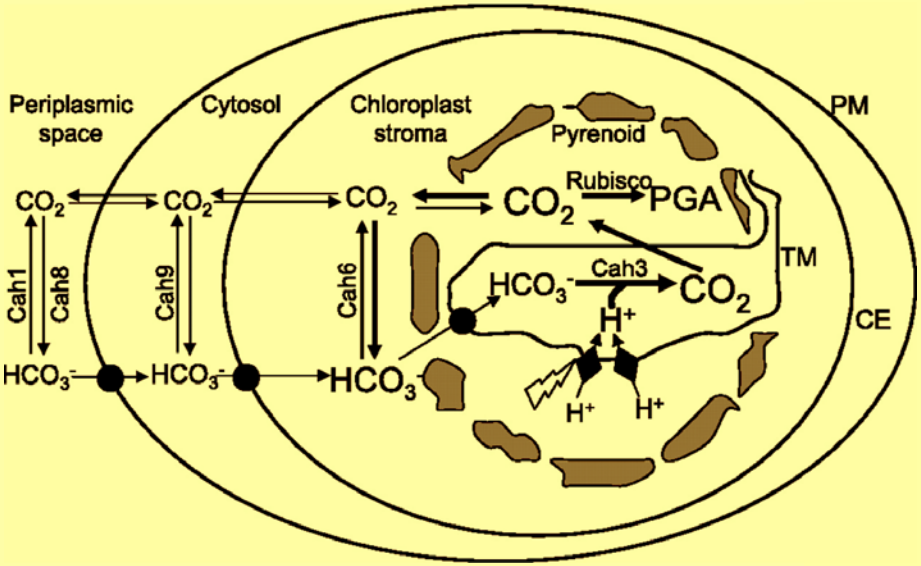
Proposed pathway for direct formation of acetyl-CoA in the algal plastid.

In contrast to seed plants, there is no need to import sucrose for conversion to acetyl-CoA. This avoids the loss of up to 1/3 of the fixed carbon through PDH.

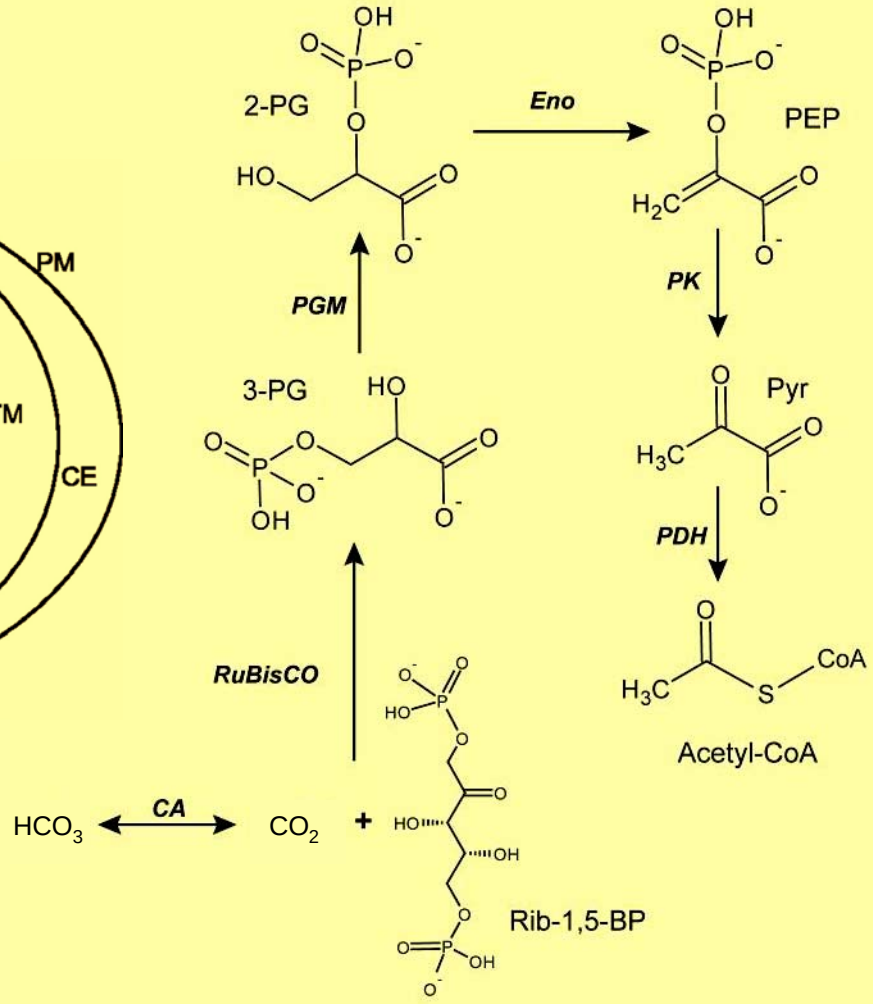
Contributions to acetyl-CoA pool by different pathways can be determined by metabolic flux analysis.

1. Production of Acetyl-CoA

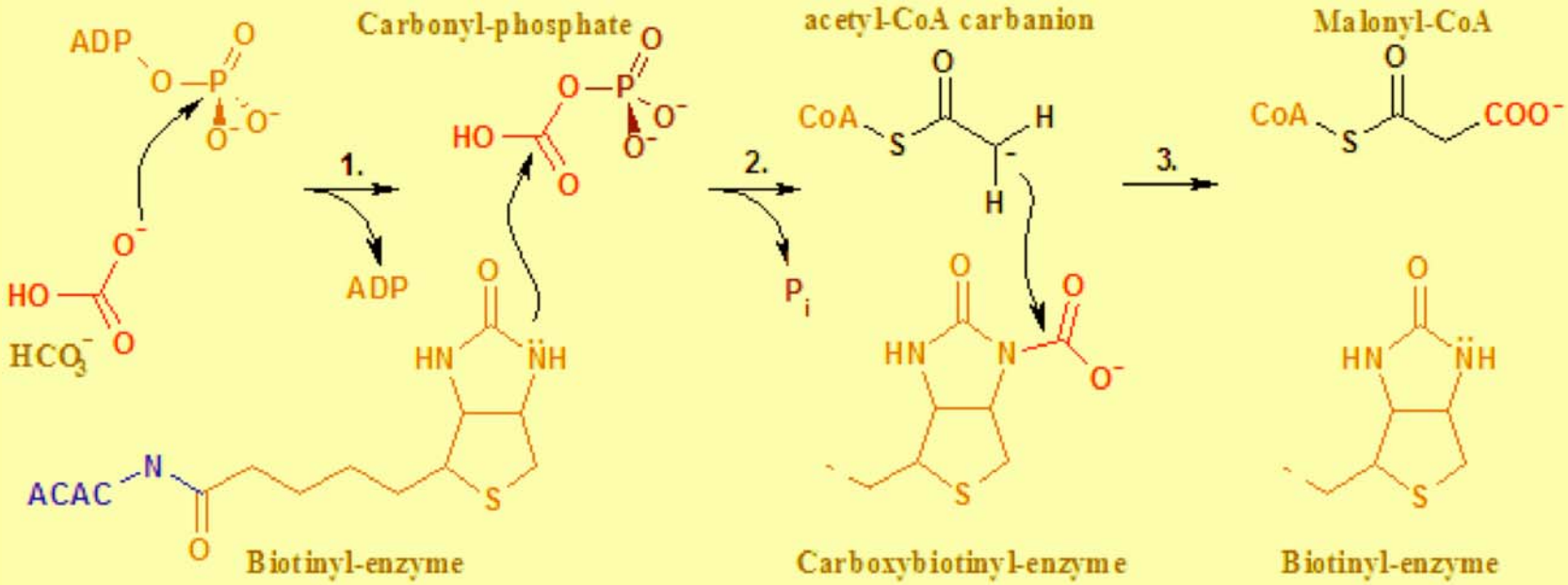
The carbon concentrating mechanism is essential under atmospheric pCO_2:



From Moroney and Ynalvez, Euk Cell, 2007



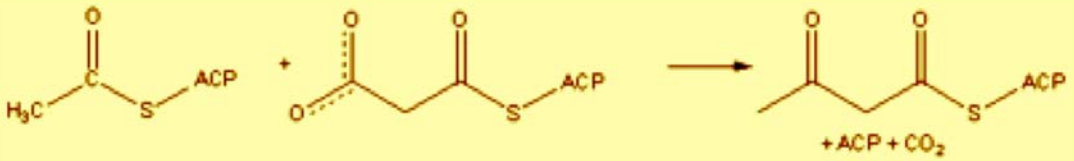
2. Acetyl-CoA carboxylase



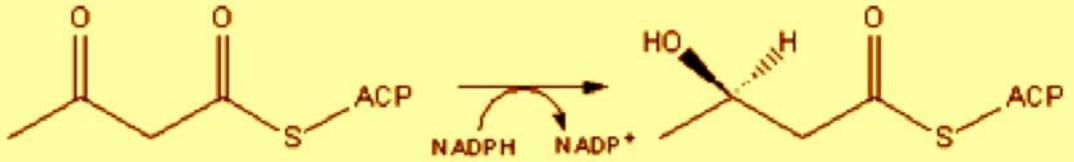
-ACCase from *Chlamydomonas*, like that from higher plants, is a heterotetrameric plastid enzyme.
-A cytosolic monomeric ACCase was not identified in the genome.

2. Fatty Acid Synthase

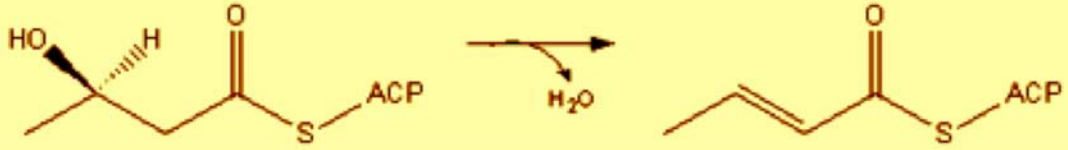
Plastids use a type II multimeric (bacterial type) FAS enzyme.



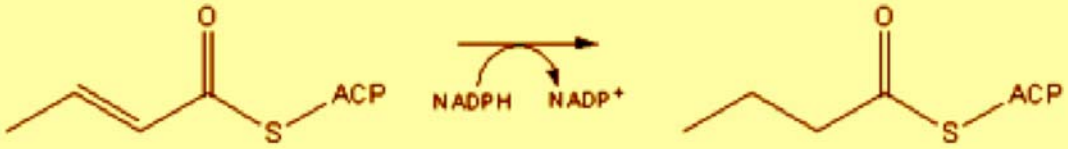
A. Claisen condensation: KAS1, KAS2, KAS3



B. Reduction of β-keto: KAR1

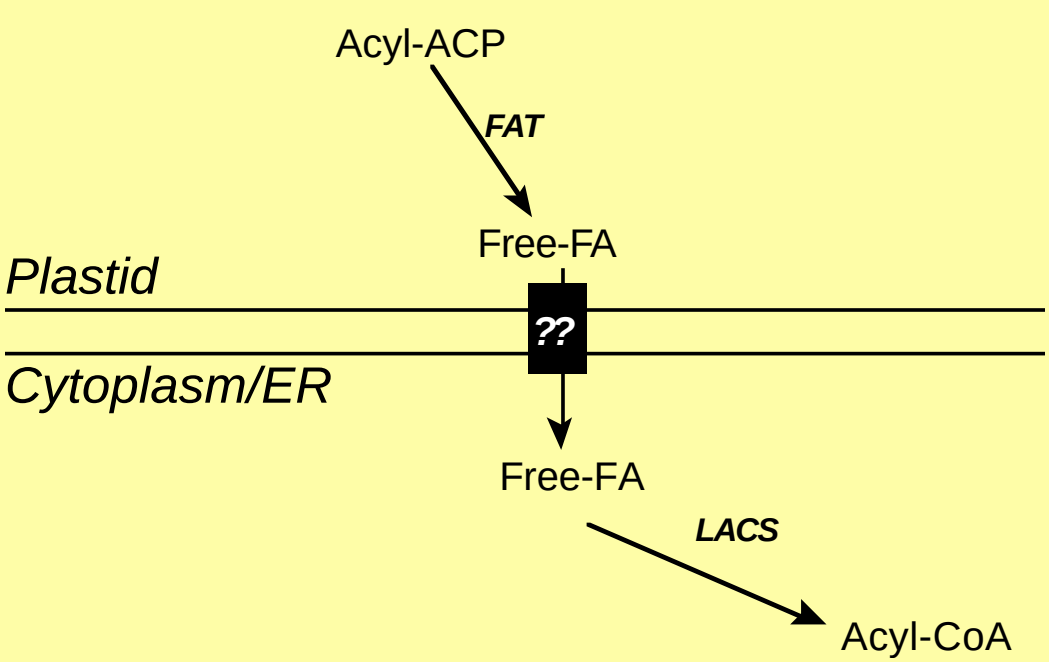


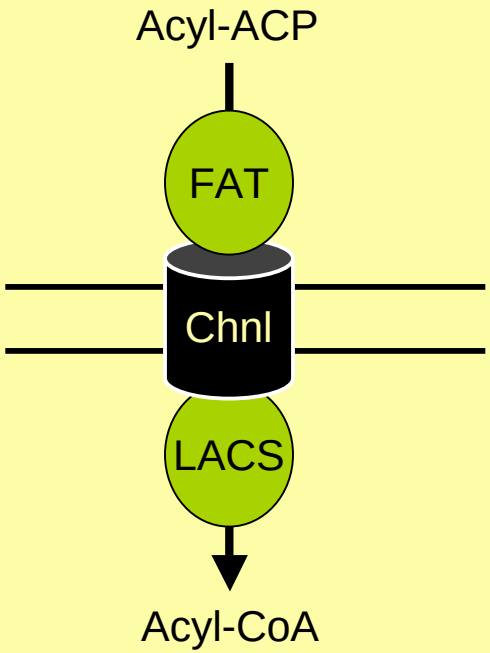
C. Dehydration of β-hydroxy: HAD1



D. Reduction of α-β double bond: ENR1

3. Export of Fatty Acid From Plastid

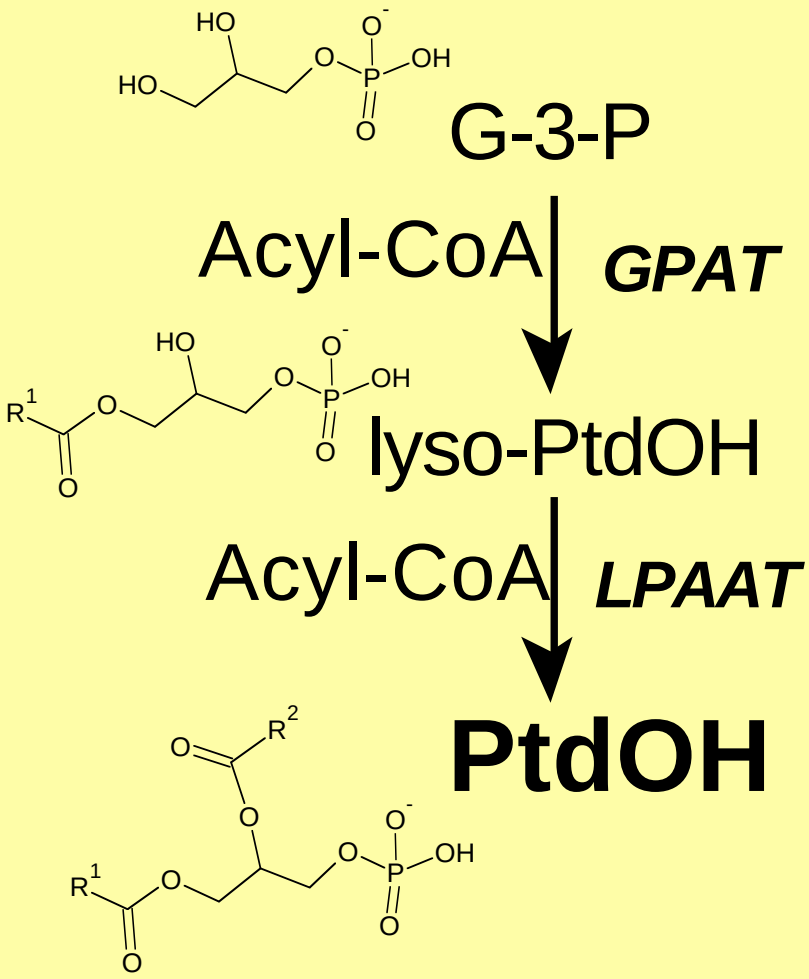




Kinetic studies with isolated spinach Cp give evidence of substrate channeling, but the nature of the channel is not well defined.

Chlamydomonas might be an ideal candidate with which to dissect this process genetically.

4. Glycerolipid Assembly



In mammalian liver and adipose tissue, GPAT is a major control point for flux into TAG.

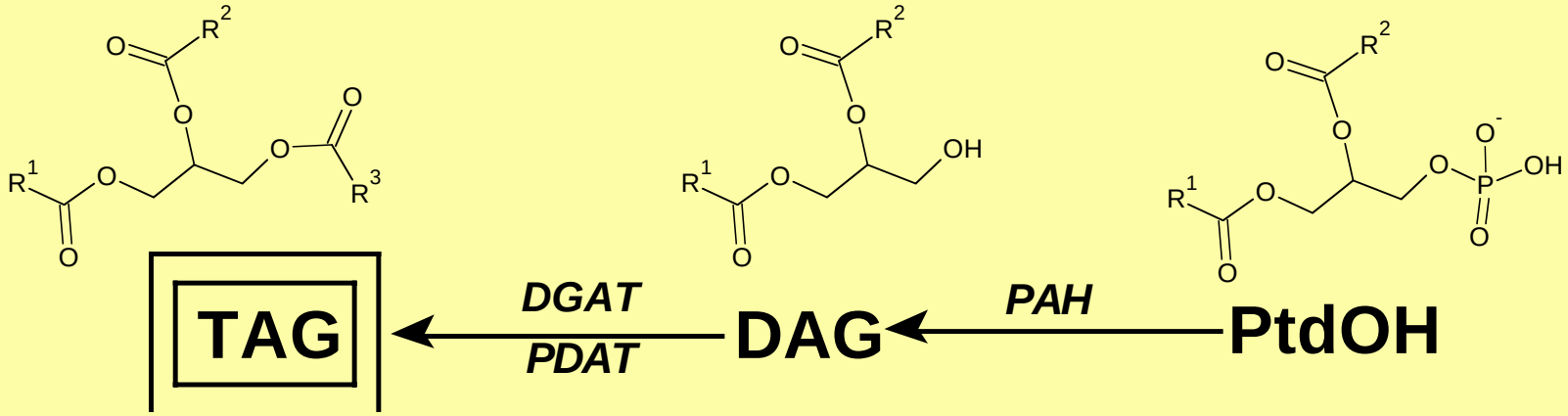
No GPAT homolog is present in the Chlamydomonas genome.

A recently identified lysophospholipid acyltransferase enzyme with LPAAT activity was recently described in Yeast (Riekhof et al., JBC, 2007, and others.)

This gene has a homolog in Chlamydomonas, and may represent the unidentified LPAAT activity.

5. Formation of Triacylglycerol

PtdOH hydrolase is a major control point for TAG synthesis in adipose tissue. Disruption causes lipodystrophy (lack of body fat) in mice.

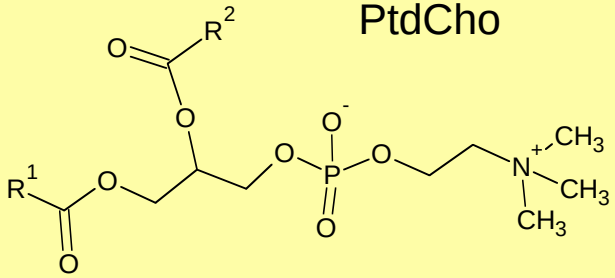


Major question: Does betaine lipid (a non-hydrolyzable ether lipid) get turned over as a source of DAG for TAG synthesis?

Betaine Lipid Vs. PtdCho

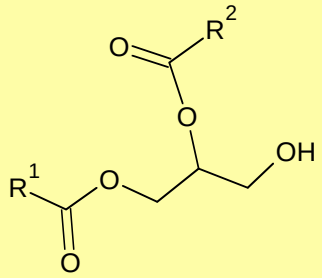
Plant-type pathway: PtdCho is an intermediate in TAG synthesis, subject to FAD.

PtdCho



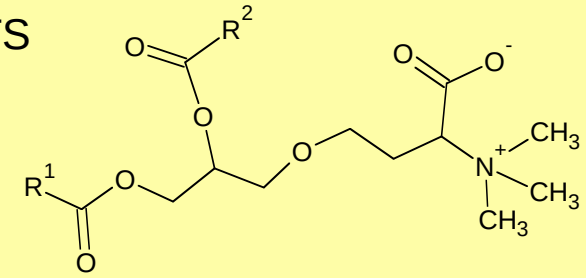
PLC/CT

DAG



Chlamy. pathway: Role of betaine lipid as TAG intermediate is unknown

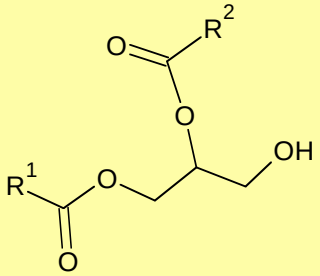
DGTS



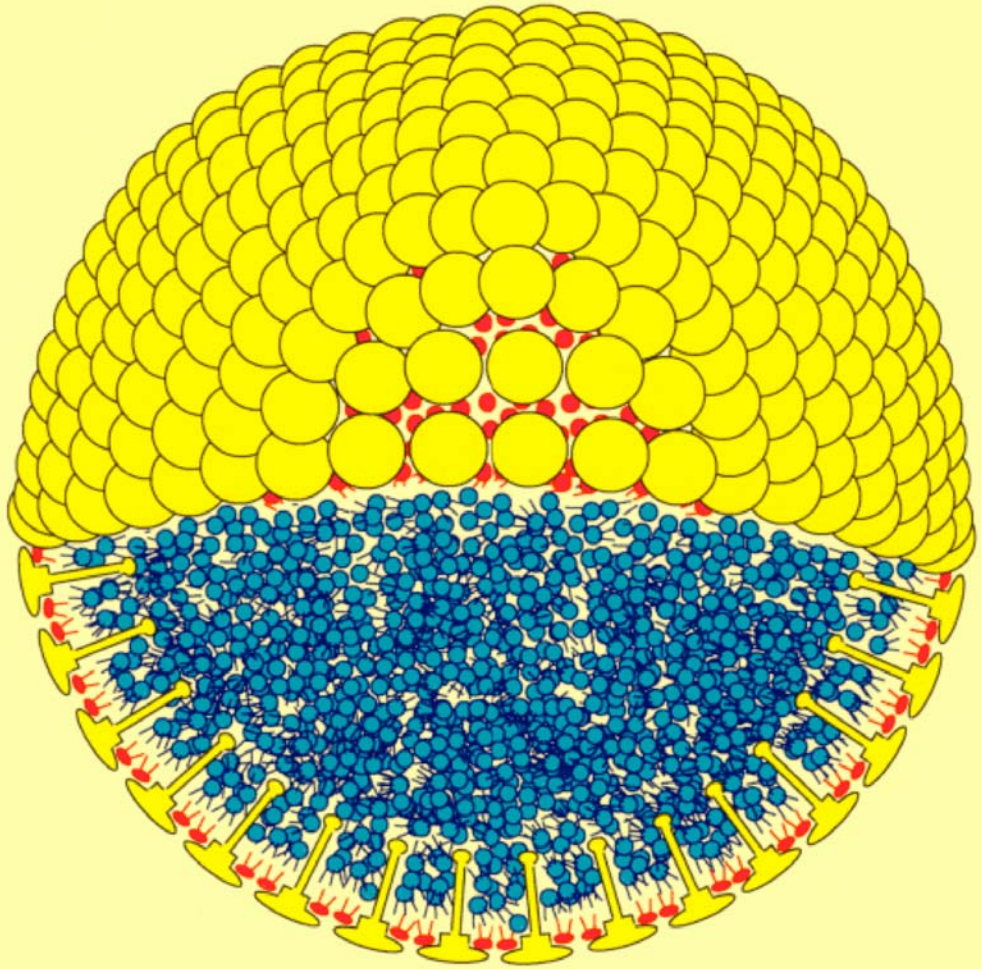
BTA1

???

DAG



Packaging of TAG Into Lipid Droplets

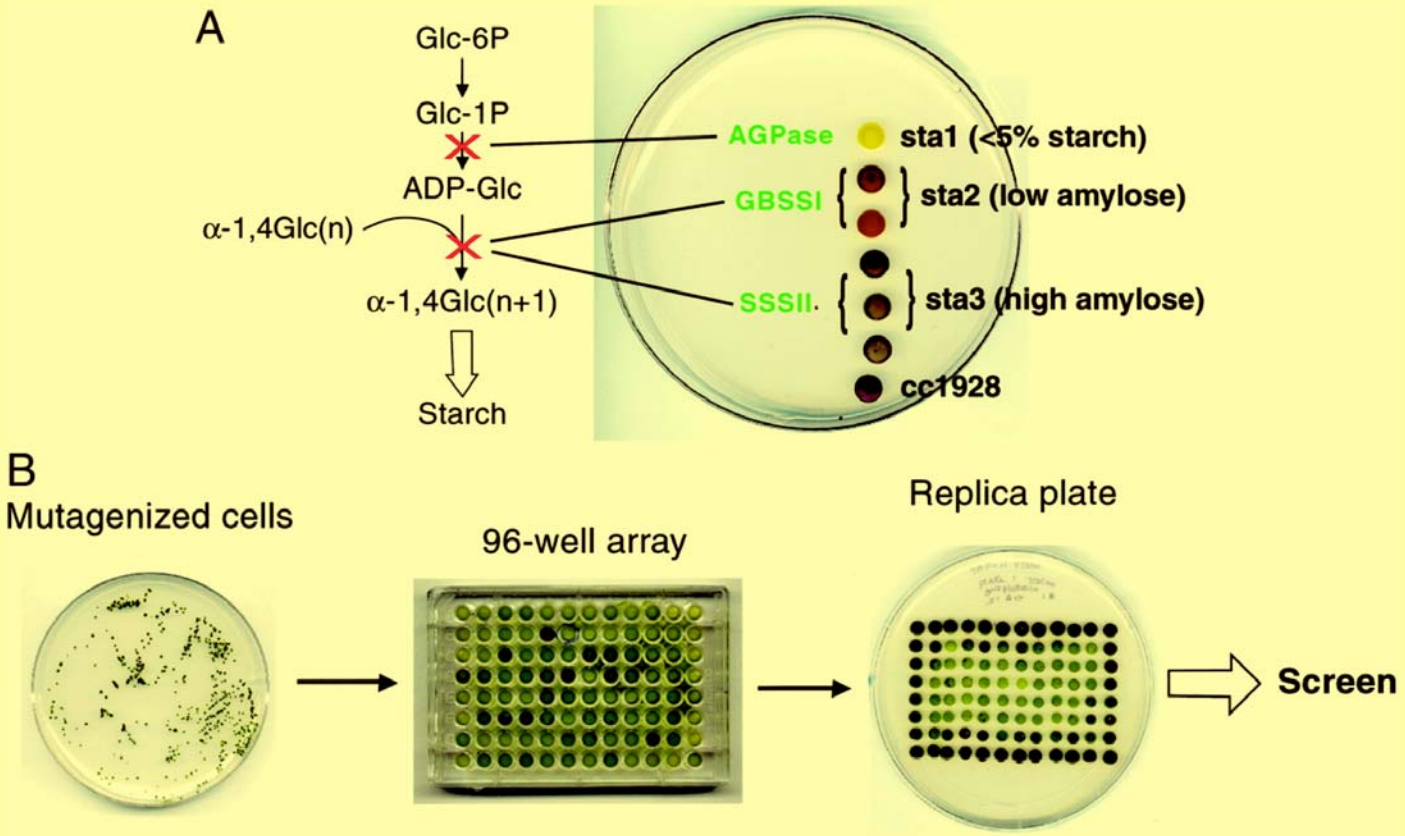


To avoid coalescence upon seed desiccation, the surface of the lipid droplet in oilseeds is coated in oleosin proteins.

No known lipid droplet surface proteins have been identified in the Chlamydomonas genome.

Protein composition of algal lipid droplets is unknown.

Starch Versus TAG Accumulation



From Hicks et al., Plant Physiol, 2001

Starchless mutants of Chlamydomonas are available. Do they make more oil under inducing conditions?

Summary

- TAG and starch synthesis are induced by nutrient limitation.
- Acetyl-CoA can be produced directly from 3-PGA in the algal plastid.
- ACCase and FAS are multmeric bacterial-type enzymes of the plastid.
- Fatty acids are exported from the plastid for synthesis of glycerolipids in the ER.
- TAG is synthesized by ER enzymes and packaged into lipid droplets.
- Protein synthesis and carbohydrate storage compete with TAG for fixed carbon.

Unresolved Questions and Future Research

- How is fatty acid exported from the plastid?
- Is betaine lipid an intermediate in the TAG synthesis pathway of Chlamydomonas and other Volvocales?
- How is carbon partitioning between starch and TAG controlled, and can it be manipulated?
- What are the changes in gene expression and enzyme activities during oil accumulation, and can we turn these pathways on under nutrient replete conditions?
- What is the protein composition of the algal lipid droplet?
- Do starchless mutants push more photosynthate into lipid than the wild-type?