5. Lipid biosynthesis overview

6. Fatty acid synthesis

7. Lipid assembly, modification, acyl editing and trafficking

8. Methodology of lipid metabolism research
Today’s topics on the Arabidopsis node map
Maize Endosperm
(mostly starch accumulation)

Maize Embryo
(accumulates 35% oil)


Plant membrane glycerolipid biosynthesis – overview

**PLASTID**

- Pyruvate → Acetyl-CoA → ACCase
- Malonyl-CoA → FAS
  - Acyl-ACP (16:0;18:0)
  - Desaturase
    - Acyl-ACP (18:1)
  - Thioesterase
- Malonyl-CoA → ACCase
- Acyltransferase
- Head group transferases

**ENDOPLASMIC RETICULUM**

- G3P → LPA
  - Acyltransferases
  - Phosphatase (PAP)
- FA SYNTHESIS
- G3P → PA → DAG → PE
- FA MODIFICATION
  - Lipid linked desaturases
  - Acyl-CoA elongases
- LIPID TRANSFER
- LPA → PA → DAG → PE
- PC
- ACYL EDITING
- G3P → LPA → PA → PC
- PS
- PG
- PI

**LIPID ASSEMBLY**

- MGDG
- DGDG
- SQDG
Acetyl-CoA carboxylase


**Two step reaction:**

1: Biotin carboxylase: HCO$_3^-$+ATP+Enzyme-biotin $\rightarrow$ Enzyme-biotin-CO$_2^-$+ADP+P$_i$

2: Carboxyltransferase: Enzyme-biotin-CO$_2^-$+Acetyl-CoA $\rightarrow$ Enzyme-biotin+Malonyl-CoA

![Diagram of Acetyl-CoA carboxylase reaction]

**Homomeric ACCase**

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**Heteromeric ACCase**

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<th>α-CT</th>
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Arabidopsis and other dicots

heteroACCase

localized in plastids

Wheat and other Graminaceae

Lack heteroACCase

Two homoACCase isoforms

(plastids and cytosol)

Light/dark responses at the posttranslational level:

Regulated by phosphorylation/ dephosphorylation

Feedback inhibition by excess fatty acids
Fatty acid synthase

Plants:
Fatty acid synthase localized to plastid (other eukaryotes: cytosol)

Type I fatty acid synthases:
Animals - various subunits are discrete domains of a single protein
Yeast, fungi - two genes produce polypeptides, which then coalesce to form a multifunctional complex.

Type II fatty acid synthases:
Plants, bacteria - separate proteins encoded by different genes, can be dissociated and purified, although they normally operate in concert.
Synthesis of a C\textsubscript{18} fatty acid requires 48 reactions and 12 different proteins.

Type III fatty acid synthetases (elongases):
Catalyze the addition of C2 units to preformed acyl-CoAs in the cytosol.
Acetyl-COA is the basic building block of the fatty acid chain and enters the pathway both as a substrate for acetyl-COA carboxylase (reaction 1) and as a primer for the initial condensation reaction (reaction 3).

Reaction 2, catalyzed by malonyl-CoA:ACP transacylase, transfers malonyl from COA to form malonyl-ACP, which is the carbon donor for all subsequent elongation reactions.

After each condensation, the 3-ketoacyl-ACP product is reduced (reaction 4), dehydrated (reaction 5), and reduced again (reaction 6), by 3-ketoacylACP reductase, 3-hydroxyacyl-ACP dehydrase, and enoyl-ACP reductase, respectively.

End products of plastid fatty acid synthesis and export

http://arabidopsisacyllipids.plantbiology.msu.edu/pathways/fatty_acid_elongation_desaturation_export_from_plastid
Stearoyl-ACP Δ9 desaturase

(Lindqvist et al. (1996) EMBO Journal 15: 4081-4092)

Soluble
(unlike animal enzyme which is membrane-bound)

Acts on 18:0-ACP
(unlike animal enzyme which acts on 18:0-CoA)
Lipid assembly

(Bates et al. (2007) Journal of Biological Chemistry 282: 31206)

Prokaryotic pathway – within the plastid

Eukaryotic pathway – within the endoplasmic reticulum

Enzymes:
GPAT: glycerol-3-phosphate acyltransferase
LPAT: lyso-phosphatidic acid acyltransferase
PAP: phosphatidic acid phosphatase
CDP-DAGS: CDP-diacylglycerol synthase
Lipid linked fatty acid desaturases
http://arabidopsisacyllipids.plantbiology.msu.edu/pathways

Fatty acid synthesis produces 16:0, 18:0 and 18:1 fatty acids however most plant membrane lipids contain mostly polyunsaturated fatty acids, these are produced by membrane bound desaturases that act on the fatty acids esterified within the membrane lipids.

Arabidopsis desaturases

Plastid localized

ER localized

Li-Beisson Y et. al. (2010) In The Arabidopsis Book 8:e0133. doi:10.1199/tab.0133
Type III fatty acid synthetases (elongases):
Catalyze the addition of C2 units to preformed acyl-CoAs in the cytosol.

Production of VLC-PUFA requires moving the fatty acid between the phospholipid pool for desaturation and the acyl-CoA pool for elongation.
Acyl editing, also termed *remodeling*, is defined as any process that exchanges acyl groups between polar lipids (mostly different PC molecular species) but that does not by itself result in the net synthesis of the polar lipids.

The PC acyl editing cycle involves rapid deacylation of PC, generating lyso-PC and releasing the FA or acyl-CoA to the mixed acyl-CoA pool. Reacylation of lyso-PC with a different acyl-CoA from the mixed pool completes the cycle.

Facets of acyl editing
1) PC is the site of extra-plastidic FA desaturation, therefore acyl editing allows PUFA to enter the acyl-CoA pool
2) The high rate of the acyl editing cycle, implies that most nascent 18:1 exported from the plastid is first incorporated into PC
Pyruvate → Acetyl-CoA → ACCase → Malonyl-CoA → Acyl-ACP (16:0;18:0) → FAS → Desaturase → Acyl-ACP (18:1) → Thioesterase → Acyl-CoA → LACS → G3P → LPA → Acyl-CoA → PAP → Acyltransferases → PA → DAG → LPA → Acyltransferases → PC → PE → PS → QC → DGDG → MGDG → SQDG

ENDOPLASMIC RETICULUM

ACYL EDITING

FA SYNTHESIS

LIPID ASSEMBLY

FA MODIFICATION
• Lipid linked desaturases
• Acyl-CoA elongases

LIPID TRANSFER

PLASTID
Galactolipid biosynthesis - compartmentation

(Mongrand et al. (1998) Phytochemistry 49: 1049)

Galactolipids produced from prokaryotic pathway DAG have sn-2 16 C fatty acids

Galactolipids produced from eukaryotic pathway DAG have 18C sn-2 fatty acids

Plants that only use the eukaryotic pathway are termed “18:3” plants

Plants that use both pathways are termed “16:3” plants

141 botanical families analyzed for presence of 16:3 fatty acids

Concluded ~12% of Angiosperms are 16:3 plants
Relative flux of fatty acids through the prokaryotic and eukaryotic pathways of membrane lipid synthesis in Arabidopsis


(Browse et al. (1986) Biochemical Journal 235: 25-31)
The Prokaryotic and Eukaryotic pathways of lipid biosynthesis were determined through metabolic labeling experiments in many different plants:

**Plants and tissues**
- \textit{In vivo} labeling
  - Algae
    - \textit{Chlorella vulgaris}
  - Leaf
    - Maize, Spinach, Pea, Broad bean, Pumpkin, Oat, Clover
    - Developing cotyledons
      - Linseed, Soybean, Safflower
  - Isolated chloroplasts labeling
    - Spinach and Pea
  - Microsome enzyme assays
    - Spinach leaf, Avocado mesocarp, Castor bean endosperm
Determining precursor-product relationships through metabolic labeling experiments

Analysis of lipid biochemistry through Arabidopsis genetics: The right time and place

(Browse et al. (1985) *Science* 227: 763)
Forward genetic screen identified mutants that changed Arabidopsis leaf fatty acid composition


Adapted from: Somerville et al. (1991) Science 252: 80
Mutational genetics and biochemical analysis allow in vivo analysis of the function of individual enzymes


Addapted from:

Browse et al. (1986) Biochemical Journal 235: 25

Kunst et al. (1988) Proceeding of the National Academy of Sciences in the USA 85: 4143