Unmasking the substrate specificity of AtGPAT4: Relevance to the Kennedy Bypass

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GPATs initiate glycerolipid biosynthesis
sn-2 GPATs are unique to land plants

Evidence that acyl oxidation precedes acyl transfer

Insight into the Cutin/Suberin Biosynthetic Pathway:

- sn-2 acylation pathway for membrane lipids allow synthesis of polyester polyesters.
- Even this question is clearly hindered by the unknowns of the biosynthetic pathway to these products.
- Migration between the two positions produces products with primary and secondary positions.
- Does biosynthesis at a ratio of approximately 4:1 in favor of a primary position over the secondary position? Does this difference from the precursors to specific precursors remain to be determined.
- The enzyme nomenclature committee of the International Union of Biochemistry and Molecular Biology (IUBMB) is involved in the nomenclature and standardization of enzymes in the biosynthetic pathway.
- The underlying reasons for the distinctive properties impart to cutin and suberin a remnant recognition signal.
- The cutin and suberin biosynthesis, controlling both composition and structure, have all been shown clearly to be required for cutin and suberin biosynthesis, controlling both composition and structure.
- Nevertheless, largely through the study of the underlying reasons for the distinctive properties impart to cutin and suberin a remnant recognition signal.
- The cutin and suberin biosynthesis, controlling both composition and structure, have all been shown clearly to be required for cutin and suberin biosynthesis, controlling both composition and structure.
- The two Arabidopsis GPATs (GPAT4 and -8) that are involved in leaf and stem cutin biosynthesis exhibited the following acyl specifcity for targeting polymer transport and assembly processes.
- An unanswered question in cutin and suberin biosynthesis is the exact biosynthetic pathway to these MAG products, which are thermodynamically less stable in animals, fungi, or microorganisms (Yang et al., 2010).
- Because close homologs of sn-2 regiospecific GPATs are unique to land plants, they can also be defined for different pathways. These unknowns await further biochemical and cellular evidence.

Figure 7.

- gpat7 mutants are impaired in wound response. A to C, GPAT7 expression is induced by wounding, and gpat7-1 mutants fail to exclude toluidine blue after wounding. Rosette leaves (6 weeks old) were wounded with tweezers and kept in standard growth conditions for 48 h. Leaves were then detached from plants and stained with toluidine blue (0.05%) to test tissue-sealing capacities. D, Freshly wounded wild-type leaves are permeable to toluidine blue. E, After 48 h, wild-type leaves are impermeable to toluidine blue, indicating suberin-type wound-sealing response. F, After 48 h, leaves from gpat7-1-GPAT2 to provide acyl-CoA substrates for acyl transfer reactions.
- The enzymology of sn-2 acylation pathway for membrane lipids allow synthesis of polyester polyesters. Even this question is clearly hindered by the unknowns of the biosynthetic pathway to these products.
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sn-2 GPATs utilise unusual fatty acids

Unsaturated

Saturated

Hydroxylated

Dicarboxylic

sn-1 LPA

sn-2 LPA

sn-2 MAG
sn-2 Monoacylglycerols are acylated during dietary fat absorption in mammals
Project Aims:

- Identify N and C domain catalytic residues.
- What determines GPAT4 substrate preference?
  - Complement MGAT2
- Novel TAG biosynthetic pathway
Assays of GPAT4 activity through $^{14}$C G3P labelling and autoradiography

GPAT expressed in yeast microsomes

Separate via TLC, expose to phosphorimaging plate

Load
EV WtGPAT4 Phosphatase mutant

sn-2 MAG sn-2 LPA
Domain specific knockouts display expected catalytic effects

**Hexadecanedioic** acyl CoA *(in vivo substrate)*

- Relative Expression
- Glycerolipid Formation (nCi/min)

<table>
<thead>
<tr>
<th>GPAT4 Variant</th>
<th>EV</th>
<th>WT</th>
<th>D27E</th>
<th>D29E</th>
<th>K178N</th>
<th>D197S</th>
<th>D201S</th>
<th>H311N</th>
<th>R380M</th>
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<td><strong>± std error</strong></td>
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- sn-2 MAG
- sn-2 LPA

CO₂

H₃N-HA TAG

Acyltransferase domain

Phosphatase domain
sn-2 MAG from oleoyl-CoA is degraded by expression systems for recombinant GPATs

Degradation of nascent sn-2 MAG reduces apparent glycerolipid production
Acyl chain identity influences degradation of sn-2 MAGs by endogenous yeast lipases

**sn-2 MAG**

R is dicarboxylic

**sn-2 LPA**

R is oleoyl/stearoyl

Slow degradation

Rapid degradation

Acylation to stable PA

Phosphatase Knockout

WT GPAT4

GPAT4
Knockout of \textit{YJU3} increases MAG formation in GPAT assays

Heier et al., \textit{BBA}, 2010, 1801, 1063-1071
Acylation outcompetes hydrolysis in \textit{yju3}Δ recombinant MGAT2 microsomes

\[ \text{HO}^{14}\text{C}_{14}\text{C}^{14}\text{C} \text{OH} \]

\[ \text{O} \]

\[ \text{O} \]

\[ \text{18:1 CoA} \]

\[ \text{DGAT} \]

\[ \text{MGAT2} \]

\[ \text{Add sn-2 MAG} \]

\[ \text{Yeast} \]

\[ \text{Acyl Migration Lipase} \]

\[ \text{Residual} \]

\[ \text{Load} \]

\[ \text{Glyc} \]

\[ \text{MAG} \]

\[ \text{DAG} \]

\[ \text{TAG} \]

\[ 5 \]

\[ 10 \] \textbf{Time (min)}
Knockout of *YJU3* permits DAG synthesis via an sn-2 GPAT-MGAT pathway

\[
\begin{array}{cccccccccccc}
\text{yju3Δ} & - & - & - & - & - & - & + & + & + & + & + & + \\
\text{GPAT4} & - & + & M & - & + & M & - & + & M & - & + & M \\
\end{array}
\]

*M=D197S mutant

- DAG
- MAG
- PA
- Glycerol
- LPA
- Load
-  *
DAG biosynthesis competes with sn-2 MAG hydrolysis

D197S GPAT4 with MGAT2 (either background)
DAG biosynthesis competes with sn-2 MAG hydrolysis

WTGPAT4+MGAT2 in gat1Δ background
DAG biosynthesis competes with sn-2 MAG hydrolysis

WTGPAT4+MGAT2 in yju3Δgat1Δ background
MAG Lipases – Hindering the Kennedy bypass

- Artifact in previous reports of sn-2 GPAT substrate specificity
- Lipases in engineered tissues may hinder oil accumulation
- Bifunctional MGAT/DGAT proteins = Two enzyme pathway to TAG
  - Fusion proteins – single protein TAG factories?
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