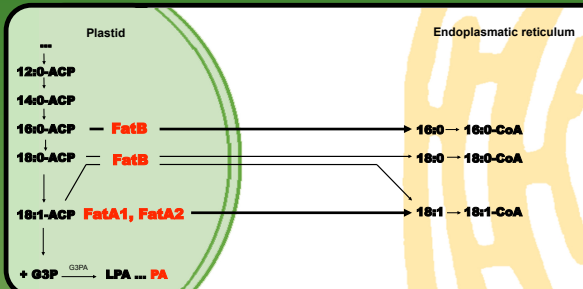


# Export of Oleic Acid from the plastid is essential for embryo development in Arabidopsis

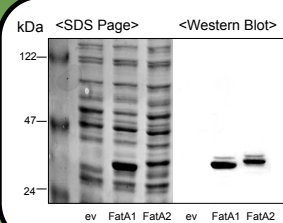
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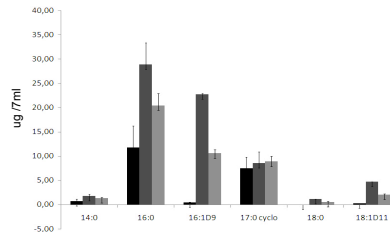
<sup>2</sup> Institute for the Reproduction of Farm Animals Schönow Inc., Germany (current address)



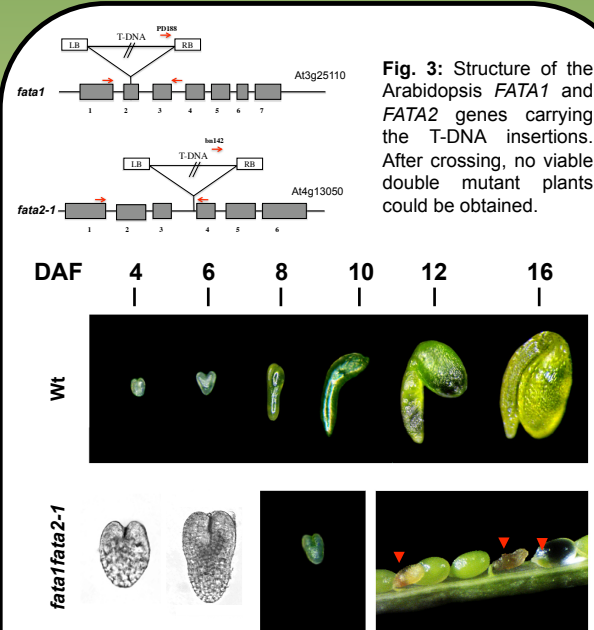
Acyl-acyl carrier protein (ACP) thioesterases hydrolyze Acyl-ACP intermediates releasing free fatty acids. The free fatty acids are exported out of the plastid and re-esterified to Coenzyme A to be incorporated into glycerolipids at the ER. In higher plants, two different isoforms of acyl-ACP thioesterases exist, FATA and FATB (Jones *et al.*, 1995), which differ in amino acid sequence and substrate specificity. FATA shows highest activity with C18:1-ACP. In Arabidopsis, there are two genes encoding type A acyl-ACP thioesterases, FATA1 and FATA2. There is one FATB gene in Arabidopsis. The FatB thioesterase has the highest activity with C16:0-ACP and C18:0-ACP but also shows some activity for C18:1-ACP.



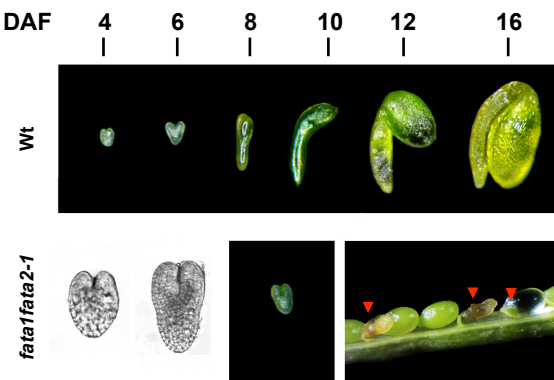
**Fig. 1:** SDS Page and Western Blot of recombinant protein after heterologous expression of FatA1 and FatA2 in *E. coli fadD* mutant. A strong band at 35 kDa and 37 kDa was obtained for the two gene products.



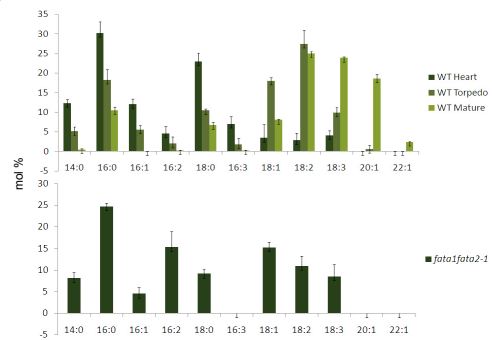
**Fig. 2:** Fatty acid composition of the neutral lipid fraction (mostly free fatty acids) after recombinant expression of FATA1 and FATA2 in *E. coli*. FatA1 and FatA2 expressing strains show accumulation of 16:1<sup>A9</sup> and 18:1<sup>A11</sup>.



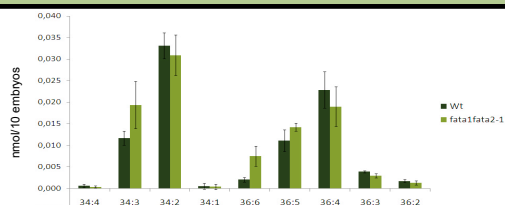
**Fig. 3:** Structure of the Arabidopsis FATA1 and FATA2 genes carrying the T-DNA insertions. After crossing, no viable double mutant plants could be obtained.



**Fig. 4:** Comparison of embryo development in *fata1fata2-1* and wild type. The images show progressive development of wild type embryos from heart stage at 4 days after flowering (DAF) through torpedo as well as early, mid and late cotyledon stages. The *fata1fata2-1* embryo development is arrested after reaching late heart stage. Images are not to scale.



**Fig. 5:** Fatty acid composition of different developmental stages in Arabidopsis wild type embryos and *fata1fata2-1* embryos at heart stage. The *fata1fata2-1* embryos show a fatty acid pattern comparable to torpedo stage in wild type embryos.



**Fig. 6:** Phosphatidylethanolamine content in Arabidopsis wild type embryos and *fata1fata2-1* embryos (heart stage). Molecular species of PE were measured by Q-TOF MS. Note that 34:3 (16:0-18:3) and 36:6 (18:3-18:3) molecular species are increased, while 36:4 (18:2-18:2) is decreased.

## Summary:

Heterologous expression in the *E. coli fadD* mutant of FatA1 and FatA2 show accumulation of 16:1<sup>A9</sup> and 18:1<sup>A11</sup>. The content of C16:0-containing molecular species of PE (extraplastidial lipid) is increased.

In the double homozygous *fata1fata2* seeds, embryo development is arrested at the (morphological) heart stage. However, fatty acid composition of the embryos is most similar to that of wild type torpedo stage embryos. The morphological embryo development comes to a halt when lipid export from the chloroplast becomes significant.