Engineering and optimization of a novel oil biosynthesis pathway

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Introduction: Plant triacylglycerols (TAGs) are an important dietary component of food and can be used as an alternative to fossil fuels for the production of certain chemicals. TAGs are usually extracted from the fruit or seeds of lipid rich crops, however metabolic engineering has the potential to greatly enhance TAG production by extending TAG production to other plant tissues (such as the leaf). Increasing total TAG production would be beneficial from food security, industrial, and environmental perspectives. One possible route to leaf TAG biosynthesis is via engineering of acyltransferases (enzymes that transfer fatty acids to glycerol to eventually produce triacylglycerol) in plants.

Aims: I aimed to customise *sn*-2 GPATs and bifunctional MGAT/DGAT proteins to generate triacylglycerols of specified composition *in vitro* or *in planta*, to increase production of industrially or nutritionally important fatty acids by transgenic crops. To facilitate this, characterization of these enzymes is an important step. Elucidating structural features and the various mechanisms of substrate recognition and catalysis of membrane bound acyltransferases is particularly important from a metabolic engineering perspective.

Results: The primary focus of this project was the glycerol-3-phosphate acyltransferase (GPAT4) from *Arabidopsis thaliana*. To allow precise *in vitro* assays of this enzyme, I tagged the protein with a haemagluttinin epitope tag. This allowed me to precisely quantify the level of protein expression from the yeast *Saccharomyces cerevisiae*, which enabled me to test activity using gas chromatography from variants expressed in yeast, allowing medium throughput analysis of mutants. GPAT4 is interesting because it has semi-independent phosphatase and acyltransferase activities. Using sequence analysis, we identified two separate domains in this enzyme, and used site directed mutagenesis to confirm that the N-terminal domain was responsible for the phosphatase activity. Further mutagenesis identified the specific residues that are responsible for this activity. We then focussed on the C-terminal domain, which is considerably less well understood in the literature. Mutagenesis of key residues located in the putative active site of the C-terminal domain confirmed not only that the C-terminal domain is important for acyltransferase activity, but provided new insight into the probably enzymatic mechanism. Finally, I have undertaken preliminary studies directed towards structural characterization of the two domains, with heterologous expression being trialled in *Escherichia coli.*

Outcome: The work undertaken as part of this PhD Scholarship, involving collaboration between the CSIRO and the ANU, has shed new light on the mechanism and function of plant acyltransferases, particularly those enzymes of the GPAT family. As part of this work, I have also developed a sensitive and accurate assay methodology that allows many variants to be tested in a matter of days (*vs* weeks *in planta*). Altogether, these results provide a foundation for future work to engineer new and improved biosynthetic pathways for oils in plants.