Production of Prostanoids in the Plant Arabidopsis thaliana

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A. Introduction

- Prostanoids (prostaglandins (PGs) and thromboxanes) are lipid mediators derived from 20-carbon polyunsaturated fatty acids, which are found in nearly all mammalian tissues and fluids. They contribute to a number of physiological, pharmacological and pathological processes, such as inflammation, thrombosis, and gastro-intestinal tract secretions.
- Transgenic plants provide cheap platforms for the production of high quality products in high yield. Their use eliminates the risk of contamination with human or animal pathogens such as HIV, hepatitis etc.
- We previously achieved the production of di-homo-γ-linolenic acid (DGLA), arachidonic acid (AA) and eicosapentaenoic acid (EPA) in *Arabidopsis thaliana* (Qi *et al.*, 2004) via, so called, Δ^8 pathway by sequential transformation with a Δ^9 elongase from *Isochrysis galbana* (Qi *et al.*, 2002), a Δ^8 desaturase from *Euglena gracilis* (Wallis and Browse, 1999) and a Δ^5 desaturase from *Mortierella alpina* (Michaelson *et al.*, 1998). These 20-carbon fatty acids are the necessary feedstuffs for PG production via various prostanoid synthases.

B. Testing the activity of prostaglandin H synthase (PGHS) in yeast

- Mouse PGHS coding regions were first amplified from cDNA of the two PGHS isoforms, PGHS-1 and PGHS-2.
- The amplified isoforms were cloned in a yeast expression vector. Yeast cultures supplemented with DGLA and AA were induced and repressed by galactose and glucose respectively.
- The cells and cultures were assayed by enzyme immunoassay (EIA) to compare the activities of the different PGHS isoforms.
- **Results:**
  - PGHS genes were active and PGs were produced.
  - PGHS-2 showed low activity relative to PGHS-1.
  - Most PG was found in the culture medium rather than in the cells.
  - PGHS-1 showed the greatest activity with both substrates, while PHGS-2 displayed relatively good activity with DGLA as substrate (69 % of the PGHS-1 level) but very little activity above background with AA (11 % of the PGHS-1 level).

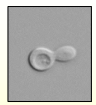
Mouse PGHS cDNAs

PCR amplification

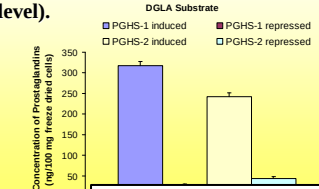
PGHS-1

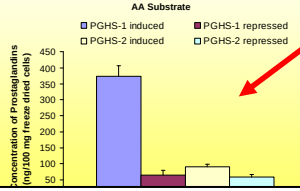
PGHS-2

Yeast Transformation

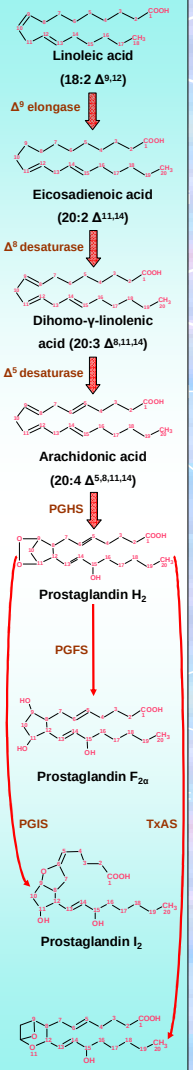


- Protein Expression
- DGLA and AA substrate
- Analysis of cells and culture supernatant by EIA





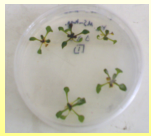
Δ^8 Pathway



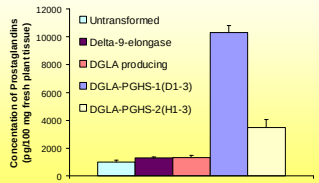
C. Production of Prostaglandin Series 1 in *A. thaliana*

DGLA-producing *A. thaliana*

- Agrobacterium-mediated transformation
- Selection for hygromycin resistance



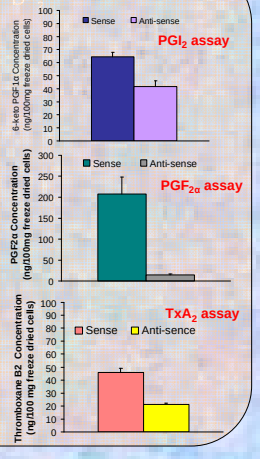
- EIA analysis of lines D1-3 and H1-3 homozygous lines



- DGLA-producing plants were transformed with PGHS-1 and PGHS-2.
- Transformed seeds were germinated on hygromycin-containing medium.
- Healthy seedlings were tested for the presence of the PGHS, Δ^8 desaturase and Δ^9 elongase genes by PCR.
- Plants showing positive results were transferred to soil and allowed to grow on and segregate. Individual homozygous lines were analyzed for PG production by EIA.
- **Results:** PGs were produced in PGHS-1 and to a lesser extent in PGHS-2 transformants. The most active PGHS-1 and PGHS-2 lines (D1-3 and H1-3, respectively) were propagated and 6 individuals of each line were analysed by EIA for PG production. The results (see below) show that the use of PGHS-1 is better than PGHS-2 for this work. These lines are now being grown on a large scale for seed production and for extraction and purification of the prostaglandin produced so that it can be fully analysed.

D. Production of other types of prostanoids

- Mouse prostaglandin I synthase (PGIS) and thromboxane A synthase (TxAS) coding regions were amplified from cDNA.
- *Trypanosoma brucei* prostaglandin F synthase (PGFS) was amplified from genomic DNA.
- The three amplified genes were cloned into a yeast expression vector in sense and antisense orientations with respect to the GAL promoter, and then expressed in yeast together with PGHS-1 using AA as a substrate.
- **Results:** The three genes were active and prostaglandin I_2, thromboxane A_2 and prostaglandin $F_{2\alpha}$ were produced in various amount (see opposite)
- DGLA-producing plants are now being transformed with a Δ^5-desaturase gene (to produce AA and EPA) together with PGHS-1 and each of PGIS, TxAS in order to produce the pharmaceutically active I_2 and $F_{2\alpha}$ or thromboxane A_2, respectively.



References:
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