Lipid Catabolism: Fatty Acid Breakdown

Abstract
Developing *Brassica napus* embryos are primarily concerned with the accumulation of storage products, namely oil, starch and protein. The presence of fatty acid catabolic pathways in the background of this biosynthetic activity was investigated. Enzymes involved in the process of lipid mobilization, such as malate synthase and isocitrate lyase, are detectable towards the late stages of embryo development. 

Introduction
Lipid mobilization is a process vital to germination, senescence and starvation. In seeds, the switch from metabolic inactivity to activation of catabolic processes occurs upon imbibition. Complete breakdown of lipid to sucrose and ATP involves consecutive pathways of lipolysis, \( \beta \)-oxidation, the glyoxylate cycle and gluconeogenesis. Proteins involved in these pathways were once viewed to be synthesized *de novo* during germination. However, various studies on cucumber [1], cotton [2] and rape seed [3] embryos indicate that glyoxysomal enzymes such as malate synthase (MS) and isocitrate lyase (ICL) are present during late stages of embryo maturation. The occurrence and function of fatty acid catabolic enzymes during periods of maximal oil deposition is unclear.

In developing embryos of *Brassica napus*, carbon flux is partitioned towards the main storage products of lipid, protein and starch. Lipid in the form of triacylglycerol is a major component of oilseed storage reserves. In this paper we present preliminary results on the developmental expression of MS, ICL and phosphoenolpyruvate carboxykinase (PEPCK) proteins in *B. napus*. We have also addressed their *in vivo* function by feeding \([^{14}C]\)acetate to whole isolated embryos and studying its incorporation into metabolites and products.

Experimental
*B. napus* cv. Topas were grown to maturity in the glasshouse at 18 \( ^\circ \)C and under a 16 h/8 h light/dark cycle. Embryos were harvested at five stages of development with fresh weights of between 1 and 5 mg/embryo. The first three stages (early-, mid- and late-cotyledon) were as defined previously [4]. Two additional stages used here were desiccating (pale-yellow green embryo, dark brown testa, green silique) and mature (dry) seed. Siliques were chosen randomly from a minimum of five individual plants and the testa removed from each embryo before analysis. Protein extraction and Western-blot analysis were carried out by standard methods. \([^{14}C]\)Acetate feeding experiments to whole embryos were performed according to [5]. Lipid extraction by phase separation was carried out using the method of [4] with the aqueous phase retained for further analysis.

Results and discussion
MS and ICL are enzymes exclusive to the glyoxylate cycle. Previous work [3] has shown that
Immuno-detection of MS, ICL and PEPCK protein in developing embryos

A control lane (Cot.) is included which contains proteins from a cotyledon extract 3 days after imbibition. 25 μg of protein from crude homogenates was separated by 10% SDS-PAGE and bands visualized by the ECL detection system.

Table I
Developmental study of ^4C recovery in organic and aqueous fractions of whole embryos incubated with 40 kBq of ^4C acetate

<table>
<thead>
<tr>
<th>Embryo stage</th>
<th>Aqueous (A)</th>
<th>Organic (O)</th>
<th>A/O ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid-cotyledon</td>
<td>0.15±0.01</td>
<td>0.34±0.01</td>
<td>0.5</td>
</tr>
<tr>
<td>Late-cotyledon</td>
<td>0.39±0.01</td>
<td>0.32±0.01</td>
<td>1.2</td>
</tr>
<tr>
<td>Desiccating</td>
<td>1.01±0.08</td>
<td>0.08±0.01</td>
<td>12.6</td>
</tr>
</tbody>
</table>

At mid-cotyledon stage, the relative partitioning of ^4C acetate into the organic phase and the aqueous fraction was very similar. As embryo development continued and desiccation occurred, the relative proportion of ^4C recovered in the aqueous phase increased while that in the organic phase declined. The ratio of aqueous- to organic-phase recovery in the desiccating embryos was 12.6 compared with 0.5 at mid-cotyledon stage. The levels of ^4C recovered in the late-cotyledon stage were similar in both organic and aqueous fractions. This preliminary result indicates a shift in carbon usage from fatty acid synthesis to a process of fatty acid breakdown as the embryo develops. Preliminary analysis of the aqueous phase reveals a large proportion of ^4C recovered in the form of glucose (results not shown). Taken together, these results suggest that fatty acid-breakdown pathways are increasingly functional as the embryo accumulates oil and other storage products.

References

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