The role of plastidial transporters in developing embryos of oilseed rape (Brassica napus L.) for fatty acid synthesis
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Abstract
The phosphoenolpyruvate transporter (PPT) is one of several important transporters for channeling carbon intermediates utilized for fatty acid synthesis and other plastidial pathways from the cytosol into the plastid. In this paper we show results on how the activity of the PPT changes between two important, physiologically different developmental stages of oilseed rape embryos.

Introduction
In plants fatty acid synthesis occurs exclusively in the plastid and in heterotrophic organs it is dependent on the supply of carbon intermediates from the cytosol. Previous studies have shown that a range of metabolites is used for fatty acid synthesis by plastids from heterotrophic tissues of different plant species [1-4]. However, their relative utilization shows considerable variation depending on developmental stage, organ and species. The differences in efficient usage for fatty acid synthesis are largely determined by the presence and activity of specific transporter proteins on the plastid envelope [1]. These transporters play an important role in channelling various carbon substrates into the plastids and they can also exert control over fatty acid synthesis.

Earlier studies have shown that glucose 6-phosphate (Glc-6-P) and pyruvate are the major substrates for fatty acid synthesis in plastids of oilseed rape embryos [1]. However, their rates of incorporation into fatty acids change significantly during embryo development, with uptake and utilization of Glc-6-P declining and that of pyruvate increasing strongly. These changes in uptake reflect the changing activities of the corresponding transporters rather than glycolytic metabolism in the plastid. Our recent work has included an evaluation of the role of phosphoenolpyruvate (PEP) as a carbon source for fatty acid synthesis and the measurement of the activity of the PEP transporter (PPT) during embryo development.

Experimental
Plastids were prepared from developing embryos of oilseed rape (Brassica napus L. cv Topaz) as described previously [3] with the following modification: peeled embryos (500–550) were homogenized with two single-edge razor blades five times for 1 min in 2 ml of buffer. Each time the extract was filtered through two layers of Miracloth (Calbiochem). For measurements of the activity of the PPT [1-14C]PEP (Amersham) was used. Short-term uptake experiments were performed using the silicone-oil filtration method [5]. The silicone oil consisted of a 1:2 (w/w) mix of AP100:AR200 oils (Wacker Chemicals). Eppendorf reaction vessels (400 µl) were filled with 50 µl of oil mix. Incubations were started by mixing 170 µl of washed plastids with 30 µl of uptake mix...
Concentration dependence of PEP uptake by plastids from developing embryos

A range of substrate concentrations was used to measure uptake of PEP by plastids isolated from embryos at stages A and C. Uptake was measured using the silicone-oil centrifugation technique. Each value represents the mean±S.E.M. of measurements made on three separate plastid preparations.

Table I

Comparison of uptake of Glc-6-P, PEP and pyruvate at stages A and C under saturated conditions (1 mM)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Stage A</th>
<th>Stage C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glc-6-P*</td>
<td>1.25</td>
<td>0.85</td>
</tr>
<tr>
<td>PEP</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Pyruvate*</td>
<td>0.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

*Data are from [1].

stages A and C PEP uptake into plastids increased about 2.0–2.5-fold (Figure 1). At both stages the rate of uptake was saturated at a concentration of 1 mM with \( K_m \) values of about 0.1 mM.

Uptake of PEP is comparable with that of Glc-6-P and pyruvate (Table I). Whereas the activity of the Glc-6-P transporter declines between stages A and C, the transporters for both PEP and pyruvate show increasing activities. The increase in activity of the PPT between stages A and C is not as great as that of the pyruvate transporter.

Further studies are underway to assess the relative fluxes of carbon from Glc-6-P, pyruvate and PEP into fatty acids and other plastidial pathways.

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References


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