Protein interactions of fatty acid synthase II

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Abstract
We have used a yeast two-hybrid approach to detect direct protein interactions between fatty acid synthase components. Enoyl-acyl carrier protein (ACP) reductase was found to interact with stearoyl-ACP desaturase and acyl-ACP thioesterase, but none of these proteins interacted with ACP in the yeast nucleus.

Introduction
De novo fatty acid synthesis is carried out by a set of sequential enzymic reactions. The organization of the fatty acid synthase (FAS) I complex is well characterized as a multifunctional polypeptide with all the enzymic activities resident on one or two polypeptides. The organization of the FAS II enzymes of bacteria and plants, on the other hand, has been variously described as a collection of separate soluble discrete entities and, in complete contrast, as a multi-enzyme complex that channels the enzymic precursors and products, i.e. a metabolon [1,2]. However, although the case for a metabolon is strong there is little evidence to reveal that the enzymes in FAS II interact directly.

In this study we used the yeast two-hybrid system [3] to detect any potential interactions between enzymes of FAS II in an in vitro environment. This is important, as under typical in vitro conditions the proteins being studied may be in a solution > 1000 times more dilute than in their in vivo environment. The dilute environment decreases the probability of interactions, and therefore weak or transient interactions may not be detected. Here, we present the first evidence of direct interaction between three enzymes of FAS II in vitro.

Methods
The work used the Clontech Matchmaker II (yeast two-hybrid) system. Briefly, using PCR, restriction sites were attached to cDNAs of the following FAS II components: acyl carrier protein (ACP), β-ketoacyl-ACP reductase, stearoyl-ACP desaturase (DES) and acyl-ACP thioesterase (TE). The cDNA of interest was then directionally cloned into the yeast two-hybrid vectors pAS2-1 and pACT2. All constructs were sequenced along their full lengths in both 5' and 3' directions.

Yeast strains Y187 (-leu, -trp, lacZ) and CG1945 (-leu, -trp, -His, lacZ) were grown to a D of 0.6 and pairwise transformations were carried out. Transformed Y187 were plated on to -Leu/-Trp media and transformed CG1945 on to -Leu/-Trp/-His media containing 5 mM 3-amino-1,2,4-triazole. In Y187, interactions are detected by monitoring β-galactosidase activity. In CG1945, interactions are detected using growth of colonies (histidine expression) and β-galactosidase activity.

Results
ACP carries the growing acyl chain during fatty acid synthesis. Enoyl-ACP reductase (ENR) carries out the final reductive step before acyl chain growth termination. The acyl chain then has two possible fates: (i) hydrolysis from ACP and export out of the plastid, or (ii) desaturation. The fate depends on whether the chain is hydrolysed and released by TE or desaturated by DES. The nature of the relationship between these three terminal enzymes is not known. In order to investigate this relationship, these enzymes were used pairwise
Table I

Table I

FAS II interactions detected using the yeast two-hybrid system

The interactions were detectable with the stronger lacZ expression in Y187. ENR subunit interactions were detectable after 12- to 16-h of incubation. TE-ENR and DES-ENR interactions took 24 h to be detected. Strength of interaction is indicated on a scale of + to +++++: --, no interaction. Number of experiments: ENR-ENR, n = 6; ENR-TE, n = 5; ENR-DES, n = 3.

<table>
<thead>
<tr>
<th>DNA-binding domain vector (pAS2-I)</th>
<th>Activation domain vector (pACT2)</th>
<th>ACP</th>
<th>ENR</th>
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<tr>
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Figure 1

PCR analysis of constructs present in yeast strains

DNA was extracted from transformed Y187 and the presence of ACP and ENR sequences identified using specific primers. The ACP insert was 0.3 kb in size and ENR was 1 kb. Lane 1, pACT2/ACP and pAS2-I/ENR; lane 2, pACT2/ENR and pAS2-I/ACP.

Discussion

No interactions were detected between ACP and any other component of FAS II tested. This could be because the proteins are directed to the yeast nucleus and post-translational modifications have not occurred, e.g. attachment of a phosphopantetheine group or acylation. Specific cofactors may be required for interactions to occur, e.g. NADPH. Such cofactors can be critical, as shown by recent work where it was found that crotonyl-ACP will not bind ENR in the absence of NADPH [4].

ENR is a tetramer, which explains the homo-interaction detectable in these assays. The strength of this interaction was reflected in the ease of detection of ENR pairs. ENR was also found to interact with the chain-terminating enzyme TE and with the DES.

These three enzymes operate at a critical point of fatty acid synthesis. TE and DES both use the product of the ENR reaction, saturated acyl-ACP, as their substrate. The competition between TE and DES dictates the fate of the acyl chain. This work shows that both TE and DES can interact directly with ENR. Such interactions between ENR, DES and TE may play a crucial role in the regulation of fatty acid synthesis.

Conclusions

For the first time, interactions between three enzymic components of FAS II, ENR, TE and DES, have been detected in vivo. The enzymes ENR and DES both utilize the product of the ENR reaction as their substrate. As they both interact directly with ENR, this may mean that they compete at a common binding site for the ENR product and therefore that they are critical in the regulation of fatty acid and lipid biosynthesis.

References


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