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Genetic modification of essential fatty acids biosynthesis in *Hansenula polymorpha*

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Abstract

The Δ^6-desaturase gene isoform II involved in the formation of γ-linolenic acid (GLA) was identified from *Mucor rouxii*. To study the possibility of alteration of the synthetic pathway of essential fatty acids in the methylotrophic yeast, *Hansenula polymorpha*, the cloned gene of *M. rouxii* under the control of the methanol oxidase (*MOX*) promoter of *H. polymorpha*, was used for genetic modification of this yeast. Changes in flux through the *n*-3 and *n*-6 pathways in the transgenic yeast were observed. The proportion of GLA varied dramatically depending on the growth temperature and media composition. This can be explained by the effects of either substrate availability or enzymatic activity. In addition to the potential application for manipulating the fatty acid profile, this study provides an attractive model system of *H. polymorpha* for investigating the deviation of fatty acid metabolism in eukaryotes.

Keywords: Essential fatty acid; Δ^6-desaturase; *Mucor rouxii*; *Hansenula polymorpha*

1. Introduction

Two main types of polyunsaturated fatty acids (PUFA), *n*-3 and *n*-6 fatty acids, have been shown to exert a regulatory function on cellular processes [1,2]. The nutritional implications of the consumption upon human health are enormous when the optimal ratio of these two PUFA classes is consumed [3]. These two PUFA classes are precursors for synthesizing the beneficial substances known as eicosanoids including leukotrienes, prostaglandins and thromboxanes, which have important biological functions in various systems such as cardiovascular, respiratory and immune systems [3,4]. The *n*-3 eicosanoids are either weak agonists or more usually, strong antagonists of the potent *n*-6 metabolites [1]. γ-Linolenic acid (C18:3 $\Delta^{6,9,12}$, GLA), which is recognized as *n*-6 essential fatty acid (EFA), is the result of the body's first biochemical step in the transformation of the main essential fatty acid, linoleic (C18:2 $\Delta^{9,12}$, LA) into the *n*-6 eicosanoids. The pathway of the formation of another eicosanoids comes from desaturation and elongation of an EFA in *n*-3 series, α-linolenic acid (C18:3 $\Delta^{9,12,15}$, ALA). Imbalances in the PUFA metabolism are thought to be linked to a

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number of pathological conditions in humans [5]. The realization of the beneficial impact on medical and biotechnological uses of EFAs has led to the development of various approaches to meet the increased demands [6,7]. In addition to seek the potential sources of EFA rich oils, the study of fatty acid metabolism and its regulation at the molecular level including gene function, expression, gene interactions and regulatory networks are combined for full understanding and consequently for effective manipulation of the PUFA profile by pathway engineering. Recent advances in multidisciplinary research such as molecular biology, biochemistry and bioinformatics have expanded opportunities to create new transgenic organisms in accordance with rational design for production of specific fatty acids [8–10].

The growing number of the fatty acid desaturase genes identified from different organisms allows analyses of the evolutionary relationships and substrate utilization among these desaturase enzymes [11–13]. It has been well known that the formation of GLA and ALA is associated with the bioconversion of substrate LA by position-specific desaturases, Δ^6- and Δ^{15}-desaturases, respectively (see Fig. 1). The variable existence of biosynthetic routes of such EFAs has been found in the microbial kingdom [14]. The amazing diversity of fatty acid profile in yeasts and fungi provides a means for elucidation of fatty acid metabolism in higher eukaryotes and for the study of the possibility of manipulation of cellular fatty acid. The methylotrophic yeast *Hansenula polymorpha*, a non-conventional yeast with biotechnological potential, has been reported to contain enzymes involved in *n*-3 fatty acid biosynthesis [15]. Like higher plants, this yeast accumulates a high content of LA, an important intermediate substrate in both *n*-3 and *n*-6 PUFAs biosynthesis (see Fig. 1). As such, *H. polymorpha* was of considerable interest as a eukaryotic model organism for engineering the metabolic pathway of PUFA biosynthesis by genetic modification and for studying the effect of deviation of fatty acid metabolites on aspects of utilization of intracellular substrates and existing enzymes under different growth conditions. The Δ^6-desaturase gene isoform II was cloned from *Mucor rouxii*, a filamentous fungus capable of GLA synthesis. This is the first report of the exploitation of *H. polymorpha* as a host for heterologous expression of the enzyme involved in fatty acid synthesis.

2. Materials and methods

2.1. Culture strains and growth conditions

M. rouxii ATCC 24905, used as a genetic resource, was routinely maintained and subcultured as previously described [16]. *H. polymorpha* strain KYC625 (*leu1-1 ade11-1*), provided by Dr. Y. Kaneko of Osaka University, was used for the analysis of expression of the cloned gene and as a model organism for engineering the fatty acid biosynthesis pathway. *H. polymorpha* was grown in a YPD medium containing 1% yeast extract, 2% bactopeptone, and 2% glucose at 37 °C. The synthetic medium for selection of *H. polymorpha* transformants was SD (0.67% bacto-yeast nitrogen base without amino acids and 2% glucose) and 20 mg/l of adenine sulfate was supplemented when necessary. *Escherichia coli* DH5α was grown at 37 °C in a Luria–Bertani medium (LB) supplemented with 50 mg/l of kanamycin when necessary.

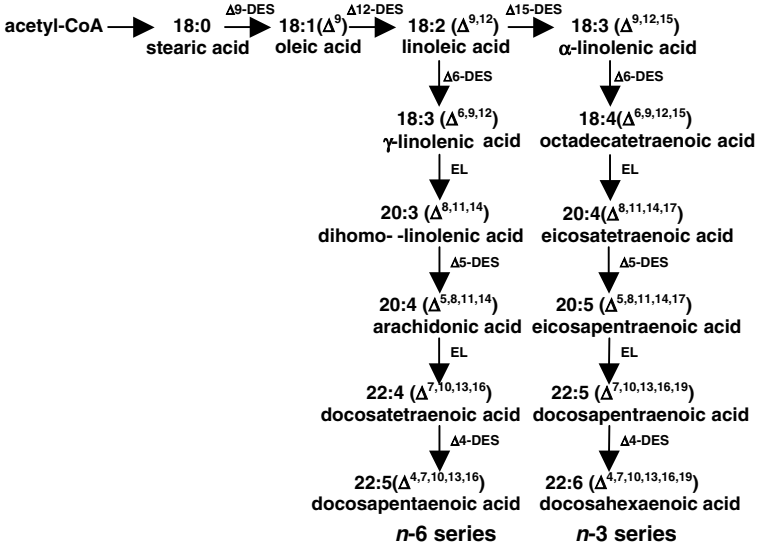

Fig. 1. Biosynthetic pathways of *n*-3 and *n*-6 polyunsaturated fatty acids in biological systems showing chain elongation and desaturation steps. Abbreviations: Δ^n-DES, n-desaturase; EL, elongase.