Leucine Biosynthesis in Yeast

Identification of Two Genes (LEU4, LEU5) that Affect α-Isopropylmalate Synthase Activity and Evidence that LEU1 and LEU2 Gene Expression is controlled by α-Isopropylmalate and the Product of a Regulatory Gene

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Summary. Tetrad analysis indicates that α-isopropylmalate synthase activity of yeast is determined by two separate genes, designated LEU4 and LEU5. LEU4 is identified as a structural gene. LEU5 either encodes another α-isopropylmalate synthase activity by itself or provides some function needed for the expression of a second structural gene. The properties of mutants affecting the biosynthesis of leucine and its regulation suggest that the expression of LEU1 and LEU2 (structural genes encoding isopropylmalate isomerase and β-isopropylmalate dehydrogenase, respectively) is controlled by a complex of α-isopropylmalate and a regulatory element (the LEU3 gene product). Similarities and differences between yeast and Neurospora crassa with respect to leucine biosynthesis are discussed.

Key words: Saccharomyces cerevisiae — Isoenzymes — Induction

Introduction

The biosynthesis of leucine via the isopropylmalate (IPM) pathway occurs in three specific steps, catalyzed by α-IPM synthase (EC 4.1.3.12), IPM isomerase (dehydratase) (EC 4.2.1.33), and β-IPM dehydrogenase (EC 1.1.1.85), respectively (Scheme 1). The regulation of this pathway has been studied most extensively in enteric bacteria (Soper et al. 1976; Calvo 1983) and in fungi (Gross 1969; Kohlhaw 1983). In Salmonella typhimurium and Escherichia coli, the three leucine pathway-specific enzymes are encoded by four contiguous genes. These genes constitute an operon which is controlled mainly by attenuation (Calvo 1983). Leucine regulation in fungi differs in two important ways from the pattern observed in bacteria. First, the genes do not constitute an operon. In fact, most of the genes that have been localized (leu-1, leu-2, leu-3, and leu-4 of N. crassa and LEU1 and LEU2

Scheme 1. Biosynthesis of the keto acid precursor of leucine. The designation of genes is for Saccharomyces cerevisiae, Neurospora crassa, and Escherichia coli, respectively (top to bottom)

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2 At the time these experiments were performed, no strong evidence for isoenzymes of α-IPM synthase existed. Therefore, no effort was made to find out whether the observed effects were due to changes in both isoenzymes or in only one of them

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of yeast), lie within different linkage groups (Gross 1969; Mortimer and Schild 1980). Although leu-3 and leu-4 of N. crassa belong to the same linkage group, they are not tightly linked (Gross 1969). Second, leucine regulation in Neurospora and yeast involves intricate combinations of the modulation of enzyme activity, repression and induction.

In N. crassa, the intracellular level of α-IPM regulates leucine biosynthesis by facilitating the expression of the leu-2 and leu-1 genes in concert with a putative regulatory protein (the leu-3 gene product) (Gross 1965; Kashmiri and Gross 1970; Polacco and Gross 1973; Reichenbecher et al. 1978). Control of leu-2 and leu-1 expression by leucine is therefore an indirect result of feedback inhibition and/or repression of α-IPM synthase.

The overall mechanism of regulation of leucine biosynthesis in yeast appears to be similar to that of N. crassa, but there are significant differences. One important point of difference is α-IPM synthase whose properties, regulation, and localization are distinct in yeast. Thus, the subunit molecular weight of the yeast synthase is 1.5 times that of its N. crassa counterpart (Kohlhaw 1983). Furthermore, the yeast enzyme is not only inhibited by leucine (Satyanarayana et al. 1968; Ulm et al. 1978), but is also inactivated by coenzyme A, a product of the reaction (Tracy and Kohlhaw 1975, 1977; Hampsey and Kohlhaw 1981). Coenzyme A inactivation, regarded to be part of a system which regulates the distribution of acetyl-CoA among anabolic and catabolic pathways, has not been reported for N. crassa α-IPM synthase. A further difference between the two enzymes is their intracellular localization. Most of the α-IPM synthase of yeast is located in the mitochondrial matrix (Hampsey et al. 1983), whereas the N. crassa enzyme appears to be cytosolic (S. R. Gross, personal communication). Differences also exist in the way these organisms regulate the synthesis of α-IPM synthase. N. crassa utilizes both repression control by leucine and regulation by the putative α-IPM-regulatory protein complex (Gross 1969).

In yeast, excess leucine in the growth medium consistently causes a significant increase in the level of α-IPM synthase (Brown et al. 1975; this paper), possibly because the enzyme is subject to the cross-pathway regulation known as “general control” of amino acid biosynthesis (Hsu et al. 1982; see Discussion). Cross-pathway regulation involving α-IPM synthase has not been described for N. crassa.

The LEU2 gene of yeast and its flanking regions have recently been sequenced (Andreadis et al. 1982). Other leu genes of yeast are now being isolated and characterized. It therefore seemed timely to gather more basic knowledge on the biosynthesis of leucine in this organism. We show in this paper that the α-IPM synthase activity of yeast is in all likelihood determined by at least two genes. Judging by the properties of mutant strains of yeast, we conclude that α-IPM induces the synthesis of IPM isomerase and β-IPM dehydrogenase, in conjunction with the product of a regulatory gene, much like in N. crassa.

Materials and Methods

Strains. The strains of S. cerevisiae used in this study are listed in Table 1.

Growth Conditions. Cells were grown aerobically at 30 °C. After at least 7–8 generations, cells were harvested in late log phase (O.D. ca. 0.85 on Spectronic 20 photometer). Cells used for determination of enzyme activities were grown in medium M-N supplemented with 0.1% ammonium sulfate and 2% glucose (Fink 1970). Complete (YE PD) and minimal media used for all other purposes were those described by Fink (1970).

Genetic Techniques. Genetic crosses, tetrad analysis, random spore plating, and complementation tests were performed as described by Fink (1970) and Sherman et al. (1979).

Cell-Free Extracts used for assaying IPM isomerase and β-IPM dehydrogenase were prepared from cells that had been frozen for at least 15 h at -20 °C. Typically, 1 g of cells (wet weight) was suspended in 1.5 ml of 0.1 M potassium phosphate buffer, pH 6.9, containing 1.25 M ammonium sulfate, 20% (v/v) glycerol, and 0.03% sodium azide. The suspension was passed twice through a French pressure cell at 138 megapascals. Extracts were clarified by centrifugation at 39,000 × g for 20 min.

Permeabilization of Yeast Cells for the purpose of assaying α-IPM synthase was performed as described by Miozzari et al. (1978).

Enzyme Assays. α-Isopropylmalate synthase activity was determined as described by Tracy and Kohlhaw (1977). Specific activity is expressed as nanomoles of product formed per min per mg of cells (wet weight). Wet weights were standardized by determining whole cell protein by a modified biuret method (Herbert et al. 1971). The “in situ” assay was chosen because of its greater reproducibility compared to assays performed with cell-free extracts.

Isopropylmalate isomerase was assayed by the method of Cho-Chung and Umbarger (1970), using disodium dimethylcitraconate as substrate. Specific activity is expressed as nanomoles of substrate utilized per min per mg of protein. β-Isopropylmalate dehydrogenase was assayed by the colorimetric method described previously (Hsu and Kohlhaw 1980). Specific activity is expressed as nanomoles of product formed per min per mg of protein. All assays were performed at 30 °C.

Determination of Amino Acid Pools. Cells from 0.3 l of culture were harvested in late log phase on a Reeve Angel glass fiber filter, washed three times with ice-cold physiological saline solution, weighed, resuspended in 10 ml ice-cold 5% trichloroacetic acid solution, and stirred on ice for 20 min. Cells were then collected on a fresh filter and washed three times with 5% trichloroacetic acid solution. The filtrate was extracted twice with anhydrous ether, and the aqueous phases pooled and lyophilized. The residue was then redissolved in 50 mM citrate buffer, pH 2.2, and subjected to amino acid analysis on a Durum D-500 analyzer. One micromole of norleucine was carried through the entire procedure as an internal standard.