

# Deoxyhypusine synthase gene is essential for cell viability in the yeast *Saccharomyces cerevisiae*\*\*

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Received 1 February 1996; revised version received 11 March 1996

**Abstract** Deoxyhypusine synthase catalyzes the first of two steps in the biosynthesis of hypusine, a modification of a specific lysine residue in the precursor of eukaryotic translation initiation factor 5A. We have purified deoxyhypusine synthase from yeast, and cloned and sequenced the corresponding gene encoding a 387-amino acid protein from *Saccharomyces cerevisiae*. Gene disruption experiments indicated that the deoxyhypusine synthase gene is essential for cell growth in yeast. This gene was shown to be an intron-free, single-copy gene, and its product can catalyze the synthesis of deoxyhypusine equally in two precursor forms of eIF-5A, derived from two distinct genes of yeast.

**Key words:** Deoxyhypusine synthase; Hypusine; Eukaryotic initiation factor 5A; Post-translational modification; Gene disruption

## 1. Introduction

Deoxyhypusine synthase catalyzes the first of two steps in the biosynthesis of an unusual amino acid, hypusine, found only in eukaryotic translation initiation factor 5A (eIF-5A) [1]. It catalyzes the transfer of the 4-aminobutyl moiety of spermidine to the  $\epsilon$ -amino group of a specific lysine residue in an eIF-5A precursor protein in the presence of NAD<sup>+</sup> [2]. This results in the formation of deoxyhypusine (*N*<sup>ε</sup>-(4-aminobutyl)lysine) which is subsequently hydroxylated by a second enzyme to form hypusine [3]. Inhibition of deoxyhypusine synthase activity by chemicals [4] or by limiting the level of the substrate spermidine [5] arrests the growth of eukaryotic cells, suggesting that this enzyme may play a pivotal role in cell growth. It has been shown by mutational analysis that preservation of the site of hypusination of eIF-5A (Lys<sup>51</sup> in the yeast precursor) is vital for the factor's function(s) [6]. Yeast contains two forms of 5A, designated eIF-5Aa and eIF-5Ab, derived from two distinct genes reciprocally regulated by oxygen [6]. It remains to be elucidated whether the

two forms of precursor are modified by different forms of deoxyhypusine synthase.

Very recently, based on the in vitro assay method, the heterologous expression of a yeast cDNA clone of the coding region with deoxyhypusine synthase activity in *E. coli* has been reported [7]. However, questions about the in vivo expression, copy number, and functional importance of the deoxyhypusine synthase gene in yeast remain to be elucidated. We have purified deoxyhypusine synthase from yeast. Based on the partial amino acid sequences of the purified enzyme, we report the cloning and sequencing of the full length deoxyhypusine synthase gene from *S. cerevisiae* genomic DNA and show that it is an intron-free single-copy gene and essential for yeast cell growth. The purified recombinant protein expressed from the cloned gene was shown to have the same level of activity as the yeast enzyme.

## 2. Materials and methods

### 2.1. Materials

*S. cerevisiae* strain 131 (MATa/MAT $\alpha$  ade2/ade2) [8] was kindly supplied by Dr. Yasuo Hotta (Nara Advanced Institute of Science and Technology). The diploid yeast strain KA31 (MATa/MAT $\alpha$  ura3/ura3 leu2/leu2 his3/his3 trp1/trp1), and the plasmids YIplac128 and YCplac22 [9], were generous gifts from Drs. K. Matsumoto and K. Irie of this department. [<sup>14</sup>C]Spermidine trihydrochloride (*N*-(3-aminopropyl)[1,4-<sup>14</sup>C]tetramethylene-1,4-diamine trihydrochloride) with a specific activity of 114 mCi/mmol was from Amersham, UK.

### 2.2. Enzyme assay

Deoxyhypusine synthase activity was measured based on the incorporation of radioactivity as 3.8% PCA precipitate from [<sup>14</sup>C]spermidine into the eIF-5A precursor protein, produced by expressing the yeast eIF-5A genes in *E. coli*. Typical assay mixtures contained 4  $\mu$ M [<sup>14</sup>C]spermidine, 0.5 mM NAD<sup>+</sup>, 3  $\mu$ M ec-eIF-5A, 1 mM DTT, and purified deoxyhypusine synthase in 50  $\mu$ l of 0.25 M glycine-NaOH buffer, pH 9.5, and were incubated for 30 min at 25°C. Labeled deoxyhypusine was precipitated onto a Whatman 3 MM paper disk by incubation at 90°C in 3.8% PCA for 10 min. The paper disk was washed with 0.2 N HCl, and ethanol, and dried. Radioactivity was measured with a liquid scintillation spectrometer.

### 2.3. Purification of deoxyhypusine synthase from yeast

Frozen *S. carlsbergensis* cells (~400 g) were ground with quartz sand and extracted with buffer A containing 50 mM Tris-HCl (pH 7.5), 30 mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM 2-ME, 10% (v/v) glycerol, 1 mM PMSF and 5  $\mu$ g/ml antipain. After ultracentrifugation of the extract at 100 000 g for 4.5 h, the ammonium sulfate precipitate (between 50 and 100% saturation) from the supernatant was subjected to conventional column chromatography using DEAE-Sephacel, DEAE-Toyopearl, and hydroxyapatite columns. Two major peaks with enzyme activity (peak 1, 200–235 mM KCl; peak 2, 275–310 mM KCl) were observed on the DEAE-Sephacel column. Both the activity peaks were purified separately. The final column was an aminobutyl-agarose (Sigma) affinity column equilibrated with buffer B (250 mM glycine-NaOH, pH 9.5, 10 mM KCl, 10 mM 2-ME, 0.1 mM EDTA, 0.1 mM PMSF, 0.5 mM NAD<sup>+</sup> and 25% glycerol). After loading, the column was washed with 5.5 column volumes of buffer B, and eluted with

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\*\*The nucleotide sequence data reported in this paper has been submitted to the DDBJ/EMBL/Gene Bank nucleotide sequence data bases with accession number D78185.

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**Abbreviations:** ec-eIF-5A, the precursor of eIF-5A expressed in *E. coli* from yeast eIF-5A genes; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; IPTG, isopropyl-1-thio- $\beta$ -D-galactopyranoside; PMSF, phenylmethylsulfonyl fluoride.



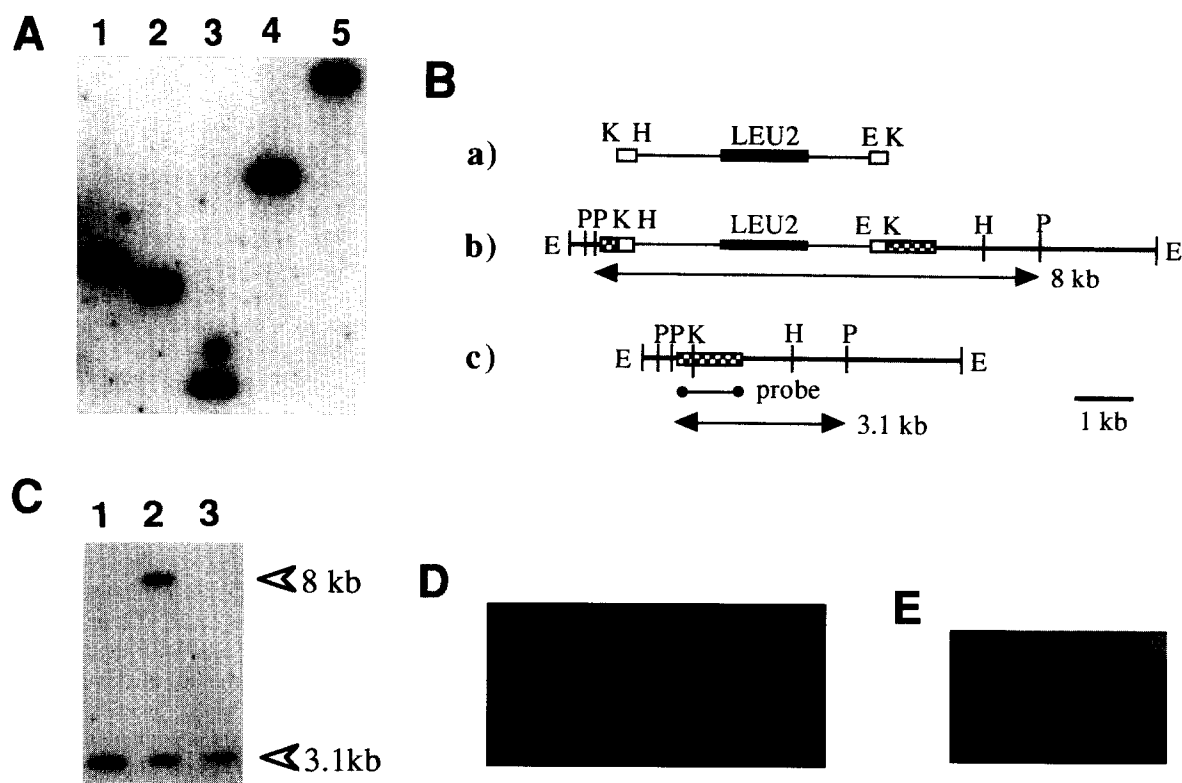


Fig. 2. *DYS1* is a single-copy and essential gene. (A) Genomic DNA from strain KA31 was digested with various restriction enzymes and probed with  $^{32}\text{P}$ -labeled *DYS1*. Lanes: 1, *Pst*I; 2, *Eco*RI-*Hind*III; 3, *Kpn*I-*Hind*III; 4, *Eco*RI; 5, *Bam*HI. (B) Illustration of integrative disruption (white boxes, plasmid-borne coding region of *DYS1*; stippled boxes, coding region of chromosomal *DYS1*; thick line, chromosomal DNA; thin line, plasmid DNA): (a) 4.9 kb linearized integrative plasmid YIplac128 carrying 0.3 kb *DYS1* fragment at each end; (b) the disrupted *dys1* with LEU2 marker; and (c) restriction map of chromosomal *DYS1*. E, *Eco*RI; P, *Pst*I; K, *Kpn*I; H, *Hind*III. (C) Southern blot: *Pst*I digested total DNA from parental diploid KA31 (lane 1), transformed *Leu*<sup>+</sup> diploid RAKA31 (lane 2), and *Leu*<sup>-</sup> haploid (lane 3). (D,E) tetrad dissections: after sporulation of a transformed diploid, asci were dissected. Spores from a single ascus were aligned vertically and allowed to germinate at 30°C for 3–5 days. (D) RAKA31 (*DYS1*/*dys1::LEU2*); (E) RAKA31 carrying pRA1 (*DYS1*).

### 3.3. *DYS1* is a single-copy and essential gene

A single band of the expected size was observed when yeast genomic DNA was digested with *Pst*I, *Eco*RI, *Bam*HI, and a combination of *Eco*RI and *Hind*III enzymes, and probed with the coding region of *DYS1* (Fig. 2A). Unexpectedly, digestion with *Kpn*I (having its restriction site in the coding region) combined with *Hind*III (Fig. 2A, lane 3) gave a single band. This may be due to the absence of any *Kpn*I and the second *Hind*III sites outside the coding region in the close vicinity of the *DYS1* gene. These findings indicated that the *DYS1* gene is a single-copy gene in yeast.

The essentiality of the *DYS1* gene for yeast growth was examined by gene disruption and its rescue with the normal gene as shown in Fig. 2. A *Leu*<sup>+</sup> diploid strain RAKA31, heterozygous at the *DYS1* locus, was obtained (section 2). It carried normal *DYS1* (panel c) on one allele, and the disrupted *dys1::LEU2* (panel b) formed by homologous recombination at the *Kpn*I site of the chromosomal gene on the other allele. Gene disruption was confirmed by southern hybridization as shown in Fig. 2C (lane 2). Upon sporulation, tetrads were dissected (Fig. 2D). Only two of the four spores of each tetrad were viable (7 out of 9 dissected asci) and all viable spores were *Leu*<sup>-</sup>, indicating that the disruption of *DYS1* gene is lethal in haploid. Furthermore, RAKA31 (*DYS1*/*dys1::LEU2*) was transformed with the centromeric plasmid pRA1 (section 2). One of the *Leu*<sup>+</sup>*Trp*<sup>+</sup> transformants was allowed to sporulate for tetrad analysis (Fig. 2E).

All the viable spores were either *Leu*<sup>+</sup>*Trp*<sup>+</sup> or *Leu*<sup>-</sup>*Trp*<sup>+</sup>, indicating that the disrupted *DYS1* on a chromosome was rescued by an intact *DYS1* on pRA1. No *Leu*<sup>+</sup>*Trp*<sup>-</sup> spores were obtained. The *Leu*<sup>+</sup>*Trp*<sup>+</sup> haploid cells were stable for many generations. These results confirm that the cloned *DYS1* is expressed in yeast and the gene is essential for yeast cell viability.

### 3.4. The cloned *DYS1* gene can produce highly active deoxyhypusine synthase

In an attempt to identify the protein product from the cloned *DYS1* gene, the DNA clone was expressed in *E. coli* using pET-3a plasmid. As illustrated in Fig. 3A, SDS-PAGE analysis showed that a 43-kDa protein was overexpressed in IPTG-induced cells (amounting to 15–20% of the total soluble protein in the extracts, lane 1) and was subsequently isolated as a single band using two chromatographic steps based on deoxyhypusine synthase activity (lanes 2,3). A polyclonal antibody raised against the recombinant enzyme detected a 43-kDa protein as a single band in yeast cell extracts (data not shown). The recombinant enzyme exhibited the same kinetics as the purified yeast enzyme, and both enzymes equally catalyzed the formation of deoxyhypusine with a specific activity of 250 pmol of product/μg of enzyme per 30 min at 25°C, as shown in Fig. 3B. Similar values were obtained using either one of the two precursors, ec-eIF-5Aa and ec-eIF-5Ab, as an acceptor of the aminobutyl group.

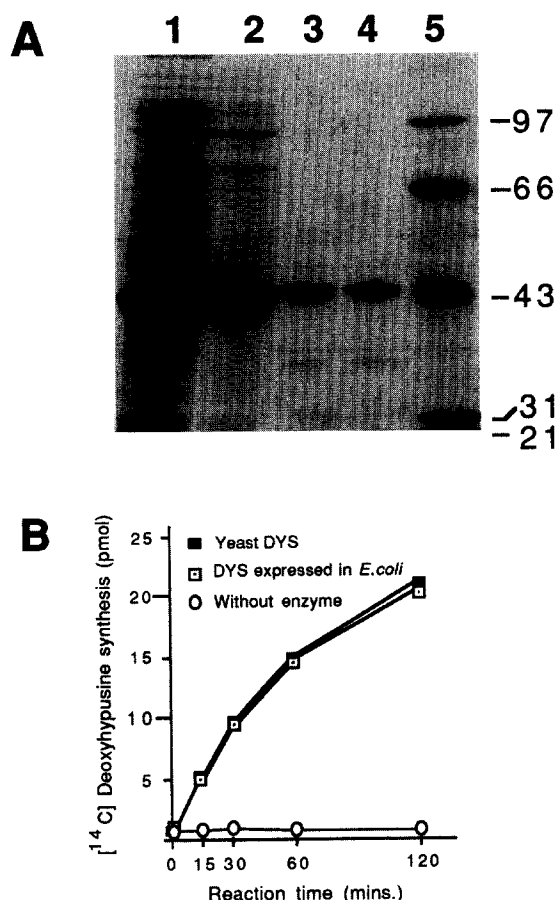


Fig. 3. Purification and kinetics of the recombinant deoxyhypusine synthase expressed in *E. coli*. (A) SDS-PAGE (10% gel). Lanes: 1, extract of *E. coli* expressing recombinant enzyme; 2, an activity peak fraction of the recombinant enzyme on DEAE-Toyopearl column; 3, a pooled fraction of the enzyme eluted from aminobutyl agarose column; 4, deoxyhypusine synthase purified from yeast; 5, molecular weight markers (kDa). (B) Kinetics of synthesis of deoxyhypusine from [<sup>14</sup>C]spermidine in yeast ec-eIF-5Aa. The amount of the enzyme used in each reaction was 40 ng, either purified from yeast or from *E. coli* (recombinant). DYS, deoxyhypusine synthase.

#### 4. Discussion

We have described here for the first time the purification of deoxyhypusine synthase from yeast. Based on the partial amino acid sequences of the tryptic peptides obtained from the enzyme, we have cloned the corresponding gene. A genomic DNA clone of *S. cerevisiae* encoding a protein of 387 amino acid with deoxyhypusine synthase activity was obtained and characterized. While this manuscript was in preparation, the purification of deoxyhypusine synthases from diverse species, e.g. *Neurospora* [14], rat [15] and human [16], and amino acid sequences of several peptides were reported. Very recently, the region YHR068w on chromosome VIII of *S. cerevisiae* [17] was indicated as a gene for a yeast deoxyhypusine synthase by cloning a cDNA obtained from PCR of the coding region, based on its (YHR068w) homology to deoxyhypusine synthases from other organisms [7,16] and the heterologous expression of its activity in *E. coli* [7]. However, they provided no in vivo evidence to indicate that this YHR068w region expressed the active enzyme within yeast cell. Our amino

acid sequence of DYS1 is identical with that of YHR068w except for 6 nucleotide changes in the coding region (all of them at third base of the codons) and 18 nucleotide changes outside the ORF, as shown in Fig. 1 (lowercase letters). These discrepancies might be due to differences in the strains used.

Having a genomic clone of deoxyhypusine synthase at hand allowed us to determine the copy number of the gene in yeast. The cloned gene (3.7 kb) with its 5'- and 3'-UTR enabled us to perform the complementation experiment by expression of the gene from its native promoter(s) in a null haploid yeast strain. In this study we have identified DYS1 as an intron-free single-copy gene in the yeast *S. cerevisiae* and provided compelling evidence that a functional copy of this gene is required for cell proliferation. To our knowledge, this is the first direct evidence showing the in vivo essentiality of deoxyhypusine synthase gene for cell viability and its copy number in any eukaryotic cell. These findings indicate that in yeast there are no alternative means for deoxyhypusination at least in the wild-type background. According to this study, protein expressed from the single-copy DYS1 gene appears to modify either one of the two precursors of eIF-5A expressed in aerobically or anaerobically grown cells with equal efficiency.

The availability of the recombinant enzyme and the ability to manipulate this gene using yeast genetics should provide powerful tools for future studies on the molecular mechanism of the biosynthesis of deoxyhypusine and the controlled expression of this essential gene.

**Acknowledgements:** We are grateful to Drs. K. Matsumoto and K. Irie for valuable advice and helpful discussions during the course of this work. We thank Dr. J. Suzuki for critical reading of the manuscript.

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