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The 110 kDa glutathione transferase of *Yarrowia lipolytica* is encoded by a homologue of the *TEF3* gene from *Saccharomyces cerevisiae*: Cloning, expression, and homology modeling of the recombinant protein

Shane McGoldrick, Tommie V. McCarthy, David Sheehan *

Department of Biochemistry, University College Cork, Lee Maltings, Prospect Row, Mardyke, Cork, Ireland

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Abstract

The *TEF4* gene of the non-saccharomyces yeast *Yarrowia lipolytica* encodes an EF1Bγ protein with structural similarity to the glutathione transferases (GSTs). This 1203 bp gene was cloned, over-expressed in *Escherichia coli*, and the recombinant protein characterized. DNA sequencing of the cloned gene agreed with the recently completed *Y. lipolytica* genome and showed 100% identity to a previously reported 30-residue N-terminal sequence for a 110 kDa *Y. lipolytica* GST, except that it encoded two additional N-terminal residues (*N*-Met-Ser-). The recombinant protein (subunit M_r 52 kDa) was found not to possess GST activity with 1-chloro-2,4-dinitrobenzene. Partial tryptic digestion released two fragments of M_r 22 and 18 kDa, which we interpret as N- and C-terminal domains. Homology modeling confirmed that the N-terminal domain of *Y. lipolytica TEF4* encodes a GST-like protein.

Keywords: Glutathione transferase; *TEF4*; EF1Bγ; Elongation factor; Yeast; *Yarrowia lipolytica*; Cloning; Sequence; Expression; Recombinant

Glutathione transferases (GSTs; EC 2.5.1.18) are detoxification enzymes which have been categorized into multiple classes (for reviews, see [1–3]). They catalyze a range of activities [1,2] and can also bind xenobiotics as non-substrate ligands [4]. Enzymes from yeast and fungi are poorly understood although multiple isoenzymes/genes have been identified [5,6]. We previously reported purification of an unusually large GST from the non-saccharomyces yeast, *Yarrowia lipolytica*, with a native M_r of 110 kDa (subunit M_r of 50 kDa) [7]. Two isoforms with identical N-terminal sequences and similar biochemical properties catalyzed conjugation to glutathione (GSH) but did not bind to GSH- or *S*-hexyl-GSH-agarose nor did they immunoblot with GST antibodies [7].

Yeast elongation factor 1 comprises subunits EF1A, EF1Bα, and EF1Bγ [8,9]. EF1A facilitates binding of aminoacyl-tRNA to the ribosomal A site and the EF1B complex acts as the guanine exchange factor for EF1A [9]. Unlike EF1A and EF1Bα, neither homologue of *Saccharomyces cerevisiae* EF1Bγ (encoded by *TEF3* and *TEF4*) is essential [10]. Tef3p was identified in a screen for calcium-dependent membrane binding proteins, and, independently, as a gene-dosage extragenic suppressor of the cold-sensitive mutant *drs2* [10,11]. *TEF4*, on the other hand, cannot suppress the *drs2* mutation and Tef4p does not co-purify with Tef3p, indicating that the proteins differ in biochemical properties and/or intracellular location [10,12]. EF1Bγ subunit is found to be associated with EF1Bα in a variety of eukaryotic organisms [13,14] and stimulates the activity of EF1Bα in *Artemia salina* [15]. Studies in human fibroblasts indicate that the EF-1 complex is associated with the endoplasmic reticulum where it may be anchored via EF1Bγ [16]. EF1Bγ association with mRNA has been reported [17] and EF1Bγ is over-expressed in adenocarcinomas and gastric carcinoma cells [18,19].

EF1Bγ has distinct GST-like N- and C-terminal domains connected by a protease-susceptible linker. A crystal

* Corresponding author. Fax: +353 21 4274034.
E-mail address: d.Sheehan@ucc.ie (D. Sheehan).

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structure for the 219-residue N-terminal domain of EF1Bγ from *S. cerevisiae* Tef3p is available [20]. Recombinant Tef3p did not bind GSH nor show conjugation activity with 1-chloro-2,4-dinitrobenzene (CDNB). Genes encoding GST-like domains of EF1Bγ and other multi-functional proteins are widespread in fungal genomes [5,6]. Complete genomes are now available for several fungi including *Y. lipolytica* [21]. Multiple sequence alignment has revealed that *TEF3*/*TEF4* sequences are especially common and confirmed that the 30-residue N-terminal sequence previously directly determined by us from purified 110 kDa GSTs [7] is identical to the N-terminal region of *Y. lipolytica* Tef4p. Tef4p is homologous to *S. cerevisiae* Tef3p (64.8%) and Tef4p (67.2%) [6].

We are interested in probing the relationship between the previously characterized GST from *Y. lipolytica* and Tef3p from *S. cerevisiae*. We have cloned *TEF4* from *Y. lipolytica* and expressed recombinant Tef4p. The protein is inactive with CDNB and susceptible to proteolytic cleavage. Homology modeling with *S. cerevisiae* Tef3p as a template confirmed that the protein belongs nonetheless to the GST structural superfamily.

Materials and methods

DNA isolation and amplification. Genomic DNA was isolated from *Y. lipolytica* E150 [22]. Yeast were streaked on 1.2% YPD agar (24 h, 30 °C) and collected by centrifugation (16,000*g*, 2 min). Pellets were resuspended in buffer B (2% Triton X-100, 1% SDS, 100 mM NaCl, 1 mM EDTA, and 10 mM Tris–HCl, pH 8) with glass beads (0.3 g), 200 μl buffered phenol/chloroform/isoamyl alcohol (25:24:1) and vortexed (3 min). TE buffer (1 mM EDTA, 10 mM Tris–HCl, pH 8) (200 μl) was then added. After vortexing (1 min) and centrifugation (16,000*g*, 5 min), DNA was ethanol precipitated from supernatant and collected in 50 μl TE buffer. Primers for amplification of *Y. lipolytica TEF4* were based on the N-terminal sequence [7] and genome ORF data [21] (Fig. 1). Primer sets F1 (5′-GAGGAATAATAAATGTCTATCGCCAAGATCTACGACCTCCAGACCCCCCGATACACTTCTATCAA-3′) and R1 (5′-AGGAGCCAGUAGGAGACGG CAACGA-3′) amplified *TEF4* exons 1 and 2 (product 1, P1; ribosome-binding site underlined). Primer sets F2 (5′-ACTGGCTCCUCTCCCAGATCCCCAACCA-3′) and R2 (5′-TTTAAGAACAGCACCGTCGACAAT-3′) amplified *TEF4* exon 3 (product 2, P2). Products were purified with QIA quick PCR purification kit (Qiagen GmbH). Glycosylase-mediated cleavage of DNA post-amplification was carried out using a modified method [23]. P1 and P2 (10 μl) were incubated with Uracil DNA Glycosylase (UDG) (5 U; New England Biolabs) and incubated (37 °C, 30 min) followed by incubation with 50 mM NaOH (95 °C, 15 min). After neutralization (30 mM TrisBase), T4 DNA ligase (2 U; New England Biolabs) was added and incubated (16 °C,

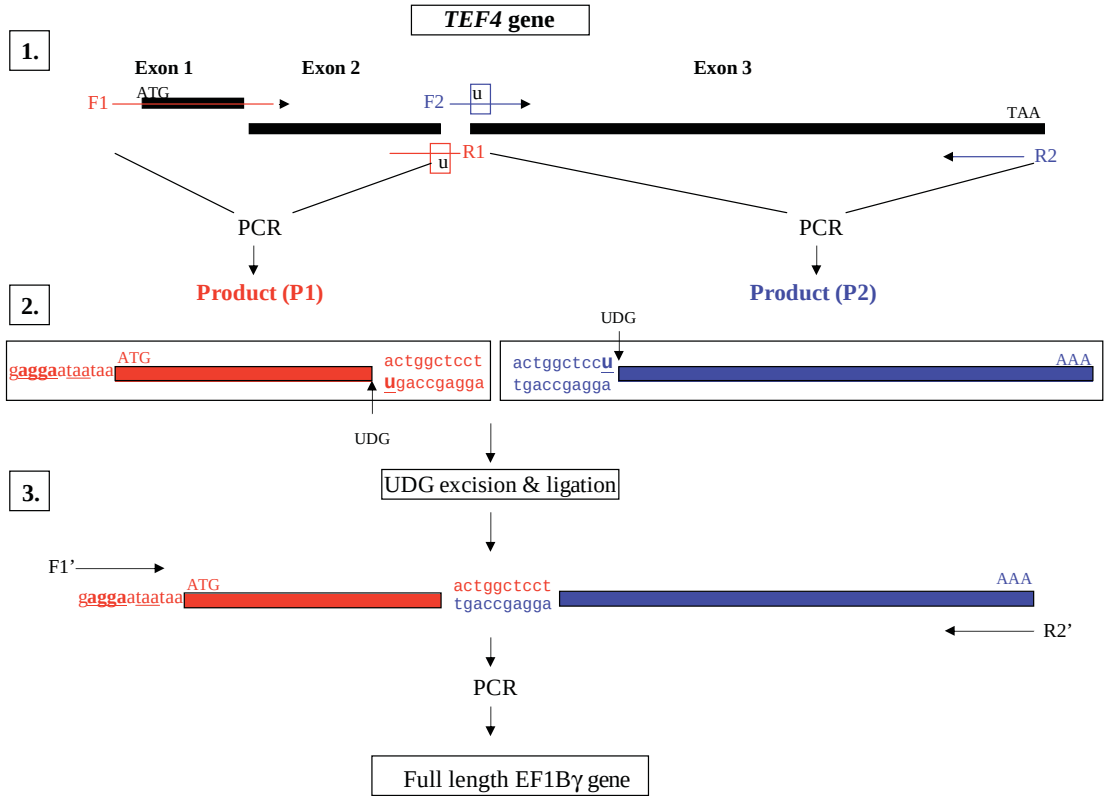

Fig. 1. Cloning strategy. (1) Forward primer (F1) incorporates an in-frame stop codon (TAA) and a ribosome-binding site (AGGA) upstream of the *TEF4* start codon (ATG). F1 comprises the complete first exon and 23 bases of exon 2. Reverse primer (R1) contains a uracil preceded by 9 bases complementary to the 5′ end of exon 3 (4 bases) and the 3′ end of exon 2 (5 bases) and preceded by 15 bases complementary to the 3′ end of exon 2. R2 to R1 generates the product (P1) comprising exons 1 and 2. Forward primer (F2) contains a uracil preceded by 9 bases (6 of exon 2 and 3 of exon 3) and proceeded by a further 18 bases of exon 3. This primer along with the reverse primer (R2), complementary to the 3′ end of exon 3 but not including the stop codon (TAA), generates a second product (P2). This product comprises exon 3. (2) Subsequent removal of uracil bases with UDG and heat/alkali generates a 10 base 3′ overhang facilitating ligation to assemble the full gene coding region. (3) After ligation, amplification of full-length gene was carried out with primers F1′ and R2′.