

GENE 07979

XDJ1, a gene encoding a novel non-essential DnaJ homologue from *Saccharomyces cerevisiae*

(PCR cloning; *Saccharomyces cerevisiae* genomic library; heat-shock gene; stress-induced gene expression; prenylation signal)

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SUMMARY

The gene encoding a novel DnaJ-like protein, termed Xdj1, has been identified by amplification of *Saccharomyces cerevisiae* genomic DNA. An open reading frame of 1380 bp was detected. Disruption of *XDJ1* did not yield any detectable new phenotype. A double-deletion strain containing a disruption of both *XDJ1* and *YDJ1*, another gene coding for a DnaJ-like protein, was still viable. Under a variety of growth conditions, no *XDJ1* transcripts could be detected by Northern blot analysis and no translation product was found by immunoblotting with antibody against Xdj1 produced in *Escherichia coli*. Thus, *XDJ1* is either expressed only under very specific conditions or represents a silent gene.

INTRODUCTION

Bacterial DnaJ has been shown to enhance the ATPase activity of DnaK, the bacterial HSP70 (Liberek et al., 1991) and thereby modulate the affinity of DnaK towards the substrate. In the yeast *Saccharomyces cerevisiae* the activity of HSP70 appears to be similarly regulated by eukaryotic DnaJ homologues (Cyr et al., 1992). So far seven DnaJ and nine HSP70 homologues have been identified in *S. cerevisiae* (Shirayama et al., 1993; Mukai et al., 1994; Rowley et al., 1994; reviewed by Lindquist and Craig, 1988). It is probable that each HSP70 member is

functionally active with its cognate DnaJ homologue. Thus, an attempt was made to clone the genes for further DnaJ homologues by taking advantage of homology domains, observed in both prokaryotic and eukaryotic DnaJ proteins. We report here on the gene encoding a new yeast DnaJ homologue, termed Xdj1, the deduced aa sequence of which is closely related to Ydj1. Disruption of the gene had no apparent phenotype and transcription of the gene was not observed under a number of different growth conditions. Since the biological function of this protein is unclear we termed the gene for this novel DnaJ homologue *XDJ1*.

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Abbreviations: aa, amino acid(s); bp, base pair(s); *E.*, *Escherichia*; ER, endoplasmic reticulum; HSP70, 70-kDa heat-shock protein; nt, nucleotide(s); oligo, oligodeoxyribonucleotide; ORF, open reading frame; PCR, polymerase chain reaction; *S.*, *Saccharomyces*; *XDJ1*, gene encoding Xdj1; *YDJ1*, gene encoding Ydj1.

EXPERIMENTAL AND DISCUSSION

(a) Cloning and sequence analysis of *XDJ1*

Two primers were designed according to the well-conserved N-terminal 'J region' and to the Cys-containing motif of known yeast DnaJ-like proteins

primer 1 GCTCTAG AAA TAC CAC CCI GAC
 XbaI G TT T
 K Y H P D

primer 2 GGAATT CGC ICC ICT ICC GTG GCA
 EcoRI GA A A
 A G S G H C

Fig. 1. Primers used for amplification of the *XDJ1* gene from *S. cerevisiae* genomic DNA. The corresponding aa sequences which were used to design the primers are given below the oligo sequences. Primer 1 is directed to a highly conserved sequence in the 'J region', the back primer is directed to a sequence in the first Cys-containing motif.

(Fig. 1). Amplification of *S. cerevisiae* genomic DNA with these primers yielded an amplification product of about 400 bp. In view of the constant domain spacing in the DnaJ homologues, exactly this size was expected. Sequence analysis showed that indeed a fragment of a potential DnaJ encoding gene had been obtained. The PCR fragment was used as a probe to screen a genomic yeast library for the full-length clone. Two clones were isolated from a YEP13 library (DNA from wild-type *S. cerevisiae* strain D273-10B) and identified as being identical. A 4.2-kb fragment was subcloned into vector pGEM4. By nt sequence analysis of both clones an ORF of 1380 bp coding for a 459-aa polypeptide was identified (Fig. 2). The polypeptide shows the same domain structure as yeast Ydj1 and *E. coli* DnaJ protein. It contains a short Gly-rich part and four Cys-containing motifs. The C-terminal aa residues CCIQ could represent a prenylation signal; a related sequence CASQ is present in Ydj1 where prenylation has been demonstrated and shown to be essential for growth at elevated temperature (Caplan et al., 1992a). The aa sequence in the highly conserved 'J region' shows 49% homology to the respective domain of *E. coli* DnaJ, and 59, 53 and 49% sequence identity with the corresponding domains of Ydj1, Sis1 and Scj1, respectively (Fig. 3).

(b) Disruption of *XDJ1*

In order to investigate whether the lack of *XDJ1* would be lethal or result in an altered phenotype, the gene was disrupted with the *URA3* gene in both a diploid and in a haploid background. The haploid cells carrying disrupted *XDJ1*, as well as the spores germinated from the diploid disrupted strain containing the *URA3* marker inserted into *XDJ1* did not show any altered growth characteristics.

The thermotolerance of the disrupted strain in comparison to that of the wild-type was investigated by growing the cells to mid-logarithmic phase in minimal medium. The cultures were then shifted to 50°C or 60°C for 60 or 15 min, respectively, either immediately or after a 30-min

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AAAAAAGACCGCAAGAGTGAAAAAACCAGTACTGTAAAGCGAAGAACGCTCTAATGG -301
GAGTGCTACTATGTAAAGATATATCATGAATGTGCTGTATTATATATGGATTATATA -241
GCCATGAATATGCGGTAATAAACATATGCAATATATGTGTGGGGCAGCGCTCTGCTGT -181
GCAATCCTTGTCTTCTTCTTCTTGGTTCGGGATTTGAGTAGCCAGCAGCCATCCTCTCG -121
AGAAAGGGAAGAAAGAAAGCAGAGAGGCAAGAAAGAGTCAAGGCTTATCTTATCTTA -61
AGAGATATGCCGCTTGGACCCAAAGAAAGGAAAGTAAAGTACCGAGGTATAGTTTGA -1

M S G S D R G D R L Y D V L G V T R D A 20
ATGATGGTAGTATAGAGGAGACCGCTTATACGATGTCTGGGGGTGACGAGAGATGGC 60

T V Q E I K T A Y R K L A L K H E P D K 40
ACCGTGCAAGAGATTAATACTGCTTACAGAAAGCTTGCCTCTGAACATCATCCGGACAA 120

Y V D Q D S K E V N E I K F K E I T A A 60
TATGTGGATCAAGACTCAAAGGAGTAAATGAAATCAAATTCAAAGAGATCACTGCCGCT 180

Y E I L S D P E K K S H Y D L Y G D D N 80
TACGAGATCTTGAGCGATCCGGAGAAGAAATCACATTCAGCACTGTATGGTGTATGATA 240

G A A S S G G A N G F G D E D F M N F F 100
GGTCCGCTAGCAGCGGTGGCGCTAATGGCTTGGAGATGAAGATTTTATGAATCTCTTT 300

N N F F N N G S H D G N H F P G E Y E A 120
AACRAATTTCTCAATATGGAGTCAAGATGAAATATTTCCCTGGCGAGTATGAAAGCG 360

Y E E G N S T S S K D I D I D I S L T L 140
TACGAGAGGGCAACTCTACGAGTCTTAAGATATCGATATCGATATATCTCTTACTTTG 320

K D L Y M G K K L K F D L K R Q V I C I 160
AAGGATTGTACATGGGCAAGAGCTGAAGTTTGATTTAAGAGAGACGGTCTACTATA 480

K C H G S G W K P K R K I H V T H D V E 180
AAGTGCCACGGTTCTGGCTGGAAACCAAGAGGAAATATCACGTTACACAGATGTGGAA 540

C E S C A G K G S K E R L K R E G P G L 200
TGTGAATCATGCCGTGAAAGGGTTCAAAGGAACTCTCAAGAGGTTTGGTCCCGCTTG 600

V A S Q W V V C E K C N G K G K Y T K R 220
GTAGCTTCGCAATGGGTGGTCTGTGAGAAATGTAATGGTAAGGGGAAGTACATAAAGA 660

P K N P K N F C P D C A G L G L L S K K 240
CCCAAGATCCAAAGAACTTTTCCCGGATTCGGCAGGCTTGGGGCTCCTGTCAAAGAAG 720

E I I T V N V A P G H H F N D V I T V K 260
GAAATCATCACAGTGAACGTGGCTCCGGGACACCACTTTAAGCAGCTAATTCAGTCAAG 780

G M A D E E I D K T T C G D L K F H L T 280
GGGATGGCGGACGAGGAATCGATAAGACCATGTGGTGATTTAAGTTCATCTCACT 840

E K Q E N L E Q K Q I F L K N F D D C A 300
GAAAACAAAGAAATTTGGAGCAGAAAGCAATCTTTTGAAGATTTTGGAGCAGCGGCC 900

G E D L Y T S I T I S L S E A L T G F E 320
GGGAAAGTTGTATACAAAGCATTACCATATCGTTAAGCGAGGCGCTTACGGGATTTGAG 960

K F L T K T F D D R L L T L S V K P G R 340
AAATTTTGCACAAAACCTTCGACGACAGGTACTAACATTAGCGGTTAAACCTGGCAGA 1020

V V R P G D T I K I A N E G W P I L D N 360
GTAGTAAGACTGGTGACACCATCAAATTCGGCAATGAAGGTTGGCCCATCTAGATAAC 1080

P H R C G D L Y V F V H I E F P P D N 380
CCTCATGGCGGGCGGATCTGTATGTTTCTGTTCAATTAAGATTTTACACCAGATAAC 1140

W F N E K S E L L A I K T N L P S S S S 400
TGGTTCATGAAAATCAGAACTACTAGCAATAAAAACGAATCTGCCGTCATCTTCATCT 1200

C A S H A T V N T E D D S N L T N N E T 420
TCTGCCCTCACATGCCACTGTAAATACTGAAGATGACAGCAACCTGACTTAACAGGAACT 1260

I S N F R I I H T D D L P E G I R P F K 440
ATATCAAATTTCCGGATCATTACAGGAGCATCTCCAGAGGGATAAGGCCCTTCAAG 1320

P E A Q D S A Y Q K A R S S Y C C I Q 459
CCAGAGCAGAGGATTCAGCGTATCAGAAAGCAAGAGTTCGTACTGCTGTATCCAATGA 1380

TGGCATCAATACTTTTATCTATTTTTTTTTTTTCA 1420
    
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Fig. 2. Nucleotide sequence of the *XDJ1* gene from *S. cerevisiae*. EMBL Data Library accession No. X76343. The deduced aa sequence is shown in one letter code.

induction at 37°C. When scoring the survival rate of both strains no significant difference could be detected. This result implies that expression of *XDJ1* is not required for thermotolerance.

(c) Construction of the *ydj1, xdj1* double-null mutant

Ydj1 has been shown to be involved in protein translocation into the ER and mitochondria (Atencio and Yaffe, 1992; Caplan et al., 1992b). Deletion of *YDJ1* results in

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