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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

SSDI 0378-1119(94)00190-4

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                XbaI    G  TT   T
primer 1    GCTCTAG AAA TAC CAC CCI GAC

                        K   Y   H   P   D

                EcoRI           GA      A   A
primer 2    GGAATT CGC ICC ICT ICC GTG GCA

                        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. cerevisae* 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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AAAAAAAGACGCAAGAGTGAAAAAAAAACCAGTACTGTAAAAGCAGAAAACGTCTAATGG  -301
GAAGTGCTACTATGTAAGATTATATCATGAATGTGCTGTGTATTATATATGGATTATATA  -241
GCCATGAATATGCGGTAAATAAACATATGCATATATGTGTGTGGGGCAGCGCTCTGCTGT  -181
GCAATCCTTGTTTCTTTGTTTCTTGGGTCGGGATTTGAGTAGCGACGAGCCATCCTCTGC  -121
AGAAGGGAAGAAGAAAGAAAGGCAGAGAGGCAAAGAAAGAGTCAGGCTTATCTTATCTTA   -61
AGAGATATGCCGCTTGGACCCAAAAGAAAGGGAAAAGTAAGTACGCAGGTATAGTTTTGA    -1

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

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

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

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

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

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

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

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

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

 C  E  S  C  A  G  K  G  S  K  E  R  L  K  R  F  G  P  G  L    200
TGTGAATCATGCGCTGGAAAGGGTTCAAAGGAACGTCTGAAGAGGTTTGGTCCCGGTTTG  600

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

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

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

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

 E  K  Q  E  N  L  E  Q  K  Q  I  F  L  K  N  F  D  D  G  A    300
GAAAAACAAGAAAATTTGGAGCAGAAGCAAATCTTTTTGAAGAATTTTGACGACGGCGCC  900

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

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

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

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

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

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

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

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

TGGCATCAATAACTTTTTATTCTATTTTTTTTTTTTCAT                       1420
```

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

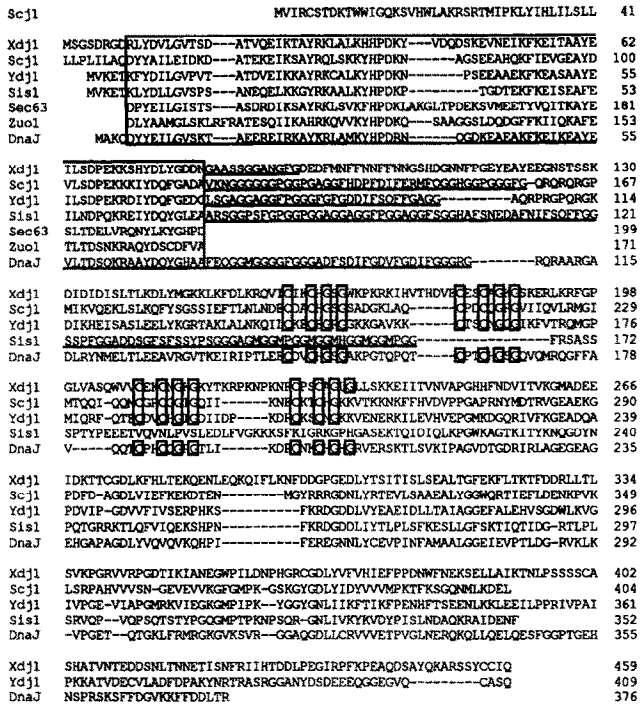

Fig. 3. Alignment of Xdj1 sequence with the other *S. cerevisiae* DnaJ homologues and *E. coli* DnaJ protein. The highly conserved 'J region' is boxed, the Gly-rich stretches are underlined, and the conserved residues of the four Cys-containing motifs (CXXCXGXG) are boxed (X, any aa). Sec63 and Zuo1 share homology only in the 'J region', therefore only this region (residues 125–199 and 97–172, respectively) is shown. References for the primary sequences of the *S. cerevisiae* DnaJ proteins: Scj1, Blumberg and Silver (1991); Ydj1, Atencio and Yaffe (1992) and Caplan and Douglas (1991); Sis1, Luke et al. (1991); Sec63, Sadler et al. (1989); Zuo1, Zhang et al. (1993); *E. coli* DnaJ, Bardwell et al. (1986).

a reduced growth rate at 30°C and inviability at 37°C (Caplan and Douglas, 1991; Atencio and Yaffe, 1992). In order to test whether *XDJ1* and *YDJ1* double-null mutants show synthetic lethality a double-deletion strain for *XDJ1* and *YDJ1* was constructed by crossing both haploid deletion strains. Tetrad analysis showed that all spores were viable at 30°C. When the haploid progeny was shifted to 37°C a 2:2 segregation was observed, which is typical for *YDJ1* disrupted cells. Thus, deletion of both, *XDJ1* and *YDJ1*, did not result in lethality.

(d) Expression analysis of *XDJ1*

Since synthesis of many known DnaJ proteins is heat inducible, the regulation of *XDJ1* expression was examined by Northern blot analysis. Cells were grown in YPD or minimal medium containing either glucose or potassium acetate as a carbon source (Sherman, 1991) and subjected to either heat or cold shock during exponential growth phase prior to RNA extraction. In another experiment shift to sporulation medium (Sherman, 1991) was tested for induction of *XDJ1* expression. Northern analysis, however, revealed that neither under physiological nor under the stress conditions tested any transcript was detected. These data were confirmed by the observation that antibody which had been raised against the recombinant *XDJ1* expressed in *E. coli* did not react with total protein isolated from heat shocked or control yeast cells. From these results we conclude that *XDJ1* expression requires either very specific induction conditions or that the gene is not expressed at all.

(e) Conclusions

(*1*) With the isolation of the *XDJ1* gene a novel DnaJ homologue from *S. cerevisiae* has been obtained.

(*2*) Since no transcripts could be detected, though various induction conditions such as heat shock, cold shock and nitrogen limitation have been applied, we conclude that either induction of the *XDJ1* gene is highly specific or this gene is not expressed at all. If the latter possibility holds true, *XDJ1* to our knowledge represents the first silent gene for a DnaJ-like protein.

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