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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*.

EXPERIMENTAL AND DISCUSSION

(a) Cloning and sequence analysis of XDJ1

Two primers were designed according to the wellconserved N-terminal 'J region' and to the Cyscontaining motif of known yeast DnaJ-like proteins

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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.

primer	1	Xbai GC <u>TCTAG</u>	-				GAC	:
			ĸ	Y	H	P	D	
primer	2	<i>Eco</i> ri G <u>GAATT (</u>						
			A	G	S	G	H	с

Fig. 1. Primers used for amplification of the *XDJ1* gene from *S. cerevis-iae* 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

AAAAAARGCCCAAGAGTGAAAAAAAAACCAGTACTGTAAAAGCAGAAAACGTCTAATG GAAGTGCTACTATGTAAGATTAATACATGAATGGCTGTGTATTATAATGGGTAGTATAATA GCCATGAATATGCGGTAAATAAACAATGACATAATGTGTGTG	-301 -241 -181 -121 -61 -1
M S G S D R G D R L Y D V L G V T R D A	20
ATGAGTGGTAGTGATAGAGGAGACGGGTTATAGGATGGGGGTGAGGGGGAGAGGAGGGG	60
T V Q E I K T A Y R K L A L K H H P D K	40
ACCETECARGAGATTAAAACTECTTACAGAAAGCTTECTEARACATCATCEEGACAAG	120
Y V D Q D S K E V N E I K F K E I T A A	60
TATGTGGATCAAGACTCAAAGGAGGTAAATGAAATCAAAGAGATCACTGCCGCT	180
Y E I L S D P E K K S H Y D L Y G D D N	80
TACGAGATCTTGAGGGATCCGGAGAAGAAATCACATTACGACTTGTATGGTGATGATAAT	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
AACAATTTCTTCAATAATGGAAGTCACGATGGAAATAATTTCCCTGGGGAGTATGAGGGG	360
Y E E G N S T S S K D I D I D I S L T L	140
TACGAAGAGGGCAACTCTACGATATCGATATCGATATCTCTTACTTG	320
K D L Y M G K K L K F D L K R Q V I C I	160
AAGGATTTGTACATGGGCAAGAAGCTGAAGTTGATTTAAAGAGACAGGTCATCTGTATA	480
K C H G S G W K P K R K I H V T H D V E	190
ANGTGCCACGGTTCTGGCTGGAAACCAAGAGGAAAATTCACGTAACACGATGTGGAA	540
C E S C À G K C S K E R L K R P G P G L	200
TGTGAATCATGCGCTGGAAGGGTTCAAAGGAACGTCTGAMGAGGTTTGGTCCCGGTTTG	600
V À S Q W V V C E K C N G K G K Y T K R	220
GTAGCTTCGCARTGGGTGGTCTGTGAGAARTGTAATGGTAGGGGAAGTACACTAAAAGA	660
PKNPKNFCPDCAGLGLLSKK	240
CCCAAGAATCCAAAGAACTTTTGCCCCGATGCGCAGGCTGGGGCTCCTGTCAAAGAAG	720
E I I T V N V A P G E E F N D V I T V K	260
GARATCATCACAGTGACGTGGGTGGCTCCGGGGACACCACTTTAACGACGTGAATTACAGTGAAG	780
G M A D E E I D K T T C G D L K F H L T	280
GGGATGGCGGACGAGGAAATCGATAAGACCACATGTGGTGATTTAAAGTTCCATCTCATC	840
EKQENLEQKQIFLKNFDDGA	300
GAAAAACAAGAAAATTTGGAGGAGGAGGAAATCTTTTGAGGAGGGGGGGG	900
G E D L Y T S I T I S L S E A L T G F E	320
GGGGAAGATTTGTATACAAGCATTACCATATCGTTAAGCGAGGCCTTGACGGGATTTGTA	960
K F L T K T F D D R L L T L S V K P G R	340
ANATTTTGACAAAAACCTTCGACGACAGGTTACCATGAGGGTTAAACCTGGCAGA	1020
V V R P G D T I K I A N E G W P I L D N	360
GTAGTAAGACCTGGTGACACCATCAAAATCGCCAATGAAGGTTGGCCCATTCTAGATAAC	1080
PEGRCGDLYVFVHIEFPPDN	380
CCTCATGGCCGGTGCGGCGATCTGTATGTTTCGTTCATATTGAATTTCCACCAGATAAC	1140
W F N E K S E L L A I K T N L P S S S	400
TGGTTCAATGAAAAATCAGAACTACTAGCAATAAAACGAATCTGCCGTCATCTTCATCT	1200
C A S H A T V N T E D D S N L T N N E T	420
TETECCTCACATECEACTERARATACTERAGATERCEACCTERACTACERACE	1260
I S N F R I I H T D D L P E G I R P F K	440
MTATCHAATTTCOGGATCATTCACACGGACGATCAAGGCCGTTCAAG	1320
PEAQDSAYQKARSSYCCIQ	459
CCAGAAGCACAGGATTCAGCGTATCAGAAGCAAGGAAGTTCGTACTGCTGTATCCAATGA	1380
IGGCATCAATAACIIIIITATTCTATTITITITITITTTCAT	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

\$cj1	MVIRCSTDKTWWIGQKSVHWLAKRSRTMIPKLYIHLILSLL	41
	MSGSDRGDRLYDVLGVTSDATVOEIKTAYRKLALKHHPDKYVDQDSKEVNEIKFKEITAAYE	62
Xdji	MSGSDRGDRLIDVIGVISDATVGEIKIAIRALALAHAPDKIAGSEEAHOKFIEVGEAYD	100
Scj1	MVKETKFYDILGVPVTATDVEIKKAYRKCALKYHPDKNPSEEAAEKFKEASAAYE	55
Ydjl	MYKETKLYDILGVPYTATOVETKATKKCALKTHPDKNTGDTEKFKEISEAFE MYKETKLYDILGVSPSANEQELKKGYRKAALKYHPDKPTGDTEKFKEISEAFE	53
Sisl	MYKLIKLIDILGVSPSARQLLANGIRMAALAINPORPALITEN IGOTERFREISEAFE DPYEILGISTSASDRDIKSAYRKLSVKFHPOKLAKGLTPDEKSVMEETYVQITKAYE	161
Sec63	DLYAAMGLSKLRFRATESOIIKAHRKQVVKYHPDKQSAAGGSLDQDGFFKIIQKAFE	153
Zuo1	MAKODYYEILGVSKTAFEREIRKAYKRLAMKYHPDRN	55
DnaĴ	MARQUYTETTAVSKTAPERETRAVARDARTHEDER	33
Xdji	ILSDPERKSHYDLYGDDRGAASSGGANGFGDEDFMNFFNNFFNNGSHDGNNFPGEYEAYEDGNSTSSK	130
Scj1	VLSDPEKKKI YDOFGADI VKNGCCCCCCPGGPGAGGFHDPFDI FERMFOGGHGGPGGGFG-ORORORGP	167
Ydji	ILSDPEKRDIYDOFGEDOLSCAGGFPGGGFGFGDDIFSOFFGAGGAQRPRGPQRGK	114
Sisl	ILNDPQKREIYDOYGLE#ARSGCPSFGPGGPGGAGGAGGFPGGAGGFSGGHAFSNEDAFNIFSOFFGG	121
Sec63	SLIDELVRONYLKYGHPE	199
Zuol	TLTDSNKRAQYDSCDFVA	171
DnaJ	VLTDSOKRAAYDOYGHAAFEOGGMGGGGGGGGGGADFSDIFGDVFGDIFGGGRGRORAARGA	115
Xdj1	DIDIDISLTLKDLYMGKKLKFDLKROVICIHC GGCWKPKRKIHVTHDVHCESCHCGKERLKRFGP	198 229
Scjl	MIKVOEKISLAOFYSGSSIEFTIALADECDACKSSADCKLAQOCCCCGVIIQVLANGI DIKHEISASLEELYKGRTAKLALAKOIICKSCKGCKKGAVKKCISCCCGIKFVTROMGP	176
Ydj1	DIKHEISASLEELYKERTAKIALNKOIIKKEKHCHCISKAGAVAKDISCRELIIKIVIKEAGP	
Sisl	SSPFGGADDSGFSFSSYPSGGACMGGPGGPGGMGGMGGMCGPGGFRSASS	172
DnaJ	DLRYNMELTLEEAVRGVTKEIRIPTLER DVG G CARPGTOPOT OPTG G COVONROGFFA	178
Xd 11		266
Sc11	MTQQI-QQACGHCGGCDIIKNICKICHCKKVTKKNKFFHVDVPPGAPRNYMDTRVGEAEKG	290
Yd 11	MIORF-OTROVCHORCDIIDPKOHCKSCNCKKVENERKILEVHVEPGNKDGORIVFKGEADOA	239
S1s1	SPTYPEEETVQVNLPVSLEDLFVGKKKSFKIGRKGPHGASEKTQIDIQLKPGWKAGTKITYKNQGDYN	240
DnaJ	VQOTOPHOLO GTLIKDHONIO GRVERSKTLSVKIPAGVDTGDRIRLAGEGEAG	235
xd11	IDKTTCGDLKFHLTEKQENLEQKQIFLKNFDDGPGEDLYTSITISLSEALTGFEKFLTKTFDDRLLTL	334
Scji	PDFD-AGDLVIEFKEKDTENMGYRRRGDNLYRTEVLSAAEALYGGWORTIEFLDENKPVK	349
Ydji	PDVIP-GDVVFIVSERPHKSFKRDGDDLVYEAEIDLLTAIAGGEFALEHVSGDWLKVG	296
Sisl	POTGRRKTLOFVIOEKSHPNFKRDGDDLIYTLPLSFKESLLGFSKTIOTIDG-RTLPL	297
DnaJ	EHGAPAGDLYVOVOVKOHPIFEREGNNLYCEVPINFAMAALGGEIEVPTLDG-RVKLK	292
Dilato	THE REAL PROPERTY OF A CONTRACT	
Xdji	SVKPGRVVRPGDTIKIANEGWPILDNPHGRCGDLYVFVHIEFPPDNWFNEKSELLAIKTNLPSSSSCA	402
Sc 11	LSRPAHVVVSN-GEVEVVKGFGMPK-GSKGYGDLYIDYVVVMPKTFKSGQNMLKDEL	404
Ydjl	IVPGE-VIAPGMRKVIEGKGMPIPKYGGYGNLIIKFTIKFPENHFTSEENLKKLEEILPPRIVPAI	361
Sisl	SRVQPVQPSQTSTYPGQCMPTPKNPSQR-GNLIVKYKVQYPISLNDAQKRAIDENF	352
DnaJ	-VPGETQTGKLFRMRGKGVKSVRGGAQGDLLCRVVVETPVGLNERQKQLLQELQESFGGPTGEH	355
Xd 11	SHATVNTEDDSNLTNNETISNFRIIHTDDLPEGIRPFXPEAODSAYOKARSSYCCIO	459
Ydil	PKKATVDECVLADEDPAKYNRTRASRGGANYDSDEEECOGEGVOCASO	409
DnaJ	NSPREKEFFDQVKKFFDDLTR	376
0		210

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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