

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)

Elisabeth Schwarz^a, Benedikt Westermann^a, Avrom J. Caplan^b, Gabriele Ludwig^a and Walter Neupert^a

^aInstitut für Physiologische Chemie, 80336 München, Germany; and ^bDepartment of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599-7260, USA. Tel. (1-919) 966-7573

Received by G. Bernardi: 10 October 1993; Revised/Accepted: 8 March/14 March 1994; Received at publishers: 24 March 1994

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*.

Correspondence to: Dr. E. Schwarz, Institut für Physiologische Chemie, Goethestr. 33, D-80336 München, Germany. Tel. (49-89) 5996-265; Fax (49-89) 5996-270.

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

```

AAAAAAGACCGCAAGAGTGAAAAAACCAGTACTGTAAAGCGAAAAACGCTCTAATGG -301
GNAAGTCTACTATGTAAAGATATATCATGAATGTGCTGTATTATATATGGATTATATA -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
ATGAGTGGTAGTGATAGAGGAGACCGCTTATACGATGTCTGGGGGTGACGAGAGATGGC 60

T V Q E I K T A Y R K L A L K H H P D K 40
ACCGTGCAAGAGATTAAAACCTGCTTACAGAAAGCTTGCCTCTGAACATCATCCGGACAA 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
TACGAGATCTTGAGCGATCCGGAGAAGAAATCACATTCAGCACTTGTATGGTATGATATA 240

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

N N F F N N G S H D G N H F G E Y E A 120
AACRAATTTCTCAATATGGAGTCAAGATGAAATATTTCCCTGGCGAGTATGAGCGG 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
AAGGATTGTACATGGCCAGAGAGCTGAAATTTGATTTAAGAGAGACGGTCTGCTATA 480

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

C E S C A G K G S K E R L K R F G P G L 200
TGTGAATCATGCCGTGAAAGGGTTCAAAGGAACCTCTCAAGAGGTTTGGTCCCGCTTG 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
CCCAAGAATCCAAAGAACTTTTCCCGGATTTGGCGAGGCTTGGGGCTCCTGTCAAAGA 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 G 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
GGGAAAGTTGTATACAAAGCATTACCATATCGTTAAGCGAGGCGCTTACGGGGATTTG 960

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

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

P H R C G D L Y V F V H I E F P P D N 380
CCTCATGGCGGGTCCGGCTCTGTATGTTTCTGTTCAATGAATTTACACCAGATAAC 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
TCTGCCCTCACATGCCAGCTTAATACTGAAGATGACAGCAACCTGACTTAACAGGAACT 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
CCAGAGCACAGGATTCAGCGTATCAGAAAGCAAGAGTTTCGTACTGCTGATCCAATGA 1380

TGGCATCAATAACTTTTTATCTATTTTTTTTTTTTCA 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

Scj1	MVIRCSTDKTWWIGQKSVHNLAKRSRTMIPKLYIHLISLL	41
Xdj1	MSGSDRGLYDVLGVTSD--ATVQEKIATYRKLAKHPDKY--VDQDSKEVNEIKFKRITAAVE	62
Scj1	LLSLILACDYIALEIDKD--ATEKEIKSAYRQLSKYHPDKN--AGSEEAHQKTEVGEAYD	100
Ydj1	MVKEIKFYDILGVVPT--ATDVEIKKAYRCAALKYHPDKN--PSEEAQKFKASAAYE	55
Sis1	MVKEIKLYDILGVSPS--ANEQELKAYRKAALKYHPDKN--TGDTEKFKETSEAFE	53
Sec63	DPYELGISTS--ASDRDLKSAYRKLGVKHPDKLAKGLTPDEKSVMEETVQITKAYE	161
Zuo1	DLYAAMGLSKLRFRAIESQIIKAKRQVVKYHPDKQ--SAAGSLDQDGFKTIQKAFE	153
DnaJ	MAKQDYVEIILGVSKT--AEEPRIRKAYRKLAMKYHPDKN--CGDKFAEAKFKETSEAFE	55
Xdj1	TLSDPEKRSYDLYGDDNLAASGANGAGEQEDFMNFFNFNFNGSHDGNFFGEVAYEENRSTSSK	130
Scj1	VLSDEPKKIIYDQFGADLVNAGCGGGGCGGAGGHDHDDIFERMEQSGHGGGGGGG-QRQRQNGP	167
Ydj1	TLSDPEKRDYDQFGEDDLSGAGGAGGCGGGGHDHDDIFSOFTGAGG--AQRPRGPRQKRG	114
Sis1	LLNDPKRRIYDQGLEPARSGGSPGGGGGAGGAGGFGGGAGGESSGHAESNDDANFISQFTGG	121
Sec63	SLTDELVRQNYLYGHPD	199
Zuo1	TLTDSNKRQAYDSCDFVA	171
DnaJ	VLTDSOKRAAYDQVGHAFPGCGGGGGGGGADFSDFTEGDFVGGTEGGGCG-----RQRAARGA	115
Xdj1	DIDIDI.SLTKDLYMGKGLKFDLKRQVYELRGGGKPKPKKIHVTHOVHESGKSKERLKRFGP	198
Scj1	MIKVQELSLKQFYSGSSIEFTLANLNDSDPFLRGGGKPKPKKIHVTHOVHESGKSKERLKRFGP	229
Ydj1	DIKHEISASLEELYKRYTAKIALANKQIYELRGGGKPKPKKIHVTHOVHESGKSKERLKRFGP	176
Sis1	SSPFGADDSGFSFSSVPSGGGAGGCGGAGGAGGFGGGAGGESSGHAESNDDANFISQFTGG	172
DnaJ	DLRYNMLTLEEAVRGVTKETRIPTLEADVGGGKPKPKKIHVTHOVHESGKSKERLKRFGPFA	178
Xdj1	GLVASQVAVLGGGKPKPKKIHVTHOVHESGKSKERLKRFGP	266
Scj1	MTQQL--QD--LGGGKPKPKKIHVTHOVHESGKSKERLKRFGP	290
Ydj1	MYQR--QD--LGGGKPKPKKIHVTHOVHESGKSKERLKRFGP	239
Sis1	SPTYPEELTQVWMLVLELDLIVKGGKPKPKKIHVTHOVHESGKSKERLKRFGP	240
DnaJ	V-----QD--LGGGKPKPKKIHVTHOVHESGKSKERLKRFGP	235
Xdj1	IDKTCGDILKFLHTEKQENLEKQKILFNKFDGPGGEDLYTSITISLSEALTGFEKFKTKTFDRLRLTL	334
Scj1	PDPT--AGDLVIEFKEDTEN-----MGYRRGONLYRTEVLSAAEALYGGQRTIEFLDENKPKVK	349
Ydj1	PDVIP--GDVVFVIERPHKS-----FKRGGDLYVYAEIDLLTAIAGGEFALEHVSGDWLKVQ	296
Sis1	PQTGRKRTIQVIVQEKSHFN-----FKRGGDLYVYAEIDLLTAIAGGEFALEHVSGDWLKVQ	297
DnaJ	EHGAZAGDLYVQVQKQHP1-----FERGNNLYCEVPIINFAMAALGGEIEVPTLDG--RVKLK	292
Xdj1	SVKGRVVRPGDITIKIANEGWPLLDNPHRCGDLVYVHIEFPPDNWFNEKSELLAIKTNLPSSSSSCA	402
Scj1	LSRFAHVVSM--GEVEVVKGQMPK--GSKGYGDLYIDVYVMPKTKSGQNMKLDL	404
Ydj1	LVFGE--VIAPQMKRVIEGKMP1PK--YGGYGNLIKFTIKFENHFTSEENLKLEELPPRIVFAI	361
Sis1	SRVQ--VQPSQTSITYEGGMP1PK--GSLIVKVKVQVYPLSINDAKRAIDENF	352
DnaJ	--VPEGT--QTKLFRMRGKGVKSVR--GGAQDGLLCCRVVETPVGLNERQKLLQLQESFGGPTGEEH	355
Xdj1	SHATVNTEDDSNLTNNETISNFR1IHTDDLPEGRIFPFKPEAQDSAYQRASSYCCIQ	459
Ydj1	PKKATVDECVLADFPKRYNTRASRGGANYSDEEEQGGEGVQ-----CASQ	409
DnaJ	NSPRSKSFFDGVKQKFDLITR	376

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.

ACKNOWLEDGEMENTS

We thank Wolfgang Voos for help in the oligo design and his assistance in some experiments. This work was supported through a grant by the Genzentrum München.

REFERENCES

- Atencio, D.P. and Yaffe, M.P.: *MAS5*, a yeast homolog of DnaJ involved in mitochondrial protein import. *Mol. Cell. Biol.* 12 (1992) 283–291.
- Bardwell, J.C.A., Tilly, K., Craig, E., King, J., Żylicz, M. and Georgopoulos, C.: The nucleotide sequence of the *Escherichia coli* K-12 *dnaJ*⁺ gene. *J. Biol. Chem.* 261 (1986) 1782–1785.
- Blumberg, H. and Silver, P.A.: A homologue of the bacterial heat-shock gene *DnaJ* that alters protein sorting in yeast. *Nature* 349 (1991) 627–629.
- Caplan, A.J. and Douglas, M.G.: Characterisation of YDJ1: a yeast homologue of the bacterial dnaJ protein. *J. Cell Biol.* 114 (1991) 609–621.
- Caplan, A.J., Tsai, J., Casey, P.J. and Douglas, M.D.: Farnesylation of YDJ1p is required for function at elevated growth temperatures in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 267 (1992a) 18890–18895.
- Caplan, A.J., Cyr, D.M. and Douglas, M.G.: YDJ1p facilitates polypeptide translocation across different intracellular membranes by a conserved mechanism. *Cell* 71 (1992b) 1143–1155.
- Cyr, D.M., Lu, X. and Douglas, M.G.: Regulation of eukaryotic Hsp70 function by a DnaJ homolog. *J. Biol. Chem.* 267 (1992) 20927–20931.
- Liberek, K., Marszalek J., Ang, D., Georgopoulos, C. and Żylicz, M.: *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. *Proc. Natl. Acad. Sci. USA* 88 (1991) 2874–2878.
- Lindquist, S. and Craig, E.: The heat-shock proteins. *Annu. Rev. Genet.* 22 (1988) 631–677.

- Luke, M.M., Sutton, A. and Arndt, K.T.: Characterisation of SIS1, a *Saccharomyces cerevisiae* homologue of bacterial DnaJ proteins. *J. Cell Biol.* 114 (1991) 623–638.
- Mukai, H., Shuntoh, H., Chang, C.-D., Asami, M., Ueno, M., Suzuki, K. and Kuno, T.: Isolation and characterization of *CAJ1*, a novel yeast homolog of *dnaJ*. *Gene* 145 (1994) 125–127.
- Rowley, N., Prip-Buus, C., Westermann, B., Brown, C., Schwarz, E., Barrell, B. and Neupert, W.: Mdj1p, a novel chaperone of the DnaJ family, is involved in mitochondrial biogenesis and protein folding. *Cell* 77 (1994) 249–259.
- Sadler, I., Chiang, A., Kurihara, T., Rothblatt, J., Way, J. and Silver, P.: A yeast gene important for protein assembly into the endoplasmic reticulum and the nucleus has homology to DnaJ, an *Escherichia coli* heat shock protein. *J. Cell Biol.* 109 (1989) 2665–2675.
- Sherman, F.: Getting started with yeast. *Methods Enzymol.* 194 (1991) 3–21.
- Shirayama, M., Kawakami, K., Matsui, Y., Tanaka, K. and Toh-e, A.: *MSI3*, a multicopy suppressor of mutants hyperactivated in the RAS-cAMP pathway, encodes a novel HSP70 protein of *Saccharomyces cerevisiae*. *Mol. Gen. Genet.* 240 (1993) 323–332.
- Zhang, S., Lockshin, C., Herbert, A., Winter, E. and Rich, A.: Zuotin, a putative Z-DNA binding protein in *Saccharomyces cerevisiae*. *EMBO J.* 11 (1992) 3787–3796.