Occurrence and sequence of a DnaJ protein in plant (Allium porrum) epidermal cells

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Antibodies raised against a purified fraction from microsomal membranes of leek epidermal cells were used to screen a λ zap expression library from epidermal cells of leek plants. A near full-length clone was isolated. This cDNA contains an open reading-frame of 1,191 bp coding for a DnaJ protein (leek DNAJ 1 or LDJ1). Leek DnaJ1 represents the second protein of this type described in a pluricellular organism, the first being that sequenced from human cells.

Plant; Allium porrum; DnaJ protein; Chaperone; Heat-shock protein: Prenylation; Zinc finger

1. INTRODUCTION

The first DnaJ protein to be sequenced [1] was first identified by the isolation of E. coli mutants unable to propagate bacteriophage λ . This protein is also involved in the replication of the plasmid P1 [2] and some recent studies have shown how this protein could be implicated in DNA replication [2-5]: for example, DnaJ protein interacts with DnaK (another heat-shock protein) to induce the active form of the P1 RepA initiator protein [3].

DnaJ protein also acts with DnaK protein and chaperonins (GroEL and GroES) in the folding of proteins in the cell: for example, DnaK, DnaJ and GroEL can interact with rhodanese and prevent its aggregation (induced by a denaturation) [6].

DnaJ proteins also seem to be implicated in the targeting of proteins in the cell: in yeast, two DnaJ proteins (NPL1 and SCJ1) have been isolated by analyzing mutants defective in the import of proteins to the nucleus [7,8]. One of them (NPL1) is encoded by a gene allelic to sec63, a gene that affects the transit of nascent secretory proteins across the endoplasmic reticulum membrane [7]. Another DnaJ protein from yeast (YDJ1P) was isolated by complementation of a mutant defective in mitochondrial protein import [9].

Seven DnaJ proteins have been sequenced from human cells [10], yeast [8,9,11,12] and prokaryotes

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Abbreviations. LB, Luria-Bertani medium (Bactotryptone 10 g, Bacto yeast extract 5 g, NaCl 10 g, H₂O 1 l; IPTG, isopropylthio-β-D-galactoside; PBS, phosphate-buffered saline; BCIP, 5-bromo-4-chloro-3-indolyl phosphate, disodium salt; NBT, Nitroblue tetrazolium chloride. [1,13]. No report has as yet described the presence of such proteins in plants.

2. MATERIALS AND METHODS

2.1. Preparation of antiserum

The antiserum used was raised against the acyl-CoA elongating system from leek epidermal cell microsomes as described previously [14]. Before screening the library, the serum was diluted 10-fold and successively incubated for 30 min with 4 nitrocellulose filters (preincubated with an E. coli lysate) at room temperature. The serum was then diluted a further 10-fold and incubated with eight other filters. Just before use, it was further diluted 5-fold. This serum cross-reacted with a DnaJ protein during the experiments described below.

2.2. Cloning of the LDJ1 gene

An expression library of leek epidermal cells in \(\lambdazap phage was purchased from Stratagene (La Jolla, CA, USA). An aliquot of a phage stock solution (titrated for confluence) was incubated at 37°C for 15 min with 600 μ l of an overnight culture of E coli (strain Sure) grown in LB supplemented with 1 mM MgSO₄. 3 ml of top agar (pre-warmed to 50°C) was then added and the mixture was plated out on LB plates and incubated at 42°C for 4 h. The plates were then overlayed with nitrocellulose filters pre-soaked with 10 mM IPTG and incubated overnight at 37°C. After washing in PBS, filters were incubated with BSA (1%) in PBS, 0.1% Triton, 0.2% sodium azide, and then with the antiserum prepared as described above (1/500). After 90 min at room temperature, the filters were washed in PBS, 0.1% Triton, 0.2% sodium azide, and incubated with anti-IgG alkaline phosphatase conjugate (1/5,000) for 1 h at room temperature. The protein-antibody complexes were visualized using BCIP (0.15 mg/ml) and NBT (0.3 mg/ml).

After purification of the 'positive' phages and in vivo excision (protocol from Stratagene), the 'packaged' pBluescript DNA was mixed with E. coli cells (strain XL1Blue) and spread on LB ampicillin + tetracycline plates to produce colonies. From these colonies, pBluescript plasmids containing the cDNA were further purified on a CsCl gradient.

2.3. DNA sequencing

Using the universal M13 and reverse M13 sequencing primers, as well as various other specifically synthesized oligonucleotide primers,

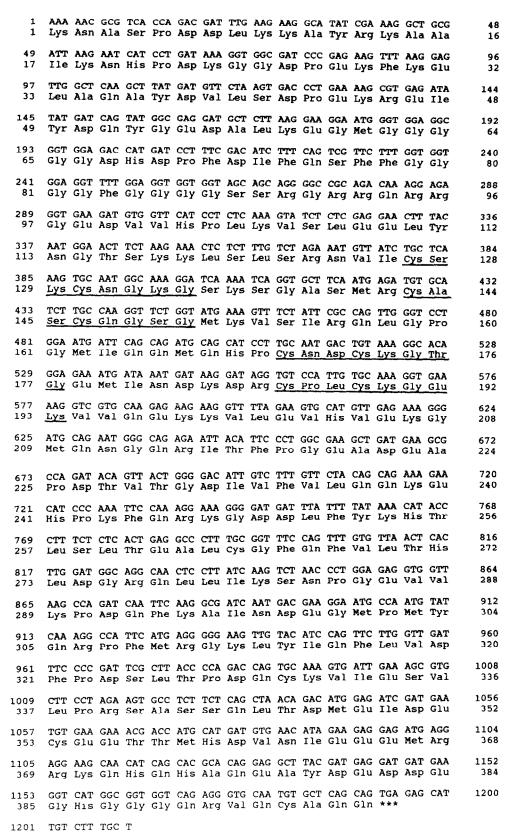


Fig. 1. Nucleotide and deduced amino acid sequence of LDJ1. The number 1 in the nucleotide sequence indicates the beginning of the clone, and the stop codon is marked by ***.

the clone was sequenced by the Sanger dideoxy chain termination method [15], using [35S]dATP as the label. The *Hin*cII- and *BstXI*-digested fragments from the purified cDNA were subcloned into pBluescript and were used to determine the complete sequence. Dideoxynucleotide triphosphates were purchased from Pharmacia and the Sequenase 2.0 DNA Sequencing Kit from United States Biochemical Corp.

3. RESULTS AND DISCUSSION

Using antibodies against a purified fraction from microsomal membranes of leek epidermal cells and screening a \$\partial zap\$ expression library from these cells, a clone coding for a polypeptide of 397 amino acids (44 kDa) was isolated (see Fig. 1). The sequence of the first 52 amino acids (N-terminal) of the protein shares homology with the consensus sequence of the previously sequenced DnaJ proteins (Fig. 2). For example, a 'HPD (-K in eukaryotes)' box is present in the eight DnaJ proteins, and sequences such as K-K/S-A-Y-R-K, EKFKE are found in both yeast DnaJ and LDJ1 proteins. Between amino acid number 77 and 87 there is a Gly(-Phe)-rich region that has also been described in several DnaJ proteins (see Table I).

Moreover, as in the case of DnaJ proteins from bacteria and in two of the four DnaJ proteins from yeast (Table I), LDJ1 contains four Cys-X-X-Cys-X-Gly-X-Gly units (underlined in Fig. 1) which can be organized into two series of direct repeats of the sequence Cys-X-X-Cys-X-Gly-X-Gly-(X)₈-Cys-X-X-Cys-X-Gly-X-Gly. These two series are the same as those described in YDJ1P and, as in the latter protein, there is also a missing final Gly, which is replaced by Lys in LDJ1. As noted by Caplan and Douglas [11], these units are similar to those described in the family of zinc finger pro-

teins [16]. To our knowledge this is the first description of such units in plants.

Among all the known DnaJ proteins, the homology of LDJ1 is highest with YDJ1P (see [11,13]). In addition to the Gly(-Phe)-rich regions and the Cys-X-X-Cys-X-Gly-X-Gly units, there are (between amino acids 23 and 75 of YDJ1P) 34 identical and 14 isologous amino acids. After the zinc finger domain, the percent homology remains high: between amino acids 210 and 409 of YDJ1P, 80 were also found in LDJ1.

Furthermore, the C-terminal sequence of LDJ1 is VQCAQQ, which is very similar to the C-terminal sequence of YDJ1P: VOCASO. As first suggested [11], and recently shown [17] by Caplan and co-authors, such a sequence corresponds to a farnesylation site in YDJ1P. The prenylation, which occurs on the cysteine residue is required for the protein to function at elevated temperature and to promote the attachment of proteins to the membranes [17,18]. Since the C-terminal sequence of LDJ1 is very similar to the C-terminal sequence of YDJ1, and since the C-terminal amino acid is a glutamine residue, it is possible that LDJ1 is prenylated and that the latter prenyl group is a farnesyl isoprenoid, rather than a geranylgeranyl group (see [19]). In any case, LDJ1 could be a membrane-bound protein (as seems to be the case for some other DNAJ proteins [1,11,18,20]) despite the absence of large hydrophobic regions (data not shown).

Some prenylated proteins (ras or GTP-binding proteins) are localized in the plasma membrane, while others (rab/YPT1) are located in the intracellular membranes (see [19] for review). Unfortunately, since the reported cDNA is not full length, it is unclear whether or not the protein is synthesized with an N-terminal

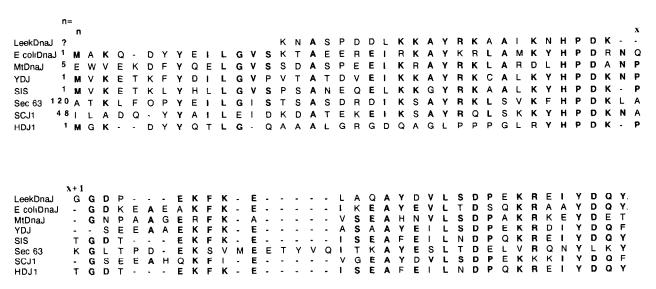


Fig. 2. Regions of known DnaJ proteins with high homologies Bold letters represent amino acids present in at least 4 of the 8 reported sequences; n is the rank in the protein sequence (starting from the N-terminal) of the first amino acids of the presented region. Dashes were introduced to optimize the homologies between the proteins; A---B signifies that there are no amino acids present between amino acids A and B in the sequence of the protein.

Table I
Some characteristics of DNAJ proteins

Name	E. coli DnaJ	MtdnaJ	NPL1/ Sec63	SIS	YDJI	SCJ1	HDJI	LDJ1
Origin	E coli	Myc tub.	Yeast	Yeast	Yeast	Yeast	Human	Leek
Number of		•						
amino acids	375	356	663	352	409	404	339	> 397
Heat-shock								
protein	Yes	?	?	Yes	Yes	?	?	9
Glycine-rich								
region	Yes	Yes	No	Yes	Yes	Yes	No	Yes
Number of								
zinc fingers	4	4	0	0	4	4	0	4
C-terminal					VQCASQ (farnesyl)	KDEL		VQCAQQ (farnesyl?)
Reference	1	13	7	12	9.11	8	10	This study

signal peptide and, therefore, from looking at the sequence, it is difficult to address the subcellular localization of the corresponding protein. Nevertheless, it can be noted that the high homology of LDJ1 with other DnaJ proteins that have less than 410 amino acids (except NPL1/Sec63), and especially with YDJ1P (409 amino acids) (Table I), strongly suggests that the sequenced cDNA (coding for 397 amino acids) is nearly full length.

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