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YGE1 is a yeast homologue of *Escherichia coli grpE* and is required for maintenance of mitochondrial functions

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Abstract

The *grpE* gene is a heat shock gene of *Escherichia coli* whose product functions as a chaperone to (re)fold proteins. We found a yeast homologue of *grpE* and designated it *YGE1*. *YGE1* can replace *grpE* in *E. coli*, indicating that *YGE1* is a functional homologue of *grpE*. Deletion of *YGE1* is lethal. During depletion of the Yge1 product, mitochondria are sequestered in mother cells thereby accumulating cells without mitochondria, suggesting that Yge1 protein plays a pivotal role in maintaining mitochondrial functions.

Key words: Mitochondria; *grpE*; *Saccharomyces cerevisiae*; Essential gene

1. Introduction

DnaK, DnaJ, GrpE and GroEL proteins of *Escherichia coli* are heat shock proteins and function as molecular chaperones to (re)fold proteins thereby playing a vital role in various cellular functions such as replication of bacteriophage λ DNA [1] and secretion of proteins [2]. DnaK is a homologue of eukaryotic Hsp70 possessing ATPase activity. DnaK, DnaJ and GroEL interact sequentially with the folding polypeptide in a reaction coupled by GrpE [3]. It has been demonstrated that GrpE and DnaJ stimulate the ATPase activity of DnaK [4]. In the yeast *Saccharomyces cerevisiae*, there are ten distinct genes encoding DnaK homologues (Hsp70s) [5–13] and four genes encoding DnaJ homologues [14–18]. Like *E. coli* DnaJ, Ydj1, a yeast homologue of DnaJ, can stimulate ATPase activity of a Hsp70 (Ssa1), leading to disruption of the Hsp70–polypeptide complex [19]. Provided that chaperones of yeast function as those in *E. coli*, GrpE homologues are expected to exert their functions in some circumstances. However, there has been no report of a eukaryotic homologue of GrpE. In this communication, we describe that a yeast essential gene, *YGE1*, encodes a GrpE homologue. From the morphological phenotype of cells from which Yge1 was depleted, we propose that Yge1 plays a pivotal role in maintaining mitochondrial functions.

2. Materials and methods

2.1. Strains

The principal yeast strains used were: R31-1D (*MAT***a** *leu2 ura3*), YPH501 (*MAT***a**/*MAT*α *leu2*/− *his3*/− *ura3*/− *trp1*/− *lys2*/− *ade2*/−). Strains derived from these strains were also used as described in the text. *Escherichia coli* DH1 (F⁻ *supE44 hsdR17 recA1 endA1 gyrA96 thi-1 relA1*) were used for construction and propagation of plasmids. DA15 (*thr leu supE pheA*::Tn*10*) and DA16 (DA15 *grpE280*) were described previously [20].

2.2 Genetic manipulation

All procedures were described previously [12].

2.3. DNA manipulation

Methods used for manipulation of DNA were those described by Sambrook et al. [21]. DNA sequencing was carried out by the dideoxy chain termination method [22] with automated DNA sequencer model 370A (Applied Biosystems).

2.4. Cytological method

For DNA staining with DAPI, cells were fixed with 70% ethanol, washed with H_2O, and then stained with 0.125 μg/ml DAPI for 10 min. Cells were washed three times with H_2O, and mounted in 10 mg/ml n-propylgallate in 90% glycerol. For mitochondrial staining with $DiOC_6$ [23], 1μl of 100 μg/ml $DiOC_6$ in ethanol was added to 1 ml of cell culture. Samples were observed by an epifluoromicroscope (Olympus BH-2).

3. Results and discussion

We made a collection of genes, when present on a multi-copy vector, conferring resistance to stauro-

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Abbreviations: DAPI, 4′,6′-diamidino-2-phenylindole; $DiOC_6$, 3,3′-dihexyloxacarbocyanine iodide; ORF, open reading frame; PKC, protein kinase C; PCR, polymerase chain reaction; YPD, yeast extract-peptone-dextrose; SD, synthetic minimal medium.

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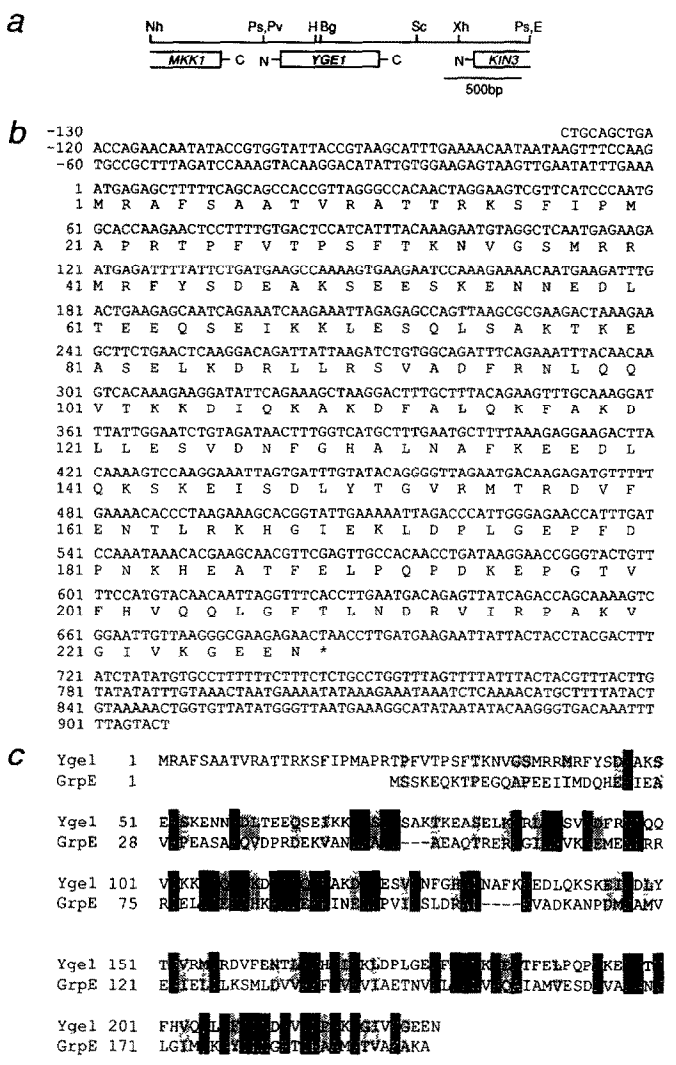


Fig. 1. *YGE1* sequence and alignment of predicted amino acid sequences of Ygel and GrpE. (a) Restriction map of the 2.5 kb *Nhe*I–*Eco*RI fragment of pRST1 containing the *YGE1* locus. An open box indicates the ORF. Symbols: Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Nh, *Nhe*I; Ps, *Pst*I; Pv, *Pvu*II; Sc, *Sca*I; Xh, *Xho*I. (b) The nucleotide sequence and the predicted amino acid sequence. The sequence data of *YGE1* is available from EMBL/GenBank/DDBJ under accession No. D26059. (c) Alignment of the amino acid sequences of Ygel and GrpE. Identical residues are in black boxes, and similar ones are in shaded boxes. Similar residues are grouped as follows: A, G, P, S, T; L, I, V, M; D, E, N, Q; K, R, H; F, Y, W; and C.

sporine, a potent inhibitor of PKC, on the wild type (R31–1D) cells to obtain some insight into the PKC pathway of yeast. We analyzed one plasmid designated pRST1 (resistant to staurosporine) (Fig. 1a) further, because it gave rather specific resistance to the drug. Inspection of the nucleotide sequence revealed one open reading frame that can encode 228 amino acid residues (Fig. 1b). Since the 1.3 kb *Pst*I–*Xho*I segment (Fig. 1a) was sufficient for staurosporine resistance and since any plasmid containing an incomplete sequence of this ORF did not confer the drug resistance, we tentatively concluded that the ORF included in this DNA segment was responsible to the drug resistance. The putative protein encoded by the ORF showed a significant homology to the *E. coli* heat shock protein GrpE consisting of 197 amino acid residues [24]; 28.9% identical and 49.7% homologous (Fig. 1c). We designated this gene *YGE1* (yeast homologue of *grpE*). Homology was found throughout the sequence between these proteins, suggesting that Ygel may substitute for GrpE in *E. coli*. To examine this possibility, we constructed plasmids carrying the *YGE1* gene (+20-228) fused to the *tac* promoter either in the right orientation (pTAC1-YGE1(+)) or in the reverse orientation (pTAC1-YGE1(−)) (Fig. 2a) and introduced each of them into DA15 (wild type) host or DA16 (*grpE280*) host. As shown in Fig. 2c, temperature sensitive mutant *grpE280* cells carrying pTAC1-YGE1(+) grew at 42°C even in the absence of IPTG, whereas those carrying pTAC1-YGE1(−) did not, indicating that *YGE1* functionally substitutes for *grpE* in *E. coli*. Induction of the *tac* promoter by IPTG made the wild type cells with pTAC1-YGE1(+) sick (data not shown). Although *grpE* is a heat shock gene of *E. coli*, transcription of *YGE1*, when examined by northern blotting, was not induced by heat shock in yeast (data not shown). Nor was found the heat shock element, a hallmark of the heat shock responsive genes, in the promoter region of the *YGE1* gene. At present we have no explanation why a high copy number of the *YGE1* gene confers resistance to staurosporine.

The *YGE1* gene is located between *HIS3* and *CDC31* on chromosome XV; 25 cM from the *HIS3* locus and 23.2 cM from the *CDC31* locus. The *YGE1* gene was flanked by the *MKK1* gene [25] and the *KIN3* [26] genes on pRST1. Since the cells carrying the disrupted *yge1* (*Δ*yge1) could not form a colony under any of the conditions we tested, we concluded that *YGE1* is essential for cell growth (data not shown).

To characterize the phenotype of cells defective in *YGE1*, EI10–3A/B (*Δ*yge1/*Δ*yge1 *GAL1-YGE1/GAL1-YGE1*) cells carrying *GAL1*-driven *YGE1* (+20-228) were grown in YPGS, in which 2% glucose was replaced with 5% galactose and 0.3% sucrose, to early log phase and then cells were transferred to YPD. These cells continued growing and remained viable up to 10 h after the shift and then they began losing their viability rapidly. When the cells completely stopped growing (28 h after the shift), approximately 80% of cells were unbudded. The cells at this stage seldom had mitochondrial DNA. The fate of mitochondria during depletion of Ygel protein was examined by staining of mitochondrial DNA with DAPI and by staining functional mitochondria with $DiOC_6$. Cells at 0 h and 10 h, contained dispersed mitochondrial DNA. In these cells, functional mitochondria appeared as strings. At 16 h after the shift, mitochondrial DNA and functional mitochondrial body gathered and stacked at the poles of the cells (arrows in Fig. 3, 16 h). As a result, daughter cells could not receive mitochon-

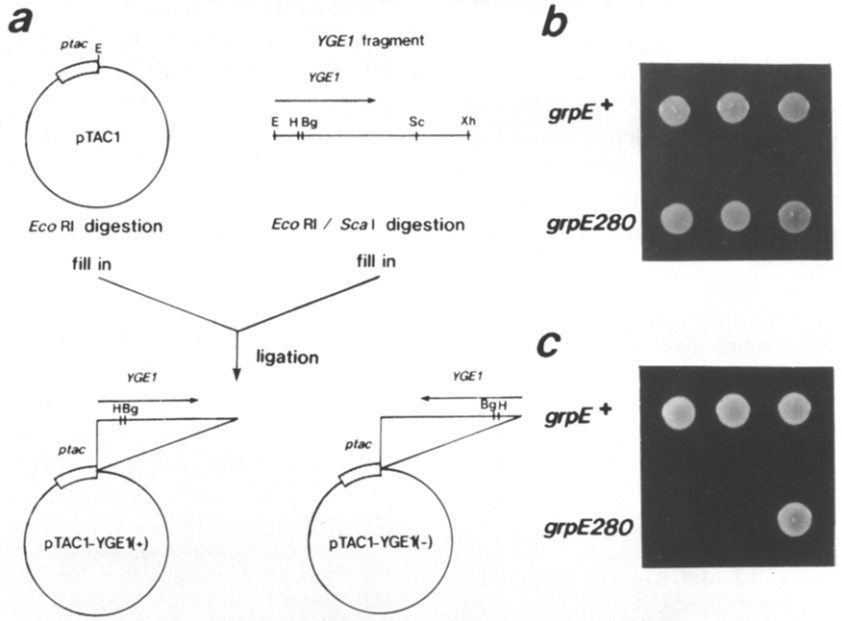
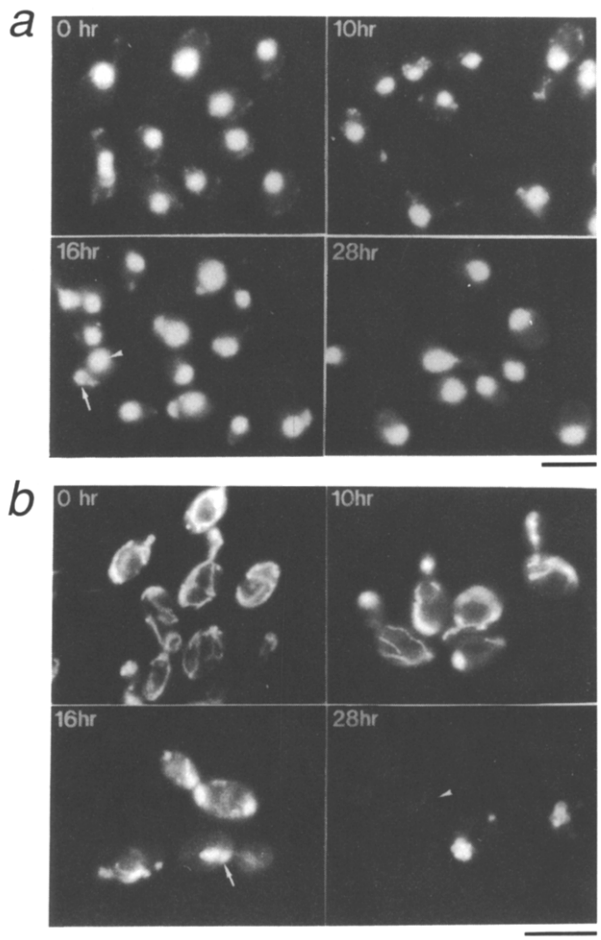


Fig. 2. *YGE1* suppresses temperature sensitivity of the *grpE280* mutation. (a) Construction of *E. coli* expression plasmids. Transformants were grown at 30°C (b) or at 42°C (c); lane Left, no plasmid; lane Middle, pTAC1-YGE1(−); lane Right, pTAC1-YGE1(+). Symbols: Bg, *Bgl*II; E, *Eco*RI; H, *Hin*dIII; Sc, *Sca*I; Xh, *Xho*I.



dria from the mother cells (Fig. 3a, 28 h; arrowhead in Fig. 3b, 28 h). The mother cells eventually lost mitochondria after the extended incubation. Morphology of nuclei was not affected by the Yge1-depletion. These observations strongly suggest that the *YGE1* gene plays an important role in the maintenance of mitochondrial functions. Elucidation of intracellular localization of Yge1 will shed some light on it function.

In the process of import of a certain set of proteins into mitochondria, Hsp70s and DnaJ homologues play essential roles [17,19,27]. In the cytoplasm, proteins to be imported into mitochondria are maintained in an unfolded state with the aid of Hsp70 proteins, Ssa [5–7,9] and a DnaJ homologue, Ydj1 [16,17,19] until the proteins are targeted to the mitochondria. In translocation across both outer and inner mitochondrial membrane,

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Fig. 3. Mitochondrial distribution is disordered in Yge1-depleted cells. The *YGE1* gene driven by the *GAL1* promoter (*GAL1-YGE1-URA3*) was constructed and integrated into the *ura3* locus of YPH501–1 (*YGE1*/*Δyge1 ura3/ura3*). By crossing between appropriate segregants from this diploid, a diploid strain, EI10–3A/B (*Δyge1*/*Δyge1 GAL1-YGE1*/*GAL1-YGE1*), was constructed. At 0, 10, 16 and 28 h after the shift, an aliquot of culture was taken out and subjected to microscopic observation. (a) Staining of nuclei and mitochondrial DNA with DAPI. An arrow indicates stacked mitochondria, and an arrowhead indicates nucleus. Bar = 20 μm. (b) Vital staining of mitochondria with $DiOC_6$. An arrow indicates mitochondria, and an arrowhead indicates a cell lacking mitochondria. Bar = 10 mm.

proteins to be imported are maintained in an unfolded state and imported into mitochondria with the aid of another Hsp70, Ssc1 [8], and another DnaJ homologue, Scj1 [15]. Since functions of Hsp70 (DnaK) and DnaJ are conserved in *E. coli* and yeast, the presence of a GrpE homologue has been expected in yeast. Our finding of *YGE1*, a homologue of *E. coli grpE*, strongly suggests that interaction among DnaK, DnaJ and GrpE is also conserved in eukaryotes.

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