The Yeast JEM1p Is a DnaJ-like Protein of the Endoplasmic Reticulum Membrane Required for Nuclear Fusion*

(Received for publication, December 18, 1996, and in revised form, March 23, 1997)

Shuh-ichi Nishikawa and Toshiya Endo‡
From the Department of Chemistry, Faculty of Science, Nagoya University, Chikusa-ku, Nagoya 464-01, Japan

DnaJ-like proteins are functional partners for Hsp70 molecular chaperones. Complete nucleotide sequencing of yeast chromosome X has revealed that an open reading frame YJL073w encodes a novel member of the DnaJ-like protein family. The open reading frame represents a protein of 692 amino acids with a J-domain and one putative membrane-spanning segment. An epitope-tagged version of the protein was anchored in the endoplasmic reticulum (ER) membrane and its J-domain faced the ER lumen. We therefore propose to designate this gene JEM1 (DnaJ-like protein of the ER membrane) and to designate its gene product JEM1p. The JEM1 gene is not essential for cell growth, but double disruption of the JEM1 gene and the SCJ1 gene causes growth arrest at elevated temperature. We also found that the Δjem1 null mutant is defective in nuclear membrane fusion.

**Materials and Methods**

Strains and Culturing Conditions—Standard recombinant techniques (14) were performed using Escherichia coli strain TG1 (supE hsd5 thi-1 Δ(lac-proAB) F′tra336 proAB lacZΔM15). Yeast strains SEY6210 (MATα ura3 leu2 trp1 his3 2α suc2) and SEY6211 (MATα ura3 leu2 trp1 his3 ade2 suc2) (15) were used in the construction of Δjem1 and Δscj1 strains. SEY621D was constructed by mating SEY6210 with SEY6211. Yeast cells were grown according to standard methods (16). Quantitative mating assay was performed as described previously (16).

Plasmids and Strain Constructions—The JEM1 gene was cloned by PCR from yeast genomic DNA using the primers based on the sequence deposited in the database: 73A (5′-GGCGAGCTCCTGACGATGAC-TATTAC-3′) and 73B (5′-GGCGCTGAGGTGGCTGGTTTGCATTAAT-3′). The amplified 2.4-kb fragment was digested with SacI and Xhol and introduced into a yeast multi-copy plasmid pYO326 (17) to generate pSNJ1. The 3HA epitope tagged three tandem repeats of the influenza virus hemagglutinin (HA) epitope (YPDVPDYA), was introduced at the C terminus of JEM1p at the DNA level by oligonucleotide-directed mutagenesis (18). The chimeric gene encoding JEM1p tagged with the 3HA epitope was subcloned into pYO326 (17) and a yeast single copy plasmid pRS316 (19) to generate pSNJ2 and pSNJ3, respectively. A H613Q mutation of the JEM1 gene was performed by oligonucleotide-directed mutagenesis (Sculptr in vitro mutagenesis system, Amer-sham Corp.) using an oligonucleotide (5′-CCACAAAAAATACCAACCAGCAAATAAG-3′).

A null allele of JEM1 was constructed by replacing the 1.2-kb BamHI/SacI restriction fragment of pSNJ1 with a 2.2-kb BamHI/SalI fragment of pJJ282 containing the yeast LEU2 gene (20). The resulting plasmid, YEPjem1, was digested with Xhol and SacI, and the 3.2-kb fragment was isolated and transformed into SEY6210, SEY6211, and SEY621D. Yeast transformation was performed by the lithium thiocyanate method (21). Leu− transformants were selected and the presence of the Δjem1 allele was confirmed by PCR using the primers 73A and 73B. The Δjem1 strains derived from SEY6210, SEY6211, and SEY621D were named SNY1028, SNY1029, and SNY1024, respectively.

The SCJ1 gene was cloned from yeast genomic DNA by PCR using the primers SCJA (5′-GGCGGAGCTCCTGACGATGAC-TATTAC-3′) and SCJB (5′-GGCGAGCTCCTGACGATGAC-TATTAC-3′). The amplified 5.5-kb fragment was digested with Xhol and SacI and subcloned into a polylinker site of pBluescript IIISK* (Stratagene) to generate pBSSCJ1. The SCJ1 gene was disrupted by inserting a 0.9-kb EcoRI/PstI restriction fragment of pJJ282 containing the yeast TRP1 gene (20) into the EcoRI/PstI sites of pBSSCJ1. The resulting plasmid was named pBSSCJ1. The 2.0-kb Xhol and SacI fragment of pBSSCJ1 was purified and transformed into SEY6210 and SEY6211, and Trp− transformants were selected. Disruption of the SCJ1 gene was confirmed by PCR using the primers SCJA and SCJB. The Δscj1 strains derived from SEY6210

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tical staining was observed with the anti-BiP antibodies (Fig. 1). This staining is typical for yeast ER proteins, and nearly identi-
sively in the ER.

Staining with the 12CA5 antibody showed perinuclear staining
with several extensions in the cytoplasm (Fig. 1).

JEM1p at the DNA level. This epitope-tagged version of JEM1p
was expressed from a multicopy plasmid, and the cells were
fixed, permeabilized, and stained with the 12CA5 antibody.

Immunofluorescence microscopy. For this purpose, three tan-
clones were attached to the C terminus of
the 12CA5 monoclonal antibody and anti-BiP polyclonal
antibodies. Panels a, b, and c show the same field of the fluorescent
images stained with the 12CA5 antibody, anti-BiP antibodies, and
DAPI, respectively. Bar, 2 μm.

and SEY6211 were named SNY1025 and SNY1027, respectively.

Double label immunofluorescent staining of yeast cells was performed as described previously (22). The 12CA5 mouse monoclonal antibody, the fluorescein isothiocyanate-conjugated sheep anti-mouse IgG antibody F(ab)2 fragment and the rhodamine-conjugated sheep anti-mouse IgG antibody F(ab)2 fragment were pur-
chased from Boehringer Mannheim Yamanouchi (Tokyo, Japan). The rabbit anti-BiP antiserum was prepared against the MalE-KAR2 fusion
protein. To analyze nuclear fusion during mating, cells of different
mating types were mated as described previously (23). Cells were fixed
with 4% formaldehyde in PBS, washed, and stained with 1 μg/ml DAPI, 2 μg/ml 4′,6-diamidino-2-phenylindole (DAPI), and 1 μg/ml rhodamine-phalloidin. Staining with the 12CA5 monoclonal antibody and anti-BiP polyclonal antibodies was detected with the FITC-labeled sheep anti-mouse IgG antibody F(ab)2 fragment and the rhodamine-
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Fluorescence Microscopy—Double label immunofluorescent staining of yeast cells was performed as described previously (22). The 12CA5 mouse monoclonal antibody, the fluorescein isothiocyanate-conjugated sheep anti-mouse IgG antibody F(ab)2 fragment and the rhodamine-conjugated sheep anti-mouse IgG antibody F(ab)2 fragment were pur-
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JEM1p Is a Yeast DnaJ-like Protein Anchored in the ER
Membrane, and Its J-domain Faces the ER Lumen—JEM1
(YJL073) on yeast chromosome X encodes a DnaJ-like protein,
which is 692 amino acids long, with a calculated molecular
weight of 80,380. The predicted amino acid sequence of JEM1p
showed a diffuse or doublet band with the apparent molecular
mass of 73–79 kDa (Fig. 2), which was not detected in
the extracts of cells that did not express the epitope-tagged JEM1p (not shown). This band shifted to a single sharp band of
71 kDa after treatment of the extracts with endoglycosidase H
(not shown), suggesting that the 3HA-tagged JEM1p contained
N-linked oligosaccharide chains and that the observed diffuse or doublet band reflected heterogeneity in glycosylation.

Because JEM1p has a stretch of uncharged hydrophobic
amino acids between residues 50 and 70 near the N terminus
(Fig. 1A), which is sufficiently long to function as a membrane
anchor, we tested whether JEM1p is an integral membrane protein in the ER. Whole cell homogenates from the strain expressing the 3HA-tagged JEM1p were treated with 1 mM NaCl, 2 mM urea, 1 mM Triton X-100, 0.1 M sodium carbonate, pH 11.5, or 1% sodium deoxycholic acid, and extractability of JEM1p from
membranes was analyzed (Fig. 2A). Because JEM1p was re-
sistant to extraction with 1 mM NaCl, 2 mM urea, or 0.1 M sodium carbonate, by which soluble and peripheral membrane proteins are extracted, JEM1p is indeed an integral membrane protein. Interestingly, JEM1p was extracted from the ER membrane with 1% deoxycholic acid, an ionic detergent, but not with 1% Triton X-100, a nonionic detergent (Fig. 2A). JEM1p may be interacting with other membrane proteins in the ER membrane.

The orientation of JEM1p in the ER membrane was exam-
ned by digestion with a proteolytic enzyme. Cell homogenates from
the strain expressing the 3HA-tagged JEM1p were treated with trypsin in the absence or the presence of
Triton X-100, and the intactness of the 3HA epitope tag of JEM1p was probed with the 12CA5 antibody. Although the 3HA-tagged
JEM1p was not digested by trypsin in the absence of detergent,
the 3HA epitope tag became susceptible to trypsin digestion when the ER membrane was lysed with Triton X-100 (Fig. 2B). Because the 3HA tag was introduced at the C terminus of

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RESULTS AND DISCUSSION

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JEM1p was not digested by trypsin in the absence of detergent,
the 3HA epitope tag became susceptible to trypsin digestion when the ER membrane was lysed with Triton X-100 (Fig. 2B). Because the 3HA tag was introduced at the C terminus of
JEM1p, the C-terminal part including the J-domain is obviously exposed on the luminal side of the ER. This transmembrane topology of JEM1p is consistent with the fact that potential sites for N-linked glycosylation are present only in the C-terminal domain (Fig. 1) and that JEM1p is glycosylated. The presence of the J-domain in the ER lumen suggests that JEM1p is a partner protein for BiP and/or LHS1p, Hsp70s of the ER lumen.

Yeast Cells Lacking JEM1p Alone Show Normal Growth, but Those Lacking Both JEM1p and SCJ1p Cannot Grow at High Temperature—To assess the roles of JEM1p in vivo, we have constructed a null allele of the JEM1 gene. A JEM1::LEU2 heterozygous diploid was constructed and subjected to sporulation. Among 25 tetrads dissected, 18 produced 4 viable spores, and 7 produced 3 viable spores. The Leu+ phenotype was segregated 2:2 in all four viable ascis. The Δjem1Δscj1 mutant strain grew as well as wild-type strains at 23 °C (left) or at 37 °C (right) for 2 days.

Disruption of the JEM1 Gene Causes a Defect in Karyogamy—In the sexual phase of the yeast, haploid cells of opposite mating types (MATa and MATα) mate each other to form diploid cells. The mating cells form projections, and the cells fuse where the two mating cells come in close contact. After cell fusion, the nuclei from both haploid cells fuse to produce a diploid nucleus. This step is called karyogamy (26). Analyses of the yeast mutants defective in karyogamy showed that this step can be divided into two steps; nuclear congression and nuclear fusion (26). Haploid nuclei move and align during the nuclear congression step, and then the two nuclei fuse. In zygotes of mutants defective in the nuclear fusion step, nuclei become closely juxtaposed but do not fuse (26). Although the Δjem1 mutant strain grows as well as wild-type strains at all temperatures tested, it is defective in karyogamy.

The presence of the J-domain in the ER lumen suggests that JEM1p is a partner protein for BiP and/or LHS1p, Hsp70s of the ER lumen (3). Disruption of the SCJ1 gene was not lethal for yeast cells. The jem1::LEU2 strain and the scj1::TRP1 strain of opposite mating types were crossed, and the resulting heterozygous diploid was sporulated and dissected. We obtained Leu+ Trp+ spores that contain disrupted alleles of both genes. The Δjem1Δscj1 double disrupted strains grew as well as wild-type strains at 14, 23, and 30 °C, but they did not grow at 37 °C (Fig. 3A). A low copy number plasmid containing the fusion gene for the 3HA-tagged JEM1p rescued the temperature-sensitive growth defect of the Δjem1Δscj1 double mutant (Fig. 3B). Therefore, the growth defect resulted from disruption of both JEM1 and SCJ1 genes. The genetic interactions between the JEM1 and the SCJ1 genes suggest that their gene products are involved in a common pathway of cellular processes, which remains to be revealed.

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The J-domain Is Required for the JEM1p Functions—Members of the DnaJ-like protein family contain a highly conserved His-Pro-Asp sequence in the J-domain, which appears to play a critical role in interactions with Hsp70 (27, 28). We attempted to test the role of the J-domain in the functions of JEM1p by
introducing an H613Q mutation in its His-Pro-Asp sequence. When \( \Delta jem1 \) mutant cells harboring the HA-tagged \( jem1 \) H613Q mutant gene were self-crossed, 75\% of the resulting zygotes showed karyogamy defects (not shown), and diploid formation was impaired (not shown). This demonstrates that the J-domain of JEM1p is essential for karyogamy.

Interestingly, the \( \Delta jem1 \) H613Q mutant gene failed to complement the temperature-sensitive growth of the \( \Delta jem1 \Delta scj1 \) double mutant (Fig. 3B). Because karyogamy is not required for normal cell growth and disruption of the \( SCJ1 \) gene alone exhibits no obvious phenotype in karyogamy, the J-domain of JEM1p, together with SCJ1p, is involved in a process that is distinct from karyogamy. In this context, it is to be noted that treatment of yeast cells with tunicamycin, which leads to accumulation of malfolded proteins in the ER and triggers the unfolded protein response, led to an increased level of \( JEM1 \) mRNA (not shown).

Acknowledgments—We are grateful to Drs. Y. Ohya and Y. Wada for plasmids, to Dr. S. D. Emr for strains, and to Dr. M. Nakai for the anti-BiP antiserum.

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