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Yeast Pdr13p and Zuo1p molecular chaperones are new functional Hsp70 and Hsp40 partners

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Abstract

The deletion of the *TOM1* gene encoding a putative ubiquitin ligase causes a temperature sensitive cellular growth in *Saccharomyces cerevisiae*. The arrested cells exhibit pleiotropic defects in nuclear division, maintenance of nuclear structure and heat stress responses. We previously identified a *zuo1* mutation as an extragenic suppressor of the *tom1* mutant. *ZUO1* encodes a DnaJ-related Hsp40. Here we show that a recessive cold sensitive mutation in *PDR13* coding for an Hsp70 suppressed the *tom1* mutation. The *pdr13* deletion mutant was sensitive to high osmolarity, just like the *zuo1* deletion mutant. A *zuo1 pdr13* double deletion mutant did not show additive phenotypes. Furthermore, a tagged-Zuo1p was co-immunoprecipitated with a tagged-Pdr13p. Taken together, we propose that Pdr13p and Zuo1p are a new pair of Hsp70:Hsp40 molecular chaperones. In addition, Pdr13p co-sedimented with translating ribosomes and this association was independent of the presence of Zuo1p.

Keywords: DnaK–DnaJ pair; Heat shock proteins; *Saccharomyces cerevisiae*

1. Introduction

Molecular chaperones are involved in many important biological processes by folding nascent polypeptide chain on ribosomes during synthesis, renaturing denatured proteins after exposure to various stresses, driving protein translocation across membranes, mediating the assembly and disassembly of complexes and facilitating protein degradation (Hartl, 1996). In *Saccharomyces cerevisiae*, 14 genes encoding Hsp70 homologues and 18 genes encoding Hsp40 proteins have been identified. Ssa and Ssb proteins are two subclasses of Hsp70s. Ssb is an abundant type of Hsp70s found associated with translating ribosomes to prevent misfolding of newly synthesized proteins (Nelson et al., 1992; Pfund et al., 1998).

Molecular chaperones often function together. For example, DnaK, a member of Hsp70 class of *Escherichia coli*, works together with DnaJ, a member of Hsp40 class. The Hsp40/DnaJ proteins contain a signature of the J domain which is required for interaction with Hsp70 and stimulates its ATPase activity (Liberek et al., 1991). Kar2p and Sec63p are known to be functional Hsp70 and Hsp40 partners, working in endoplasmic reticulum (Lyman and Sheckman, 1995). The Ssb proteins have been proposed to be partners of Zuo1p, a member of Hsp40s associated with ribosomes (Yan et al., 1998).

TOM1 encodes a putative ubiquitin ligase. The deletion of *TOM1* causes temperature sensitive growth: at a restrictive temperature the mutant exhibits pleiotropic defects in maintenance of the nuclear structure, nuclear division and heat stress responses (Utsugi et al., 1999; Sasaki et al., 2000a), as well as certain gene expressions (Saleh et al., 1998). As multi-copy suppressors of the *tom1* mutant, we previously identified *STM1* coding for a quadruplex nucleic acid-binding protein (Utsugi et al., 1995; Frantz and Gilbert, 1995) and *NPI46* encoding a nucleolar FK506-binding protein (Utsugi et al., 1999).

As described previously, we isolated various kinds of extragenic suppressor of the *tom1* mutant (Sasaki et al.,

Abbreviations: GFP, green fluorescent protein; HA, hemagglutinin; kb, kilobase(s); PCR, polymerase chain reaction; *PDR13*, pleiotropic drug resistance; *STM*, suppressor of *tom1*; *TMR*, *tom1* revertant; *TOM1*, temperature dependent organization in mitotic nucleus; *ZUO1*, Zuotin.

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2000a). Among them, molecular chaperone mutants were identified: *zuo1* and *sse1*. *SSE1* encodes an Hsp110 protein, a member of the superfamily of Hsp70s, and is involved in the function of Hsp90s as a co-chaperone (Liu et al., 1999). In most of these suppressor mutants, the general stress response was constitutively activated (Sasaki et al., 2000a).

Here we describe that a *pdr13* mutation is an extragenic suppressor of *tom1*. The *PDR13* gene encodes a member of Hsp70s and is reported to be required for enhancing the transcriptional regulatory protein Pdr1p which is involved in gene expression of ATP binding cassette transporter-encoding genes. The general stress response is constitutively activated in the *pdr13* mutant (Hallstrom et al., 1998).

In addition, we report here that the *pdr13 zuo1* double mutant does not show additive effects and Zuo1p interacts with Pdr13p. From these results, we propose that Pdr13p and Zuo1p are a new functional pair of Hsp70:Hsp40.

2. Materials and methods

2.1. Strains and genetic manipulations

The strains of *S. cerevisiae* used in this study are described in Table 1. Media, techniques for genetics and molecular biological experiments were those described (Kaiser et al., 1994; Sambrook et al., 1989).

2.2. Plasmids and gene disruptions

Plasmid vectors of YEp13, YEplac195 and YCplac33 were described previously (Broach et al., 1979; Gietz and Sugino, 1988). Plasmid pTOM5 (YCp-*TOM1*) was described previously (Utsugi et al., 1999). A plasmid containing *SSB1* was isolated from a YEp13-based gene bank and pTA106 was constructed by deleting the *Xba*I–*Mlu*I fragment from the original plasmid. A plasmid containing *SSB2* was isolated from the YEp13-based gene bank, the 3 kb *Xba*I–*Bam*HI fragment of the original isolate was cloned into YEplac195 and the resulting plasmid was pTA104. Plasmid pTM1032 carrying *ZUO1-GFP* was constructed as follows. DNA fragment was amplified by PCR (polymerase chain reaction) using a primer of −1 kb 5′-upstream region of *ZUO1* containing a *Pst*I site: 5′-GCGCCTGCAGATCCTGAGTCCCTACCAATTAA-3′ and a C-terminal primer carrying a *Bam*HI site: 5′-GCGCGGATCCCACGAAGTAGGACAACAAGCTG-3′, and genomic DNA as template. The amplified DNA was digested with *Pst*I and *Bam*HI, and inserted into a GFP-tagging vector pTS910IL, a gift from T. Sasaki. The resulting plasmid pTM1032 was digested with *Sal*I and integrated into the *ZUO1* locus of the wild type strain W303-1A. The Zuo1-GFP protein was detected by Western blotting. A plasmid pTM1051 containing *PDR13* was isolated from a genomic library constructed on YCUp4, which was given by A. Fujita of National Institute of Life Science. By deleting the 1.2 kb *Xba*I fragment from pTM1051 (see Fig. 2), pTM1053 was generated. To construct pTM1055, the ORF (open reading frame) of *PDR13* was amplified by PCR, using a primer of the N-terminal region containing a *Bam*HI site: GCGCGGATCCATGAAGTACATGGTAGTCAGCT, and a C-terminal primer carrying a *Sal*I site: GCGCGTCGACTAATTCACCCTTTACAGCATTAG. The PCR product was digested with *Bam*HI and *Sal*I and cloned into plasmid pYESHA (a gift of Y. Uesono), to express a double HA-tagged N-terminal protein under control of the *GAL1* promoter.

To disrupt *ZUO1* by replacing with *HIS3* of *Candida grablata*, the 1.2 kb DNA fragment was amplified by PCR using primers 5′-GGTTTTCCTACCGATGCTTTTATAAAATCTTCGTTTTGTCTCACATATACCAACAAGAGTAACGCACCGATCAACGTACAGTGG-3′ (the sequence of *HIS3* of *C. grablata* is underlined) and 5′-CACGAAGTAGGACAACAAGCTGGATGGTAGTTTGCCAGAATCGACAATAGTCTTTGCAGCCTGACAATCTGGCAGCTCG-3′, and *C. grablata* genomic DNA as a template and introduced into W303-1A. A cold-sensitive His^+ transformant (TMY301) was selected. The disruption was confirmed by PCR. To disrupt *PDR13* by replacing with *TRP1* of *C. grablata*, the 1.1 kb DNA fragment was amplified by PCR using primers 5′-GCTCTTTTTCTTCGTCGAATGTGATGGTGAAATTTTTTCCAAGAGATGATGAGGTCGGATAATATAGATACACACAAATCTTTTCTACG-3′ (the sequence of *TRP1* of *C. grablata* is underlined) and 5′-CCATCATGTATGTATTTTTCTATATACGTATACATACCGTTTTTCTTAGAGCGCTCTAAGTTGGTG-

Table 1
The yeast strains used in this study

Strains[a]	Relevant genotypes
W303-1A	*MATa ade2 leu2 trp1 ura3 his3*
W303-1B	*MATα ade2 leu2 trp1 ura3 his3*
TUY044	*MATa/MATα tom1-1/tom1-1 leu2/leu2 trp1/trp1 ura3/ura3* His^- (Utsugi et al., 1999)
YTS004-A	*MATa tom1-2::HIS3* (Sasaki et al., 2000a)
TMY301	*MATa zuo1::cgHIS3*
TMY051	*MATα pdr13-101*
TMY052	*MATa pdr13-101 tom1-2::HIS3*
TMY053	*MATα pdr13::cgTRP1*
TMY054	*MATa/MATα zuo1::cgHIS3/ZUO1 pdr13::cgTRP1/PDR13*
TMY055	*MATa ZUO1::GFP-LEU2* pTM1055 (pYESHA-*PDR13*)
TMY056	*MATa ZUO1::GFP-LEU2* pYESHA
TMY058	*MATα pdr13::cgTRP1 zuo1::cgHIS3*

[a] All the strains except TUY044 are congenic to W303-1A.

ATTAGTTATCAGA-3′, and *C. grablata* genomic DNA as a template and introduced into W303-1B. A cold-sensitive Trp^+ transformant (TMY053) was selected. The disruption was confirmed by PCR.

2.3. Immunoprecipitation experiments

Immunoprecipitation experiments were performed as described previously (Sasaki et al., 2000b) with some modifications. 60 ml of each cell culture of TMY055 expressing both an HA-tagged Pdr13p and GFP-tagged Zuo1p or TMY056 expressing the GFP-tagged Zuo1p was grown in SGal-Ura medium, collected at logarithmic phase and broken with glass beads in 250 μl of buffer A [100 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 10% glycerol, 0.1% Triton X-100, 1 mM dithiothreitol, 50 mM NaCl, 1 μM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml antipain, 1 μg/ml pepstatin and 2 μg/ml aprotinin]. After centrifugation (10 min, 13 000 rpm), the supernatant was mixed with 250 μl of buffer A containing 2 mg/ml BSA, incubated with 2 μl of anti-HA antibody (16B12, Boehringer Mannheim) for 2 h and further incubated with 50 μl of protein A-sepharose suspension for 2 h. Beads were washed three times with 1 ml of buffer A containing 100 mM NaCl for 10 min by end-over-end rotation. Bound proteins were eluted with SDS sample buffer by heating and detected by immunoblotting, using anti-HA or anti-GFP antibody (Boehringer Mannheim) and horseradish peroxidase conjugated secondary antibody (Chemicon).

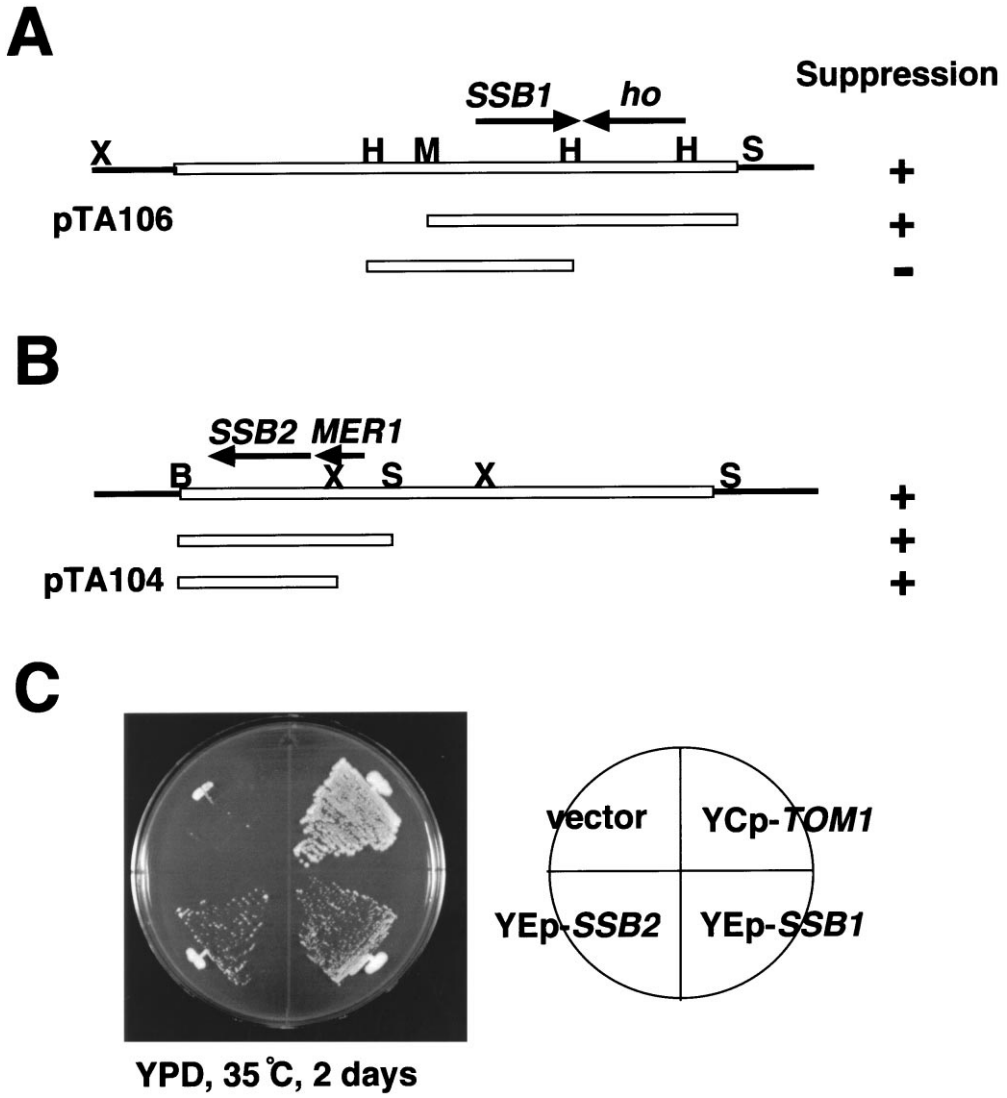

Fig. 1. The *SSB1* and *SSB2* genes are multi-copy suppressors of the *tom1* mutant. (**A**) Identification of *SSB1*. (**B**) Identification of *SSB2*. (**C**) Strain TUY044 (*tom1*) was transformed with each plasmid pTA106 (YEp13-*SSB1*), pTA104 (YEplac195-*SSB2*), pTOM5 (YCp-*TOM1*) or YEp13 (vector). The transformants were streaked on a YPD plate which was incubated at 35°C for two days. + or − indicates ability or inability of suppression. B; *Bam*HI, H; *Hin*dIII, M; *Mlu*I, S; *Sph*I, X; *Xba*I.

2.4. Sucrose density gradient centrifugation

Cell lysates were prepared according to Baim et al. (1985) and centrifuged through 7–47% sucrose density gradients in SW41 rotor at 18 000 rpm for 17 h at 5°C. Each fraction was subjected to Western blotting.

3. Results

3.1. SSB1 and SSB2 are multi-copy suppressors of tom1

The deletion of the *TOM1* gene causes a temperature sensitive growth phenotype (Utsugi et al., 1999). In order to explore the gene function, we previously isolated multi-copy suppressors which rescued the temperature sensitivity of the *tom1* mutant and identified *STM1* (suppressor of *tom1*) (Utsugi et al., 1995; Frantz and Gilbert, 1995) and *NPI46* as *STM3* (Utsugi et al., 1999).

We isolated two more multi-copy plasmids (*STM4* and *STM5*) which rescued the temperature sensitivity of a *tom1* mutant (TUY044) at 35°C. By subcloning and sequencing experiments of *STM5*, we identified *SSB1* as a multi-copy suppressor, because plasmid pTA106 contained *SSB1* and a defective *HO* gene (Fig. 1A, C). The second multi-copy suppressor *STM4* was identified as *SSB2*, since plasmid pTA104 which contained the 3.0 kb *Bam*HI–*Xba*I fragment carrying only *SSB2* suppressed the *tom1* mutation (Fig. 1B, C). These *SSB1* and *SSB2* genes encode a pair of Hsp70 molecular chaperones associated with ribosomes to fold nascent polypeptide chains and have been proposed to be partners of Zuo1p, a DnaJ-related Hsp40 (Pfund et al., 1998; Yan et al., 1998).

3.2. pdr13 is an extragenic suppressor of tom1

We previously isolated extragenic mutational suppressors (*tmr*; *tom1* revertant) which suppressed the *tom1* mutation (Sasaki et al., 2000a). As *tmr3* and *tmr6*, *zuo1* and *sse1* were identified, respectively. The *SSE1* gene encodes a member of the Hsp70 superfamily. The *sse1* mutant was temperature sensitive, whereas the *zuo1* mutant exhibited cold sensitive growth phenotype. The double mutant (*zuo1 sse1*) was temperature sensitive and cold sensitive (data not shown). So far we have not found any genetical relation between *zuo1* and *sse1*.

A high dose of *SSB1* or *SSB2* suppressed the temper-

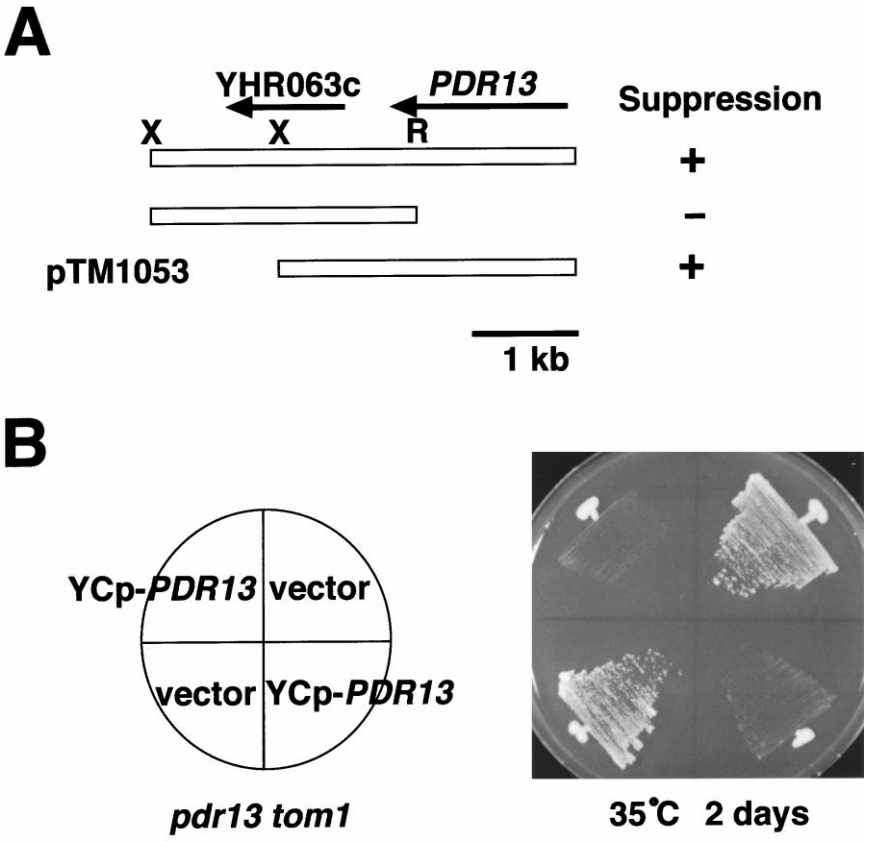

Fig. 2. A *pdr13* mutation suppresses *tom1*. (**A**) Complementation of *pdr13*. Strain TMY051 was transformed with a YCUp4-based gene bank and a plasmid which complemented its cold sensitivity was isolated. By subcloning and sequencing experiments, plasmid pTM1053 was found to contain only *PDR13*. (**B**) The double mutant TMY052 (*pdr13 tom1*) was transformed with pTM1053 (YCp-*PDR13*) or YCplac33-vector and Ura^+ transformants were streaked on a SD-Ura plate which was incubated at 35°C for two days. R; *Eco*RI, X; *Xba*I.

ature sensitivity of the *tom1* mutant, whereas a mutation in *ZUO1* is known to suppress *tom1* (Sasaki et al., 2000a), suggesting that *SSB* and *ZUO1* have opposite effects on the *tom1* mutation. Thus we anticipated that Ssb should not be an Hsp70 partner of Zuo1p, at least in this case.

By another unrelated experiment, we isolated a cold sensitive mutant TMY051. From a YCUp4-based gene bank, we isolated a plasmid which complemented its cold sensitivity. By subcloning and sequencing experiments, plasmid pTM1053 was found to contain only *PDR13* (Fig. 2A). *PDR13* encodes another member of Hsp70s and the *pdr13* disruptant was reported to be cold sensitive (Hallstrom et al., 1998).

To see if *pdr13* suppressed the temperature sensitivity of *tom1*, the strain TMY051 was crossed with YTS004-A (*tom1*) and the diploid was sporulated. A double mutant TMY052 (*pdr13 tom1*) was isolated which did not grow at 15°C, but grew at 35°C. When a single copy of *PDR13* (pTM1053) was introduced into TMY052, the transformant did not grow at 35°C, whereas the transformant containing a vector grew at 35°C (Fig. 2B). Thus we conclude that the *pdr13* mutation partially suppressed the temperature sensitivity of the *tom1* mutant.

The *pdr13* mutation complemented with the unidentified *tmr4* or *tmr7* (Sasaki et al., 2000a), indicating that *TMR4* or *TMR7* is not *PDR13* (data not shown).

3.3. The zuo1 and pdr13 mutants have similar phenotypes

As described in Materials and methods, we constructed *zuo1* (TMY301) and *pdr13* disruptants (TMY053) in the W303 background and mated them. The resulting heterozygous diploid strain TMY054 (*pdr13::cgTRP1/PDR13 zuo1::cgHIS3/ZUO1*) was sporulated and Trp^+ His^+ clones (*zuo1 pdr13*) were recovered. The *zuo1 pdr13* double mutant (TMY058) grew slowly and cold sensitive, just like the *zuo1* (TMY301) or *pdr13* mutant (TMY053) (data not shown). The *pdr13* mutant was also sensitive to high osmolarity (1 M NaCl) (Fig. 3), similar to the *zuo1* mutant (Yan et al., 1998). Furthermore, the double mutant (TMY058) did not show an additive phenotype, suggesting that *ZUO1* and *PDR13* share some function in the same pathway.

3.4. Zuo1p and Pdr13p form a complex

The physical interaction of Zuo1p with Pdr13p in vivo was tested by an immunoprecipitation experiment. Strain TMY055 expressing a GFP-tagged Zuo1p and an HA-tagged Pdr13p under control of the *GAL1* promoter, or TMY056 expressing only the GFP-tagged Zuo1p, was grown in galactose medium to mid-log phase. The cell extracts were prepared and anti-HA antibody was added to the lysates for immunoprecipitation, as described in Materials and methods. Then samples were subjected to Western blotting, using anti-GFP or anti-HA antibody. As shown in Fig. 4, Zuo1-GFP was co-immunoprecipitated with the HA-tagged Pdr13p (lane 5), while almost no bands of Zuo1-GFP was detected when the cells contained a mock vector (lane 6).

3.5. Association of Pdr13p with ribosomes does not depend on Zuo1p

By sucrose density gradient centrifugation, we fractionated the lysates of TMY055 containing the

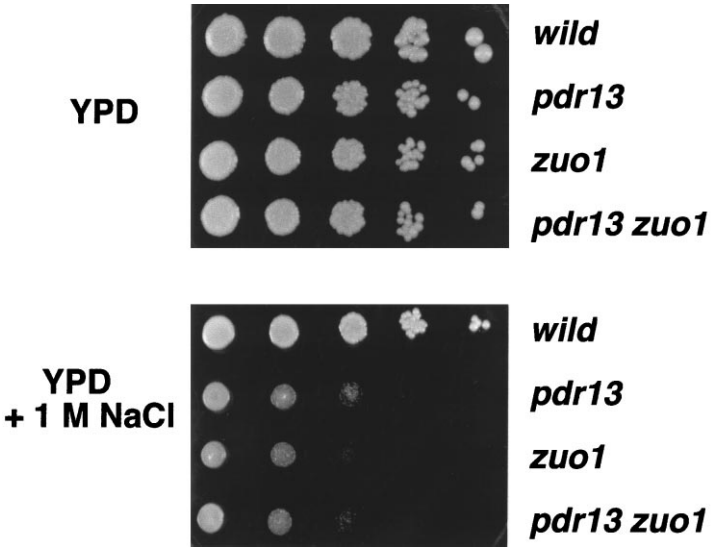

Fig. 3. The *zuo1* and *pdr13* mutants have similar phenotypes. Cells of W303-1A (wild type), TMY053 (*pdr13*), TMY301 (*zuo1*) and TMY058 (*zuo1 pdr13*) were grown at 30°C overnight and an equal number of cells were spotted as 10-fold dilution series on a YPD plate with or without 1 M NaCl. The plates were incubated at 30°C for four days.

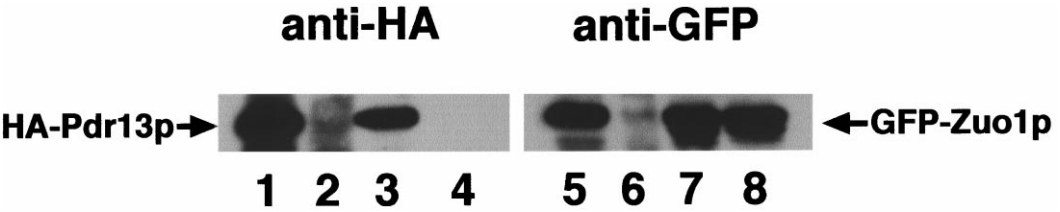

Fig. 4. Zuo1p interacts with Pdr13p. Strain TMY055 expressing GFP-Zuo1p and HA-Pdr13p or TMY056 expressing GFP-Zuo1p was grown in galactose medium to mid-log phase. The cell lysates were mixed with anti-HA antibody for immunoprecipitation. Then samples were subjected to Western blotting. Lanes 1, 5: immunoprecipitates from TMY055 lysates; lanes 2, 6: immunoprecipitates from TMY056 lysates; lanes 3, 7: total lysates of TMY055; lanes 4, 8: total lysates of TMY056. Lanes 1–4: detection by anti-HA antibody; lanes 5–8: detection by anti-GFP antibody.

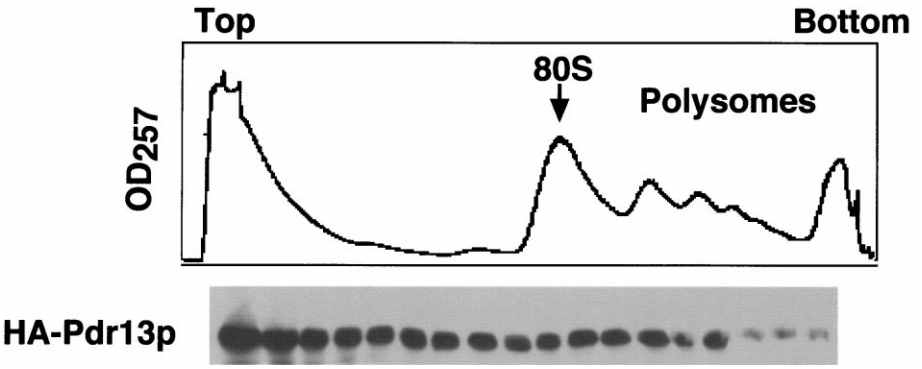

Fig. 5. Association of Pdr13p with ribosomes in the absence of Zuo1p. The strain TMY058 (*zuo1 pdr13*) expressing an HA-Pdr13p was grown in galactose medium to mid-log phase. The cell lysates were prepared according to Baim et al. (1985) and centrifuged through sucrose density gradient in SW41 rotor at 18 000 rpm for 17 h at 5°C. Each fraction was subjected to Western blotting, using anti-HA antibody.

HA-tagged Pdr13p expressed from the *GAL1* promoter and analysed by immunoblotting. As reported recently, the HA-tagged Pdr13p co-sedimented with translating ribosomes (data not shown) (Hallstrom and Moye-Rowley, 2000). In addition, it broadly sedimented in slower fractions.

To see whether this ribosome association depends on the presence of Zuo1p, strain TMY058 (*zuo1 pdr13*) was transformed with pTM1055 (pYESHA-*PDR13*) and the transformants were grown in galactose medium, to express the HA-tagged Pdr13p. The lysate was prepared and fractionated through sucrose density gradient centrifugation. Without Zuo1p, Pdr13p co-sedimented with translating ribosomes, just like the wild type lysate (Fig. 5). These results suggest that Pdr13p associates with ribosomes, independently of Zuo1p. It may bind to nascent polypeptide chains.

4. Discussion

Tom1p is a putative ubiquitin ligase and required for growth at high temperatures (Utsugi et al., 1999). During the isolation of suppressors of the *tom1* mutant, we noticed that *SSB1*/*SSB2* and *ZUO1* had opposite effects on the *tom1* mutation, since *SSB1* and *SSB2* were multi-copy suppressors (Fig. 1), while *zuo1* as *tmr3* was a recessive mutational suppressor of the *tom1* mutant (Sasaki et al., 2000a).

We showed that the *pdr13* mutation suppressed *tom1* (Fig. 2B), just like the *zuo1* mutation. Both mutants are reported to be cold sensitive and the general stress response is constitutively activated in either mutant (Hallstrom et al., 1998; Sasaki et al., 2000a). As shown in Fig. 3, the *pdr13* mutant was sensitive to high osmolarity, just like the *zuo1* mutant (Yan et al., 1998). Thus both mutants have common phenotypes. Furthermore, the double deletion mutant did not exhibit an additive phenotype, suggesting that these genes function in the same pathway.

The physical interaction between Pdr13p and Zuo1p was seen in the co-immunoprecipitation experiment (Fig. 4). Taken together, we suggest that Pdr13p and Zuo1p are new functional Hsp70 and Hsp40 partners.

Lu and Cyr (1998) suggested that the protein-folding activity of Hsp70/Ssa1p is modified differentially by two kinds of Hsp40s, Ydj1p and Sis1p. Thus functionally distinct Hsp40 proteins can specify the action of Ssa1p by generating Hsp70:Hsp40 pairs that exhibit different chaperone activities. Similarly, the biological functions of Zuo1p may be specified by changing its partner with either Ssb or Pdr13p.

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References

Baim, S.B., Pietras, D.F., Eustice, D.C., Sherman, F., 1985. A mutation allowing an mRNA secondary structure diminishes translation of *Saccharomyces cerevisiae* Iso-1-cytochrome *c*. Mol. Cell. Biol. 5, 1839–1846.

Broach, J.R., Strathern, J.N., Hicks, J.B., 1979. Transformation in yeast: development of a hybrid cloning vector and isolation of the *CAN1* gene. Gene 8, 121–133.

Frantz, J.D., Gilbert, W., 1995. A yeast gene product, G4p2, with a specific affinity for quadruplex nucleic acids. J. Biol. Chem. 270, 9413–9419.

Gietz, R.D., Sugino, A., 1988. New yeast–*Escherichia coli* shuttle vectors constructed with in vivo mutagenized yeast gene lacking six-base pair restriction sites. Gene 74, 527–534.

Hallstrom, T.C., Moye-Rowley, W.S., 2000. Hyperactive forms of the Pdr1p transcription factor fail to respond to positive regulation by the Hsp70 protein Pdr13p. Mol. Microbiol. 36, 402–413.

Hallstrom, T.C., Katzmann, D.J., Torres, R.J., Sharp, W.J., Moye-Rowley, W.S., 1998. Regulation of transcription factor Pdr1p function by an Hsp70 protein in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 18, 1147–1155.

Hartl, F.U., 1996. Molecular chaperones in cellular protein folding. Nature 381, 571–580.

Kaiser, C., Michaelis, S., Mitschell, A., 1994. Methods in Yeast Genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Liberek, K., Marszalek, J., Ang, D., Georgopoulos, C., 1991. *Escherichia coli* DnaJ and GrpE heat shock proteins jointly stimulate ATPase activity of DnaK. Proc. Natl. Acad. Sci. USA 88, 2874–2878.

Liu, X.-D., Morano, K.A., Thiele, D.J., 1999. The yeast Hsp110 family member, Sse1, is an Hsp90 cochaperone. J. Biol. Chem. 274, 26 654–26 660.

Lu, Z., Cyr, D.M., 1998. Protein folding activity of Hsp70 is modified differentially by the Hsp40 co-chaperones Sis1 and Ydj1. J. Biol. Chem. 273, 27 824–27 830.

Lyman, S.K., Sheckman, R., 1995. Interaction between Bip and Sec63p is required for the completion of protein translocation into the ER of *Saccharomyces cerevisiae*. J. Cell Biol. 131, 1163–1171.

Nelson, R.J., Ziegelhoffer, T., Nicolet, C., Werner-Washburne, M., Craig, E.A., 1992. The translation machinery and 70 kd heat shock protein cooperate in protein synthesis. Cell 71, 97–105.

Pfund, C., Lopez-Hoyo, N., Ziegelhoffer, T., Scilke, B.A., Lopez-Buesa, P., Walter, W.A., Wiedmann, M., Craig, E.A., 1998. The molecular chaperone Ssb from *Saccharomyces cerevisiae* is a component of the ribosome–nascent chain complex. EMBO J. 17, 3981–3989.

Saleh, A., Collart, M., Martens, J.A., Genereaux, J., Allard, S., Cote, J., Brandl, C.J., 1998. TOM1p a yeast hect-domain protein which mediates transcriptional regulation through the ADA/SAGA coactivator complexes. J. Mol. Biol. 282, 933–946.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sasaki, T., Toh-e, A., Kikuchi, Y., 2000a. Extragenic suppressors that rescue defects in the heat stress response of the budding yeast mutant *tom1*. Mol. Gen. Genet. 262, 940–948.

Sasaki, T., Toh-e, A., Kikuchi, Y., 2000b. Yeast Krr1p physically and functionally interacts with a novel essential Kri1p and both proteins are required for 40S ribosome biogenesis in the nucleolus. Mol. Cell. Biol., in press.

Utsugi, T., Toh-e, A., Kikuchi, Y., 1995. A high dose of the *STM1* gene suppresses the temperature sensitivity of the *tom1* and *htr1* mutants in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1263, 285–288.

Utsugi, T., Hirata, A., Sekiguchi, Y., Sasaki, T., Toh-e, A., Kikuchi, Y., 1999. Yeast *tom1* mutant exhibits pleiotropic defects in nuclear division, maintenance of nuclear structure and nucleocytoplasmid transport at high temperatures. Gene 234, 285–295.

Yan, W., Scilke, B., Pfund, C., Walter, W., Kim, S., Craig, E.A., 1998. Zuotin, a ribosome-associated DnaJ molecular chaperone. EMBO J. 17, 4809–4817.