Partial dispensability of Djpl's J domain in peroxisomal protein import in *Saccharomyces cerevisiae* results from genetic redundancy with another class II J protein, Caj1

Neha Dobriyal¹ · Prerna Tripathi¹ · Susrita Sarkar^{1,2} · Yogesh Tak¹ · Amit K Verma¹ · Chandan Sahi¹

Received: 5 January 2017 / Revised: 8 February 2017 / Accepted: 16 February 2017 / Published online: 6 March 2017
© Cell Stress Society International 2017

Abstract J proteins are obligate co-chaperones of Hsp70s. Via their signature J domain, all J proteins interact with their partner Hsp70s and stimulate their weak ATPase activity, which is vital for Hsp70 functions. The dependency of J proteins on their J domain is such that mutations in critical amino acids in the J domain often results into a null phenotype for a particular J protein. Here, we show that the J domain of Djpl, a cytosolic J protein important for peroxisomal protein import in *Saccharomyces cerevisiae*, is partially dispensable. A complete deletion of Djpl J domain resulted into only partial loss in peroxisomal protein import function. Instead, the C-terminal domain of Djpl was found to be essential for proper localization of the peroxisomal targeted GFP-PTS1. Furthermore, we show that Caj1, another cytosolic J protein, also has some role in peroxisomal protein import. Caj1 was found to be partially redundant with Djpl as cells lacking both Djpl and Caj1 resulted into a much more severe defect in GFP-PTS1 localization. Based on these results, we propose that dispensability of J domains could be attributed to genetic redundancy between different J proteins sharing common structural topology and cellular localization.

Keywords J proteins · J domain · Djpl · Caj1 · Peroxisome · Yeast

Electronic supplementary material The online version of this article (doi:10.1007/s12192-017-0779-8) contains supplementary material, which is available to authorized users.

✉ Chandan Sahi
sahi@iiserb.ac.in

¹ Department of Biological Sciences, Indian Institute of Science Education and Research Bhopal, Bhopal, Madhya Pradesh, India

² Department of Biology, Boston University, Boston, MA 02215, USA

Introduction

Heat shock proteins of 70 kDa (Hsp70s) are involved in a variety of functions within the cell (Kampinga and Craig 2010). Heat shock proteins of 40 kDa (Hsp40s) as their obligatory co-chaperones, Hsp70s maintain proper protein folding, translocation of proteins to various cellular compartments, disassembly of protein complexes, as well as degradation of unfolded proteins (Craig et al. 2006; Mayer and Bukau 2005). Hsp40s, also called DnaJ proteins or J proteins, recruit the Hsp70 chaperone to a motley of substrates, in turn, deciding the fate of the client and functionality of the Hsp70: J protein chaperone machinery (Kampinga and Craig 2010; Kampinga et al. 2009). All Hsp70s are structurally similar to each other in sharing a nucleotide binding domain (NBD) and a substrate binding domain (SBD) connected together by a flexible linker. The SBD encompasses a hydrophobic cleft with a flexible lid on top so that the substrate can be trapped and released (Mayer 2013; Mayer and Bukau 2005; Saibil 2013). The NBD of Hsp70s provides it with its intrinsic ATPase activity which is very weak underscoring the importance of J proteins in driving the functionality of Hsp70s. Via their signature J domain, J proteins stimulate the weak ATPase activity of Hsp70s, thereby stabilizing the client:Hsp70 interactions (Kampinga and Craig 2010). J domains have a tetra-helical structure with a highly conserved histidine-proline-aspartic Acid (HPD), tripeptide sequence, which is critical for the activation of Hsp70's ATPase activity. A histidine to glutamine mutation in the HPD motif completely nullifies the function of a J protein by preventing its ATPase stimulation activity (Walsh et al. 2004).

J proteins have been grouped into three different classes. Class I J proteins resemble DnaJ of *Escherichia coli* and have an N-terminal J domain, followed by a glycine/phenylalanine (G/F)-rich region, four repeats of the CxxCxxG type zinc finger and a C-terminal region which binds client proteins. Class II J proteins have an N-terminal J domain, followed by a G/F-rich region and

a C-terminal putative client binding domain. All other proteins that contain a J domain but no other domains present in class I and II J proteins have been arbitrarily placed in class III. By their ability to bind unique clients or affect sub-cellular localization, domains other than the J domain bestow upon J proteins their functional diversity as well as specificity (Kampinga and Craig 2010; Sahi and Craig 2007). The cytosol of *Saccharomyces cerevisiae* has 13 J proteins (Apj1, Xdj1, Ydj1, Caj1, Djp1, Hlj1, Sis1, Cwc23, Jjj1, Jjj2, Jjj3, Swa2, and Zuo1) and predominantly two classes of Hsp70s, Ssa (SSA1–4) and Ssb (SSB1–2) (Huang et al. 2005; Sahi and Craig 2007). The Ssa class of Hsp70s are multi-functional and are involved in myriad of cellular processes in the cytosol including protein folding, degradation, as well as translocation of protein across membranes while the ribosome-associated Ssb class of Hsp70s are specifically involved in nascent chain folding (Kampinga and Craig 2010). Twelve out of 13 cytosolic J proteins work with the Ssa class of Hsp70s for carrying out diverse cellular functions (Sahi and Craig 2007). Although some overlap is possible, most of the J proteins are highly specialized in their function (Sahi and Craig 2007). Besides structural differences, J proteins vary significantly in their expression levels which might conceal the functional redundancy between J proteins.

Peroxisomes are ubiquitous organelles important for beta oxidation of fatty acids and degradation of hydrogen peroxide. Defects in peroxisomal protein import leads to developmental defects and syndromes (Smith and Aitchison 2013). Although cytosolic Hsp70s have been implicated in peroxisomal protein import in mammalian and plant cells (Crookes and Olsen 1998; Diefenbach and Kindl 2000; Walton et al. 1994) such information is still lacking in *S. cerevisiae*. In part, this could be due to the functional redundancy between the cytosolic Hsp70s, Ssa1–4 of *S. cerevisiae*. Djp1, a non-essential class II J protein, plays an important role in peroxisomal protein import in *S. cerevisiae* as cells lacking Djp1 mislocalize peroxisomally targeted GFP-PTS1 into the cytosol and show abnormal peroxisome morphology (Hettema et al. 1998). Peroxisomal targeting signal 1 (PTS1), with the sequence Ser/Ala/Cys, Lys/Arg/His, Leu/Met/Ile is found on most proteins destined to be imported into the peroxisomal matrix (Smith and Aitchison 2013). Additionally, Djp1 is specifically required for biogenesis of outer mitochondrial proteins Mim1 and Mim2 in *S. cerevisiae* (Papic et al. 2013).

Although Djp1 has a definitive role in peroxisomal protein import, its functionality as a bonafide J protein in this process has not been addressed. In this study, we accessed the importance of different domains of Djp1 in peroxisomal protein import functions. We asked if among all other cytosolic J proteins, Djp1 is specifically required for peroxisomal protein import in *S. cerevisiae*. Our results show that unlike most other J proteins, the J domain of Djp1 is only partially required for its functions. Instead, the C-terminal region of Djp1 was found to be absolutely critical. Furthermore, we show that

Caj1, another class II J protein of the *S. cerevisiae* cytosol, has a subtle and overlapping functions with Djp1 in this process. Additionally, moderate over-expression of Djp1 also resulted into mislocalization of GFP-PTS1 in a J domain independent manner. Put together, our work highlights the importance of regions outside the J domain in determining the functional specificity of Djp1 in *S. cerevisiae* cytosol and shows that genetic redundancy between Djp1 with Caj1 can be attributed to the lack of complete loss of function upon deletion of Djp1 J domain.

Materials and methods

Yeast strains and methods used in the study

Yeast strains used in this study belong to either W303 genetic background or BY4743 genetic background. Wild-type W303 (WT), *djp1*Δ::LEU2 and *caj1* Δ::LEU2 strains have been described previously (Sahi and Craig 2007). Different cytosolic J protein deletion strains, *djp1*Δ, *caj1*Δ, *xdj1*Δ, *ydj1*Δ, *apj1*Δ, *hlj1*Δ, *jjj1*Δ, *jjj2*Δ, *jjj3*Δ, and *swa2*Δ, along with the wild-type strain with BY4743 genetic background were obtained from Thermo Yeast Knockout Collection (Open Biosystem). *cwc23*Δ cells harboring a Cwc23ΔJ plasmid (Sahi et al. 2010) and *sis1*Δ strain (Lopez et al. 2003) with Sis1 expressed from a tetracycline repressible promoter (*Tet^R-SIS1*) were utilized to study the function of essential J proteins Cwc23 and Sis1. *djp1*Δ*caj1*Δ double deletion strain was generated to study the genetic interaction between Djp1 and Caj1 by mating *djp1*Δ::KanMX strain with *caj1* Δ::LEU2 strain. The resulting diploids were sporulated to get the haploid double deletion strain. Yeast strains were transformed with plasmids following standard LiAc/PEG-mediated protocol (Gietz and Schiestl 2007) and selected on appropriate amino acid drop-out plates.

Plasmid constructions

Open reading frames (ORFs) corresponding to the full length or amino acids 57–432 (ΔJ) and 1–282 (ΔC50) fragments of Djp1 were PCR amplified from genomic DNA of wild-type strain of *S. cerevisiae* and cloned into restriction sites *SpeI*/*EcoRI* in pRS414-*TEF1* and pRS414-*ADH* vectors (Mumberg et al. 1995). The genomic clone of Caj1 (−790 to +1651 bp) was cloned in pRS314 vector as a *SpeI*/*EcoRI* fragment. Point mutations were generated by using Quick change PCR (Stratagene, USA). All constructs were verified by restriction digestion and sequencing.

Microscopy

Yeast cells were grown to logarithmic phase, harvested by brief centrifugation at 13000 rpm. Cells were then washed with sterile

distilled water and visualized under fluorescence microscope (Zeiss, Apotome at Excitation/Emission ~361/497 nm) at 100X magnification.

Preparation of protein extracts and Western blot analysis

Total cellular protein was prepared by incubating yeast cells with 0.2 N NaOH for 10 min at room temperature. Following a brief centrifugation, the cell pellet was resuspended in 5X SDS sample buffer (125 mM Tris-HCl pH 6.8, 5% SDS, 0.004% Bromophenol Blue, 1.427 M (5%) β -mercaptoethanol, 20% glycerol) and finally boiled for 10 min. Appropriate amount of the total lysate was then resolved on 10% SDS-PAGE and electro-blotted onto PVDF membrane by using a wet transfer apparatus (BioRad, USA). The membrane was probed with 1:1000 dilution of anti-Djp1 Rabbit polyclonal antibody and 1:20,000 dilution of HRP-linked anti-rabbit secondary antibody (Genei, India). The blot was placed in 6.5 ml each of ECL Developing Solutions A (100 mM Tris-HCl (pH 8.5) and 7 μ l H₂O₂) and B (100 mM Tris-HCl (pH 8.5), 60 μ l of 250 mM luminol and 53 μ l of 90 mM *p*-coumaric acid). Densitometric quantification of the bands was done by ImageJ software.

Bioinformatics analysis

J domain sequences of all cytosolic J proteins of *S. cerevisiae* were compared with that of Djp1 by aligning their sequences in EMBOSS Pairwise alignment (http://www.ebi.ac.uk/Tools/psa/emboss_needle/). Domain organization of Djp1 and Caj1 was predicted using SMART Database (Schultz et al. 1998).

Results and discussion

J domain of Djp1 is partially dispensable for peroxisomal protein import function

Djp1 has an N-terminal J domain followed by a loosely defined glycine-rich region and an uncharacterized C-terminal DnaJ-X type putative client binding domain as predicted by SMART database (Fig. 1a). Out of all other cytosolic J proteins, only Caj1 shares a similar domain organization with Djp1 (Sahi and Craig 2007). The J domain fragment of Djp1 rescues the temperature sensitivity of cells lacking Ydj1, an abundant and multifunctional J protein of the yeast cytosol, suggesting that Djp1 has a functional J domain that can stimulate the ATPase activity of the cytosolic Hsp70, Ssa (Sahi and Craig 2007). We asked if the J domain is important for peroxisomal protein import function of Djp1. The complete ORF of Djp1 was cloned under promoters of varying strengths; *ADH*, *TEF1*, and *GPD* in single copy number, centromeric (*CEN*) vectors (Mumberg et al. 1995) and tested in yeast. No colonies were obtained when wild-type

yeast cells were transformed with *CEN-GPD-Djp1* plasmid (data not shown). Colonies were obtained for yeast cells harboring *CEN-TEF1-Djp1* and *CEN-ADH-Djp1*. While cells expressing Djp1 from a *CEN-ADH* plasmid grew like wild type, *CEN-TEF1-Djp1* harboring cells grew very slowly at all the temperatures tested (Fig. 1b). Next, we estimated the Djp1 protein levels in these cells. Djp1 was expressed approximately two and four fold more than the endogenous levels from the *CEN-ADH* and *CEN-TEF1* plasmid, respectively (Fig. 1c). This indicates that wild-type yeast cells are sensitive to subtle changes in Djp1 levels. Djp1 over-expression has been previously shown to be toxic for yeast cells; however, the reasons for Djp1's toxicity are not clear (Makanae et al. 2013; Sahi and Craig 2007). It is possible that at higher expression levels, Djp1 indulges into non-productive interactions with other proteins resulting into toxicity. Since *CEN-ADH-Djp1* grew like wild type, this construct was used for testing the importance of different domains of Djp1 in peroxisome protein import function in *S. cerevisiae*.

Loss of Djp1 results into mislocalization of GFP-PTS1 into the cytoplasm (Hettema et al. 1998). GFP-PTS1 plasmid (gift from Dr. Ewald Hettema, University of Sheffield, UK) transformed into wild-type cells showed punctate fluorescence indicative of proper peroxisomal protein localization (Fig. 1d). Almost 80% of the wild-type cells showed punctate fluorescence (Fig. 1e). As expected, only 20% of *djp1* Δ cells showed punctate fluorescence indicating a defect in localization of GFP-PTS1 (Fig. 1d). Moreover, this defect was restored to wild-type condition when *CEN-ADH-Djp1* plasmid was introduced into *djp1* Δ cells, suggesting that this plasmid was fully functional (Fig. 1d, e). Next, we asked if the J domain of Djp1 is important for peroxisomal protein import. *CEN-ADH-Djp1H34Q* carrying a mutation in the critical HPD tripeptide motif, essential for J domain function was transformed into *djp1* Δ cells harboring GFP-PTS1. Surprisingly, these cells showed almost 50% of cells with punctate fluorescence (Fig. 1d, e). To rule out any residual J domain function in Djp1H34Q and to more rigorously test the importance of Djp1's J domain, a *CEN-ADH-Djp1* Δ J, construct containing an N-terminal deletion of 56 amino acids encompassing almost the complete J domain of Djp1 was generated and tested in *djp1* Δ cells harboring GFP-PTS1. This construct showed defects identical to *CEN-ADH-Djp1H34Q* (Fig. 1d, e), proving that the J domain of Djp1 is only partially required for peroxisomal protein import in *S. cerevisiae*. Normally, J domains are critical for all J protein functions and a non-functional J domain phenocopies the null phenotype of a particular J protein (Kampinga and Craig 2010). Given the partial defect of peroxisomal protein import in Djp1 Δ J mutant and evidence supporting the involvement of cytosolic Hsp70 in peroxisomal import process in mammalian and plant systems (Diefenbach and Kindl 2000), it is very much likely that Djp1 cooperates with cytosolic Hsp70, Ssa for proper peroxisomal protein import in budding yeast, although this association is not absolutely

Fig. 1 J domain of Djp1 is partially dispensable for its peroxisomal protein import function. **a** Domain organization of Djp1. **b** Tenfold serial dilution of wild-type yeast cells harboring either an empty vector (–), *CEN-ADH-Djp1* or *CEN-TEF1-Djp1* were spotted on selective media and incubated at indicated temperatures for 2 days. **c** Total protein lysate prepared from *djp1Δ* cells (Sahi and Craig 2007) expressing empty vector (*djp1Δ*), wild-type cells harboring either empty vector (WT) or *CEN-ADH-Djp1* [loading 0.5 OD₆₀₀ equivalent cells], and *CEN-TEF1-Djp1* [loading 0.25 OD₆₀₀ equivalent cells] were resolved by SDS-PAGE, electro-blotted on PVDF membrane and probed with anti-Djp1 antibody (Djp1) (gift from Dr. B. Distel, Academic Medical Center, The Netherlands). A consistently appearing nonspecific band was used as a loading control (**c**). **d** WT or *djp1Δ* cells harboring either empty *CEN-ADH* vector (–) or full-length Djp1, Djp1H34Q, Djp1_{57–432} (ΔJ) expressed from a *CEN-ADH* based plasmid were transformed with GFP-PTS1. For visualizing GFP-PTS1, cells were processed as described previously (Hettema et al. 1998). Cells were visualized under fluorescence microscope at excitation/emission 470 nm/590 nm) (Zeiss, Apotome) at 100X. The scale bars indicate 1 μ m. **e** Bar graph was created using ‘GraphPad Prism v. 5.00’ showing quantitative analysis for WT and *djp1Δ* cells with GFP-PTS1 harboring either wt Djp1 or its mutants. The experiments were done in triplicates and each time approximately 200 cells either having completely diffuse or punctate GFP fluorescence were manually counted using ImageJ software. Variations in the triplicates were depicted as error bars. **f** Expression level of Djp1 and its mutants wild-type (WT) or *djp1Δ* cells harboring either Djp1, ΔJ , H34Q expressed from a *CEN-ADH*-based plasmid was estimated by western analysis as mentioned above. **g** *djp1Δ* cells harboring either *CEN-ADH-Djp1* or *CEN-ADH-Djp1*_{1–282} ($\Delta C50$) plasmid was transformed with GFP-PTS1 and visualized under microscope as mentioned above. **h** Bar graph showing quantitative analysis for *djp1Δ* cells with GFP-PTS1 harboring either empty vector (–), Djp1 or Djp1($\Delta C50$) as mentioned above. **i** Western analysis of total protein lysate prepared from *djp1Δ* cells harboring either *CEN-ADH-Djp1* or *CEN-ADH-Djp1* ($\Delta C50$) plasmid was done as mentioned above

critical. Although interesting, this scenario is not that unique. Several J proteins have been shown to have J domains that are either partially or completely dispensable for their function, in these cases, regions other than the signature J domain are crucial for J protein functionality (Ajit Tamadaddi and Sahi 2016).

To show that the partial import defect exhibited by J domain mutants of Djp1 was not because of reduced or non-expression of mutant proteins, we checked their expression levels. Wild-type Djp1, Djp1H34Q, and Djp1 ΔJ were expressed at comparable levels, suggesting that defects in peroxisomal protein import function observed in J domain mutants were not because of reduced protein levels (Fig. 1f). Then, we asked if the C-terminal region of Djp1 had some role in peroxisomal import function of Djp1. *djp1Δ* GFP-PTS1 cells expressing *CEN-ADH-Djp1ΔC50*, carrying a truncation of last 50 amino acids, appeared like *djp1Δ* cells (Fig. 1g, h). Western analysis showed that Djp1 $\Delta C50$ was expressed at levels similar to *CEN-ADH-Djp1* after normalizing against the loading control (Fig. 1i), suggesting that the C-terminal domain of Djp1 is more important for Djp1’s function than its J domain, which was of moderate importance in peroxisomal protein import. How does the C-terminal domain of Djp1 contribute to peroxisomal protein import is not clear.

We hypothesize that the C-terminal domain of Djp1 determines the specificity of functions by its ability to interact with specific proteins or complexes that is crucial for proper peroxisomal protein import.

Caj1 has overlapping functions with Djp1

Apart from Djp1, there are 11 more J proteins that reside in the same sub-cellular compartment and potentially work with the same multifunctional Hsp70, Ssa (Sahi and Craig 2007). Among all other J proteins, Djp1 is uniquely required for the biogenesis of outer mitochondrial protein Mim1 in *S. cerevisiae* (Papic et al. 2013). We thus investigated if Djp1 is the only J protein involved in peroxisomal protein import. We checked the peroxisomal localization of GFP-PTS1 in yeast cells lacking Caj1, Xdj1, Ydj1, Apj1, Hlj1, Jjj1, Jjj2, Jjj3, and Swa2. Cwc23 and Sis1 are essential; therefore, we used a system that had *SIS1* under the tetracycline repressible promoter (*Tet^R*) that allows repression of Sis1 expression upon application of doxycycline. For Cwc23, we used a *Cwc23ΔJ* construct, described previously that lacks the J domain of Cwc23 but has no effect on viability. Compared to all other J protein deletion strains, peroxisomal import defects observed in *caj1Δ* was most significant. About 60% of *caj1Δ* cells displayed a completely diffuse GFP-PTS1 fluorescence as compared to wild-type cells in which such cells represented only 20% of the total population (Fig. 2a, b). Caj1 is a class II J protein and has domain organization similar to Djp1. Both Djp1 and Caj1 carry a DnaJ-X type domain toward their C-terminal, with 30.8% identity and 50.7% similarity. The C-terminal region of Djp1 and Caj1 is distinct from the client binding domains present in other class I and class II J proteins (Kampinga and Craig 2010). Furthermore, as compared to other J proteins, the J domains of Djp1 and Caj1 are also similar to each other with 78% similarity and 59.3% identity (Supplementary Table 1). It is possible that Caj1 has some role in peroxisomal protein import and can functionally overlap with Djp1. To further investigate if Caj1 shows a synthetic genetic interaction with Djp1 in peroxisomal protein import, *djp1Δcaj1Δ* double deletion strain was generated and analyzed for GFP-PTS1 localization. Cells lacking both Caj1 and Djp1 showed a much more severe defect in the peroxisomal targeting of GFP-PTS1. The fluorescence for GFP-PTS1 was completely diffuse throughout the cytoplasm and no punctate structures were visible (Fig. 2c, d). Next, we addressed the possibility that J domain of Caj1 is compensating for the partial loss of J domain function in Djp1. While the severe defects in the localization of GFP-PTS1 was rescued by a plasmid expressing wild-type Caj1, Caj1H34Q carrying a critical mutation in the J domain of Caj1 was null as these cells were indistinguishable from *djp1Δcaj1Δ* (Fig. 2c, d). This result clearly suggests that Caj1 has overlapping functions with Djp1 in peroxisomal protein import and in the absence

Fig. 2 Caj1 has overlapping functions with Djp1. **a** WT yeast cells, deletion strains of ten of the non-essential cytosolic J proteins (Thermo yeast knockout collection), *sis1* Δ strain (Lopez et al. 2003) with *Sis1* expressed from a tetracycline repressible promoter (*Tet^R SIS1*) in the presence of 0.25 μ g/ml of doxycycline (*sis1* Δ^*), and *cwc23* Δ cells harboring a *Cwc23* Δ construct (*cwc23* Δ^*) (Sahi et al. 2010) all expressing GFP-PTS1 were inoculated and visualized as mentioned earlier. **b** Bar graph showing quantitative analysis of cells having punctate fluorescence for GFP-PTS1 as mentioned in Fig. 1. **c** WT, *djp1* Δ , *caj1* Δ , *djp1* Δ *caj1* Δ yeast cells harboring either empty vector (–), wild-type Caj1 (Caj1), or Caj1H34Q transformed with GFP-PTS1 and visualized under microscope as mentioned in Fig. 1. **d** Bar graph showing quantitative analysis of cells having punctate fluorescence for GFP-PTS1 as mentioned previously

of Djp1, the J domain of Caj1 becomes important for GFP-PTS1 localization in *S. cerevisiae*. Cwc23 is the only other cytosolic J protein that has a dispensable J domain; however, in this case, a compensatory effect of other J proteins was not found (Sahi et al. 2010). Functional overlap between cytosolic J proteins in budding yeast is not new. The abundant class I J protein Ydj1 has overlapping functions with the essential class II J protein Sis1. Ydj1 and Sis1 also work with the Ssa class of Hsp70s; however, in this case, the C-terminal client binding

domain of either of the two is required for viability of yeast cells (Johnson and Craig 2001).

Dosage imbalance of Djp1 causes peroxisomal protein import defects

Genes that exhibit dominant negative phenotypes when their expression levels are altered are referred to as dosage-sensitive genes (Papp et al. 2003). Besides Djp1, two more J proteins of

Fig. 3 Dosage imbalance of Djp1 causes peroxisomal protein import defects. **a** Tenfold serial dilution of wild-type yeast cells harboring either empty vector (–), or *CEN-TEF1*-Djp1, *CEN-TEF1*-Djp1(ΔJ), were spotted on selective media at indicated temperature. **b** These cells were transformed with GFP-PTS1 and visualized under microscope as

mentioned previously. **c** Total protein lysate extracted from wild-type (WT) expressing either empty vector (–) or *CEN-TEF1*-Djp1 (Djp1), *CEN-TEF1*-Djp1 (ΔJ) representing the lower ΔJ^* truncated protein was resolved by SDS-PAGE and processed for western analysis as mentioned previously

the yeast cytosol are dosage sensitive, namely, Caj1 and Xdj1 (Makanae et al. 2013). While toxicity of Xdj1 which has been attributed to mitochondrial import defects requires a functional J domain (Sahi et al. 2013), the molecular basis of Caj1 toxicity is not understood. Moderate over expression of Djp1 resulted into growth defects at 30 °C (Fig. 3a). However, unlike Xdj1, this toxicity was independent of Djp1's Hsp70-dependent functions as an N-terminal 56 amino acid truncation; almost lacking the complete J domain was equally toxic in wild-type yeast cells (Fig. 3a). One hallmark of dosage-sensitive genes is that often their deletion and over-expression phenotypes are the same (Sopko et al. 2006). So we tested if over-expression of Djp1 also resulted into defects in peroxisomal protein import. GFP-PTS1 plasmid was transformed into wild-type yeast cells harboring a *CEN-TEF1*-Djp1 construct and visualized under a microscope. Cells over-expressing Djp1 showed completely diffuse GFP fluorescence suggestive of defects in peroxisomal protein import (Fig. 3b). Next, we accessed if the J domain of Djp1 had some role in peroxisomal protein import defects observed upon over-expression. So the GFP-PTS1 plasmid was transformed in wild-type yeast cells expressing a *CEN-TEF1*-Djp1 ΔJ construct and visualized under the microscope. These cells showed completely diffuse GFP fluorescence exactly like *CEN-TEF1*-Djp1 (Fig. 3b). Western analysis followed by densitometric quantization showed that approximately four fold of Djp1 over its endogenous levels was toxic to the yeast cells (Fig. 3c). These data indicate that dosage imbalance caused by either deletion or over-expression of Djp1 might be resulting into nonproductive protein complexes resulting into peroxisomal protein import defects. Further work is required to experimentally validate this assumption.

After being translated on cytosolic ribosomes, peroxisomal proteins are assisted by molecular chaperones for their proper import into different organelles. Although the chaperone requirements for peroxisomal protein import might be distinct from that of mitochondria, Djp1, which is also implicated in biogenesis of outer mitochondrial proteins, is a common chaperone important for both peroxisomal and mitochondrial function. However, unlike its highly specialized role in mitochondrial import, Djp1

shows functional redundancy with another cytosolic J protein Caj1 in peroxisomal import function. In summary, results presented in this paper show for the first time that under conditions where multiple J proteins work with a single Hsp70, dispensability of the J domain in one J protein can be compensated by other J protein(s) that also resides within the same sub-cellular compartment.

Acknowledgments We thank Professor Elizabeth Craig (UW-Madison, USA), Dr. B. Distel (Academic Medical Center, The Netherlands), and Dr. Ewald Hettema (University of Sheffield, UK) for yeast strains, plasmids, and antibodies. ND and YT thank DBT and CSIR, New Delhi for fellowships respectively, AKV thanks MHRD for GATE fellowship, PT and SS thank DST for INSPIRE Scholarship. This work was supported by funds from the Department of Biotechnology (BT/PR6885/GBD/27/488/2012 and BT/PR12149/BRB/10/1348/2014), Department of Science and Technology (EMR/2015/001213), and intramural funds from IISER Bhopal.

References

- Ajit Tamadaddi C, Sahi C (2016) J domain independent functions of J proteins cell stress chaperones doi:10.1007/s12192-016-0697-1
- Craig EA, Huang P, Aron R, Andrew A (2006) The diverse roles of J-proteins, the obligate Hsp70 co-chaperone. *Rev Physiol Biochem Pharmacol* 156:1–21
- Crookes WJ, Olsen LJ (1998) The effects of chaperones and the influence of protein assembly on peroxisomal protein import. *J Biol Chem* 273:17236–17242
- Diefenbach J, Kindl H (2000) The membrane-bound DnaJ protein located at the cytosolic site of glyoxysomes specifically binds the cytosolic isoform 1 of Hsp70 but not other Hsp70 species. *Eur J Biochem* 267: 746–754
- Gietz RD, Schiestl RH (2007) Frozen competent yeast cells that can be transformed with high efficiency using the LiAc/SS carrier DNA/PEG method. *Nat Protoc* 2:1–4. doi:10.1038/nprot.2007.17
- Hettema EH, Ruigrok CC, Koerkamp MG, van den Berg M, Tabak HF, Distel B, Braakman I (1998) The cytosolic DnaJ-like protein djp1p is involved specifically in peroxisomal protein import. *J Cell Biol* 142:421–434
- Huang P, Gautschi M, Walter W, Rospert S, Craig EA (2005) The Hsp70 Ssz1 modulates the function of the ribosome-associated J-protein Zuo1. *Nat Struct Mol Biol* 12:497–504. doi:10.1038/nsmb942

- Johnson JL, Craig EA (2001) An essential role for the substrate-binding region of Hsp40s in *Saccharomyces cerevisiae*. *J Cell Biol* 152:851–856
- Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* 11:579–592. doi:[10.1038/nrm2941](https://doi.org/10.1038/nrm2941)
- Kampinga HH et al (2009) Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress Chaperones* 14:105–111
- Lopez N, Aron R, Craig EA (2003) Specificity of class II Hsp40 Sis1 in maintenance of yeast prion [RNQ+]. *Mol Biol Cell* 14:1172–1181. doi:[10.1091/mbc.E02-09-0593](https://doi.org/10.1091/mbc.E02-09-0593)
- Makanae K, Kintaka R, Makino T, Kitano H, Moriya H (2013) Identification of dosage-sensitive genes in *Saccharomyces cerevisiae* using the genetic tug-of-war method. *Genome Res* 23:300–311. doi:[10.1101/gr.146662.112](https://doi.org/10.1101/gr.146662.112)
- Mayer MP (2013) Hsp70 chaperone dynamics and molecular mechanism. *Trends Biochem Sci* 38:507–514. doi:[10.1016/j.tibs.2013.08.001](https://doi.org/10.1016/j.tibs.2013.08.001)
- Mayer MP, Bukau B (2005) Hsp70 chaperones: cellular functions and molecular mechanism. *Cell Mol Life Sci* 62:670–684. doi:[10.1007/s00018-004-4464-6](https://doi.org/10.1007/s00018-004-4464-6)
- Mumberg D, Muller R, Funk M (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* 156:119–122
- Papic D et al (2013) The role of Djpl in import of the mitochondrial protein Mim1 demonstrates specificity between a cochaperone and its substrate protein. *Mol Cell Biol* 33:4083–4094. doi:[10.1128/MCB.00227-13](https://doi.org/10.1128/MCB.00227-13)
- Papp B, Pal C, Hurst LD (2003) Dosage sensitivity and the evolution of gene families in yeast. *Nature* 424:194–197. doi:[10.1038/nature01771](https://doi.org/10.1038/nature01771)
- Sahi C, Craig EA (2007) Network of general and specialty J protein chaperones of the yeast cytosol. *Proc Natl Acad Sci U S A* 104:7163–7168. doi:[10.1073/pnas.0702357104](https://doi.org/10.1073/pnas.0702357104)
- Sahi C, Lee T, Inada M, Pleiss JA, Craig EA (2010) Cwc23, an essential J protein critical for pre-mRNA splicing with a dispensable J domain. *Mol Cell Biol* 30:33–42. doi:[10.1128/MCB.00842-09](https://doi.org/10.1128/MCB.00842-09)
- Sahi C, Kominek J, Ziegelhoffer T, Yu HY, Baranowski M, Marszalek J, Craig EA (2013) Sequential duplications of an ancient member of the DnaJ-family expanded the functional chaperone network in the eukaryotic cytosol. *Mol Biol Evol* 30:985–998. doi:[10.1093/molbev/mst008](https://doi.org/10.1093/molbev/mst008)
- Saibil H (2013) Chaperone machines for protein folding, unfolding and disaggregation. *Nat Rev Mol Cell Biol* 14:630–642. doi:[10.1038/nrm3658](https://doi.org/10.1038/nrm3658)
- Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. *Proc Natl Acad Sci U S A* 95:5857–5864
- Smith JJ, Aitchison JD (2013) Peroxisomes take shape. *Nat Rev Mol Cell Biol* 14:803–817. doi:[10.1038/nrm3700](https://doi.org/10.1038/nrm3700)
- Sopko R et al (2006) Mapping pathways and phenotypes by systematic gene overexpression. *Mol Cell* 21:319–330. doi:[10.1016/j.molcel.2005.12.011](https://doi.org/10.1016/j.molcel.2005.12.011)
- Walsh P, Bursac D, Law YC, Cyr D, Lithgow T (2004) The J-protein family: modulating protein assembly, disassembly and translocation. *EMBO Rep* 5:567–571. doi:[10.1038/sj.embor.7400172](https://doi.org/10.1038/sj.embor.7400172)
- Walton PA, Wendland M, Subramani S, Rachubinski RA, Welch WJ (1994) Involvement of 70-kD heat-shock proteins in peroxisomal import. *J Cell Biol* 125:1037–1046