Sulphydryl Oxidation, Not Disulfide Isomerization, Is the Principal Function of Protein Disulfide Isomerase in Yeast *Saccharomyces cerevisiae*

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Protein disulfide isomerase (PDI) is an essential protein folding assistant of the eukaryotic endoplasmic reticulum that catalyzes both the formation of disulfides during protein folding (oxidase activity) and the isomerization of disulfides that may form incorrectly (isomerase activity). Catalysis of thiol-disulfide exchange by PDI is required for cell viability in *Saccharomyces cerevisiae*, but there has been some uncertainty as to whether the essential role of PDI in the cell is oxidase or isomerase. We have studied the ability of PDI constructs with high oxidase activity and very low isomerase activity to complement the chromosomal deletion of *PDI1* in *S. cerevisiae*. A single catalytic domain of yeast PDI (PDIα) has 50% of the oxidase activity but only 5% of the isomerase activity of wild-type PDI in vitro. Titrating the expression of PDI using the inducible/repressible GAL1–10 promoter shows that the amount of wild-type PDI protein needed to sustain a normal growth rate is 60% or more of the amount normally expressed from the PDI1 chromosomal location. A single catalytic domain (PDIα) is needed in molar amounts that are approximately twice as high as those required for wild-type PDI, which contains two catalytic domains. This comparison suggests that high (>60%) PDI oxidase activity is critical to yeast growth and viability, whereas less than 6% of its isomerase activity is needed.

Fast and efficient protein folding is essential for cell viability. Although spontaneous folding is adequate for some proteins, many disulfide-containing proteins require help from specific folding assistants to form the correct disulfide bonds and to prevent the formation of non-native disulfides that might stabilize misfolded structures (1).

Protein disulfide isomerase (PDI)

Protein disulfide isomerase (PDI) is an essential ER folding assistant present in all eukaryotes that facilitates the folding of disulfide-containing proteins (2). PDI catalysis of oxidative protein folding consists of two types of reactions, oxidation of free sulphydryls into disulfides (oxidase activity) and rearrangement of incorrectly formed disulfides (isomerase activity) (1).

These two activities are performed by each of the two active sites of PDI. Each active site contains two cysteines that cycle between oxidized (disulfide) and reduced (sulphydryl) forms during the PDI enzymatic cycle. The catalytic sites (CGHC) are found in two independent thioredoxin homology domains located near the N (a domain) and C termini (a' domain) of the molecule.

In the yeast *Saccharomyces cerevisiae*, deletion of the *PDI1* gene is lethal (2). A mutant PDI in which all the active site cysteines have been replaced with serines (NSGHS–CSGHS) is still an active chaperone and has other, secondary activities of PDI in vitro, but it is incapable of complementing the *PDI1* deletion. Catalysis of thiol-disulfide exchange is the required function of PDI in yeast (3). Although catalysis of thiol-disulfide exchange is essential, there has been some uncertainty about which part of this catalytic activity represents the essential *in vivo* function of PDI. PDI mutants with defects in the oxidase activity can complement the *PDI1* deletion (3, 4), but surprisingly, expression of a single PDI catalytic domain (a or a'), which is defective in catalyzing isomerization, can also rescue the *PDI1* deletion (5).

There are two problems in deciding which one of the PDI catalytic activities represents the essential function. The first problem is the extent to which the two activities can be separated in a specific PDI construct. Oxidase-deficient PDI mutants have active sites with only one of the two cysteines. The NCGHS–CNSGHS mutant has 16% of the oxidase activity of wild-type PDI and approximately 40% of the isomerase activity when assayed *in vitro* using scrambled RNase as the substrate (6). In this mutant, the ratio of oxidase/isomerase activity is 1:2.5. A single catalytic domain of PDI (a or a') has approximately 50% of the oxidase activity of wild-type PDI *in vitro* but only 3–5% of the isomerase activity (5). Here, the ratio of oxidase to isomerase activity is greater than 10:1, which is a much more effective separation of activities than has previously been available. The second problem is the absence of expression level measurements and the use of different PDI proteins (yeast and mammalian) during the complementation experiments (3–5).

We have used the large separation of activities afforded by a catalytic domain (a') of yeast PDI in combination with the inducible/repressible GAL1–10 promoter (7) to titrate the levels of oxidase and isomerase activities that are required to maintain growth, viability, and *in vivo* isomerase activity. The experiments show that *S. cerevisiae* growth rate and viability require levels near those of wild-type oxidase activity, but less than 6% of the isomerase activity is sufficient to maintain wild-type growth rates. Measurements of the *in vivo* isomerase...
activity show that significant defects in isomerization catalysis are tolerated well by the yeast ER.

EXPERIMENTAL PROCEDURES

Materials and Reagents—5-Fluoroorotic acid was obtained from RPI International. Cell culture media such as agar, yeast extract, and LB were from Invitrogen. Amino acids, dithiothreitol, and other reagents were from Sigma. Restriction enzymes and PCR reagents were from Invitrogen and Promega. Anti-His, tag monoclonal antibodies and 5% casein blocking solution were purchased from Novagen. Anti-rat PDI polyclonal antisera was produced in our laboratory, and anti-yeast PDI antisera was kindly provided by Jakob Winther (Carlsberg Laboratories). Nitrocellulose membranes were obtained from Amersham Biosciences, and West Pico Supersignal ECL development reagents were from Pierce. Anti-carboxypeptidase Y (CPY) polyclonal antisera was a gift from Jakob Winther (Carlsberg Laboratories).

Yeast Strains and Plasmids—The yeast strain carrying the PDI1 gene deletion (YPH274, Δpdi1) was obtained from Ronald Raines (University of Wisconsin, Madison, WI). All yeast strains were haploids with the appropriate genes disrupted (as described in Refs. 2 and 4). The Escherichia coli strain used for DNA manipulations was XL1Blue from Stratagene. Plasmids used for complementation were constructed on the basis of the vectors pRS414 and pRS424 (8). The plasmid pRS414 is a centromeric yeast E. coli shuttle vector with a TRP1 selectable marker; pRS424 is identical to pRS414, except that it has a multicopy 2μ yeast origin. The plasmid pRS414 was digested with KpnI and SalI, and a 780-bp restriction fragment containing GAL1–10 promoter was inserted. This was followed by digestion with BclI and NotI and the insertion of a 128-bp fragment containing the yeast PDI ER-targeting sequence, a sequence that encodes an N-terminal His, tag, a multcloning site with BamHI, SalI, SmaI, and BgII restriction sites, and an HDEL ER retention sequence followed by a stop codon. To provide a low copy expression vector driven by the PDI1 promoter, pRS414 was digested with KpnI and EcoRI, and an 885-bp fragment encoding PDI1 promoter was inserted. This was followed by digestion with EcoRI and NotI and the insertion of the 128-bp expression fragment providing the same signal or targeting sequences described above. The domain boundaries of the rat protein were described previously (5).

The catalytic domains of the yeast protein were identified by modeling the sequence of the yeast a and a′ domains into thioerodoxin family structures with 3D-FSSM software (9). The protein or domain coding sequences were inserted into the BamHI-BglII-digested plasmid. Construct fragments were generated by PCR to include a 5′ BglII site and a BamHI 3′ site (for yeast PDI BamHI site at both 5′ and 3′ ends) using the following primers synthesized by Sigma Genosys: for yeast PDI, GAG GGA TCT CCT GAA GAC TAC GCT GTC GAT and CTC GGA TCC AAT GGC TTC TTC TTC GGC ACAA; for yeast PDIa′ domain, GAG AGA CAG GGA TTT ATT GAC TCA CTG TCT CCT CTT TCT GGA TTT ATT and for rat PDIa′ domain, GAG AGA TCT GGG GCC CTG GTC GTT AAG and CTC GGA TCC CCT GGC ACC GCT C. The sequences of the entire insert were verified by DNA sequencing.

Complementation and Yeast Cell Cultures—Routine yeast complementation and culture conditions were basically as described by Guthrie and Fink (14). Complementation experiments were performed using a plasmid-shuffling technique essentially as described in Laboissiere et al. (4). Briefly, the Δpdi1 (YPH274) strain contains a deletion of the entire coding sequence of PDI1 by the insertion of a HIS marker. The strain is maintained as viable with a plasmid (URA3) expressing yeast PDI. After transformation, cells are plated on His-Ura-Trp agar plates to select transformants missing the chromosomal PDI1 gene. After several days, colonies are replicated on His-Ura-Trp plates with galactose to induce the expression of constructs under the control of GAL1–10 promoter. Resulting colonies are replicated on agar plates that contain 5-fluoroorotic acid to force a loss of the URA3 plasmid expressing yeast PDI. Aliquots of cells for storage were pre- served in glycerol at –80 °C.

Growth Rate, Viability, and Protein Level Measurements—Growth rates were measured in synthetic complete (SC) liquid medium with various concentrations of glucose and galactose. The cultures were incubated at 30 °C on a rotating wheel, and an optical density of A600 nm was measured at fixed intervals of time. Cell cultures at an optical density higher than 1.0 were diluted to ensure the linear relationship between optical density and cell number.

Suppression of Expression—The expression of PDI variants was controlled by induction or suppression of the GAL1–10 promoter. Yeast complemented with specific PDI constructs were grown in SC medium to an A600 nm of 1–1.5. Cells were centrifuged, washed with SC medium, and resuspended in fresh SC medium containing glucose. The optical density was measured, and samples of cells were taken at various times. Cells were centrifuged, disrupted with glass beads in 20% trichloroacetic acid, and washed with acetone, and the insoluble material was solubilized in buffer containing SDS. After SDS-PAGE, the proteins were transferred to nitrocellulose membranes. The PDI bands were detected with a monoclonal anti-His tag antibody followed by a horseradish peroxidase conjugated to horseradish peroxidase and developed with ECL. Autoradiograms were analyzed using Scion Image software. Exposure times and band intensities were chosen to achieve a linear correlation between band intensity and protein concentration. At the same time points, aliquots containing 1000 cells were spread on SC medium agarose plates containing galactose to evaluate the viability of yeast. Plates were incubated at 30 °C for 26–48 h, and the number of colonies/plate was counted.

Carboxypeptidase Y Pulse-Chase Labeling and Immunoprecipitation—Immunoprecipitation was performed as described previously (10). After overnight culturing in SC medium, cells were isolated, resuspended in synthetic medium without methionine, cysteine, and sulfate – 0.4, and grown for 8 h. Cells (20 OD units) were resus- pended in fresh medium containing [35S]cysteine and methionine (40 μCi/OD units). After a 15-min labeling period, 106 cells were chased with 100X cysteine, methionine, and sulfate. Cells were mixed with NaN3 and NaF to final concentrations of 5 mM each and placed on ice to stop the labeling process. Cells were pelleted and disrupted by vortexing with glass beads in 20% trichloroacetic acid. The pellet was resus- pended in immunoprecipitation buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, 0.2% SDS, pH 7.4), vortexed, and boiled for 5 min to resolubilize the precipitated proteins. Pansorbin was added, and the tubes were incubated with rotation for 20 min. After centrifugation, the supernatant was diluted (5-fold) with IP dilution buffer (60 mM Tris-HCl, 190 mM NaCl, 1.25% Triton X-100, pH 7.4), and the CPY was immunoprecipitated with anti-CPY antibody. The immunoprecipitated protein was separated using 4–20% SDS-PAGE and transferred to nitrocellulose membranes. The labeled proteins were detected using a phosphor screen from Amersham Biosciences (24–36 h exposure). Screens were scanned using a StormScan scanner.

RESULTS

The coding regions for the various domains of rat and yeast PDI were individually inserted into either a high copy (2μ) plasmid under the control of the inducible/repressible GAL1–10 promoter or into a low copy plasmid (cen) under the control of the normal yeast PDI1 promoter. The expression vector, containing a TRP1 selectable marker, also added the yeast signal sequence, an N-terminal His, tag, and a C-terminal ER retention signal (HDEL). The plasmids were introduced using a plasmid-shuffling protocol into a yeast strain, YPH274, containing a full deletion of the PDI1 gene (Fig. 1). In the presence of galactose to induce expression and on a high copy plasmid, yeast PDI and its a and a′ domains, like the a and a′ domains of the rat protein (5), support growth (Table I); however, strains transformed with vectors containing the non-catalytic domains (b or b′) or a control vector with no insert are not viable. When expression of the wild-type PDI and catalytic domains of yeast or rat PDI is driven by the normal PDI1 promoter from a low copy (cen) plasmid, only wild-type rat PDI, wild-type yeast PDI, and the yeast a′ domain will complement the pdi1A mutation.

Because only the wild-type yeast and rat proteins and the yeast PDIa′ domain support growth under the control of the PDI1 promoter, these proteins were examined further for the effects of variable expression levels on the growth and viability of yeast and their ability to support in vivo isomerization activity. The GAL1–10 promoter is inducible by galactose but repressible by glucose, which provides the opportunity to te- trate the expression of PDI or its individual catalytic domains.
Quantitative Western blots using an anti-yeast PDI antibody were used to compare the expression levels of wild-type yeast PDI from its chromosomal location to the expression levels of yeast PDI from a fully induced GAL1–10 promoter (Fig. 2). This comparison using the yeast antibody provides a direct measure of the expression levels provided by the strong GAL1–10 promoter relative to that of the PDI promoter. The expression driven by the fully induced GAL1–10 promoter on a low copy (cen) plasmid produces PDI at a level that is 7.8 times higher than the normal expression level of PDI in wild-type yeast (Fig. 2). To compare the expression levels of the various PDI constructs, the expression levels of wild-type yeast PDI and PDiA were also compared using Western blots with an antibody to the common His6 tag. The expression levels of all the species are comparable when each is driven by the GAL1–10 promoter (Fig. 3).

When yeast strains expressing rat or wild-type PDI or the a’ catalytic domains are transferred from galactose to glucose media to suppress expression from the GAL1–10 promoter (7), the PDI concentrations decrease because of protein degradation and dilution through cell division. As the protein levels fall (Fig. 3), the cells expressing yeast wild-type PDI continue to grow, although the growth rate becomes quite slow. Cells expressing the yeast PDiA domain or wild-type rat PDI, however, grow progressively more slowly after transfer to glucose and finally stop doubling after undergoing a few divisions (Table II).

The doubling time, relative level of PDI protein, and viability were all measured at various times after suppressing PDI expression. Protein levels were estimated by quantitative Western blotting using an anti-His6 antibody (Fig. 3). The response of the antibody to an equal molar amount of each protein (based on sequence molecular weight) is comparable. When fully suppressed, the concentration of wild-type yeast PDI drops from 7.8 times the normal level of yeast PDI to ~60% of the normal level (Fig. 4A). At this level of Pdi1p, the cells continue to divide, albeit at a slow rate. By contrast, significant overexpression of yeast PDiA’ from the fully induced GAL1–10 promoter causes a slow growth phenotype (Fig. 4B) that is eliminated at lower expression levels. For PDiA’ after suppression with glucose, the growth rate initially increases then decreases as the amount of protein falls. Ultimately the cells stop doubling and lose viability as the concentration of PDiA’ falls to levels that are comparable with the levels of Pdi1p normally expressed in wild-type strains. Strains supported by rat PDI also stop growing after glucose suppression (Fig. 4C).

To determine whether the switch from galactose to

| TABLE I | Rescue of the lethal phenotype of pdi1Δ strains by expression of various PDI constructs |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Protein         | Domain          | Overexpression  | Normal expression |
| Yeast           | wtPDI           | +               | +               |
|                 | a’ domain       | +               | +               |
| Rat             | wtPDI           | +               | +               |
|                 | b’ domain       | –               | –               |
|                 | a’ domain       | +               | +               |
|                 | a domain        | +               | –               |

Expression was driven by the GAL1–10 promoter on a multicopy plasmid (2-μm) in the presence of galactose to fully induce the promoter. +, the strain was viable when plated on 5-fluoroorotic acid in the presence of galactose. –, strains were not viable when plated onto 5-fluoroorotic acid in the presence of galactose.

Expression was driven by the PDI1 promoter from a low copy (cen) plasmid. +, the strain was viable when plated onto 5-fluoroorotic acid.

| TABLE II | Properties of pdi1Δ strains complemented with various PDI constructs |
|-----------|-------------------------------------------------------------|
| Construct | Doubling time in galactose (h) | Number of doublings in glucose media | Protein level after suppression (unitless) |
| Yeast Pdi1p | 2.8 ± 0.4 | Indefinite | 0.64 ± 0.4 |
| Yeast a’ domain | 2.4 ± 0.1 | 3.5 ± 0.2 | 1.1 ± 0.2 |
| Rat PDI (cen) | 8.7 ± 0.2 | 1.4 ± 0.3 | 1.7 ± 0.3 |

Averages of at least triplicate measurements.

* Expressed protein levels are given relative to the amount of wild-type yeast Pdi1p expressed from its chromosomal location as determined from Western blotting. Protein level measurements were made after growth slowed or stopped when the protein level was at its lowest amount. When fully induced, the GAL1–10 promoter produces 7.8 times the amount of Pdi1p as the endogenous PDI1 promoter (Fig. 2).
glucose medium affects the requirement for PDI activity, yeast cells were grown continuously in liquid media that contained a range of glucose to galactose ratios from 100 to 0% of total carbohydrate represented by galactose. This approach allowed us to achieve a variable steady-state level of the PDI proteins so that the decreasing concentrations of protein during the assay of viability and changes in the growth rate were less problematic to the measurement of doubling time and viability. Because of the slow growth phenotype and higher levels needed for the heterologous rat PDI, experiments are shown for only yeast PDI and yeast PDIa/H11032. By varying the carbohydrate source, we were able to vary the protein levels over a wide range, from 7.8-fold higher than the normal expression level of PDI to 60% of that value. As with suppression over time, these experiments show that dropping the steady-state level of wild-type yeast PDI results in a slow growth rate when the protein level falls below 60% of the normal amount of yeast PDI expressed from its chromosomal location. The fully suppressed GAL1–10 promoter, however, still produces enough yeast PDI to maintain viability, although the growth rate is significantly slower (Fig. 5). For the single catalytic domain PDIa the cells grow slowly and lose viability when the expression level falls below the amount of yeast PDI normally expressed from its chromosomal location (Fig. 5).

To more directly assess how replacing wild-type yeast PDI with a single catalytic domain or the heterologous rat protein affects protein folding in vivo, pulse-chase experiments were performed to follow the maturation of endogenous yeast CPY and evaluate the isomerization of a disulfide-containing protein in vivo. CPY is a non-essential yeast vacuolar protease that contains five disulfide bonds. Its folding relies heavily on the ER thiol-disulfide folding machinery. It is initially synthesized as a pro-protein that is oxidized and glycosylated in the ER to a precursor form, p1 (Fig. 6). After additional carbohydrate processing in the Golgi, a second precursor, p2 (Fig. 6), is finally cleaved to mature (m) CPY in the vacuole (11). The exit of correctly folded precursors from the ER requires correct disulfide formation. Consequently, CPY maturation from the precursor (p1) to mature (m) form reflects the competency of thiol-disulfide exchange catalysis in the ER. Complementation of the PDI1 deletion with yeast PDI expressed from the plasmid under the control of GAL1–10 promoter provides for normal
Disulfide Formation by PDI

**Fig. 6. Maturation of CPY in strains supported by PDI variants.** Cell were pulse-labeled with [35S]cysteine and methionine for 15 min and then chased for the indicated times with an excess of unlabeled cysteine and methionine. CPY was purified by immunoprecipitation and resolved on 4–20% SDS-PAGE and visualized by autoradiography. The slowest migrating bands represent the precursor (p) forms of CPY, p1 and p2 (see text). The fastest migrating band represents mature (m) CPY.

CPY maturation, as expected (Fig. 6). Strains complemented with yeast PDIa, on the other hand, show defective maturation of CPY (Fig. 6) in either 50 or 30% galactose media, conditions that support wild-type growth rates. Compromising the isomerase activity supplied by PDI does compromise CPY maturation. Strains complemented with full-length rat PDI also show compromised CPY folding (Fig. 6).

**DISCUSSION**

Although the catalysis of thiol-disulfide exchange by PDI is essential to *S. cerevisiae*, there has been some uncertainty about whether the oxidase or the isomerase activity of PDI (or both) is the principal function of PDI in *in vivo* assays to 20% SDS-PAGE and visualized by autoradiography. The slowest migrating bands represent the precursor (p) forms of CPY, p1 and p2 (see text). The fastest migrating band represents mature (m) CPY.

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**DISCUSSION**

Although the catalysis of thiol-disulfide exchange by PDI is essential to *S. cerevisiae*, there has been some uncertainty about whether the oxidase or the isomerase activity of PDI (or both) is the principal function of PDI in *in vivo*. Both oxidase-deficient (3, 4) and isomerase-deficient (5) mutants of PDI will, under the appropriate conditions, complement the lethal deletion of PDI1. PDI mutants with only one cysteine per active site (NCVGH3-CGUD1) are deficient in oxidase activity *in vitro*, but they will complement the PDI1 deletion in yeast (4); however, the single catalytic domains (α or α'), which are isomerase-deficient, will rescue the PDI1 deletion as well (5). The previous reports of complementation by various PDI species have been complicated by a variety of factors including the use of heterologous proteins (i.e., mammalian), various expression vectors (multicopy and low copy), and different promoters.

All the mutant PDI species that support yeast growth possess at least some level of both isomerase and oxidase activities. Without a large ratio of oxidase to isomerase activity and without defining the relationships among expression levels, growth rate, and viability, it is difficult to make a determination about how much of each activity is required. Assayed *in vitro*, the individual catalytic domains of PDI have significant (50%) oxidase activity but only 5% of the isomerase activity (12, 13).

The individual catalytic domains of PDI exhibit a 10-fold difference in their catalytic activities, which is the largest separation of the two activities that is available.

Titrating the levels of PDI by suppressing the GAL1-10 promoter provides a way to manipulate the levels of PDI *in vivo* and to correlate PDI protein expression levels with growth and viability (Fig. 5). As the amount of PDI protein decreases, the growth rate is relatively constant up to a threshold after which the growth rate declines rapidly. When the expression level of wild-type PDI falls below ~60% (~20%) of normal wild-type levels, growth is compromised (Fig. 5A). A somewhat greater molar amount (100% ~ 20% of normal wild-type levels) of PDIa is needed to maintain growth and viability. At the minimal level that will support normal growth rates, wild-type yeast PDI should supply 60% of the oxidase and isomerase activities that are available under normal conditions. On the other hand, the minimal amount of yeast PDIa that will support normal growth supplies approximately 50% of the normal oxidase activity (50% oxidase activity ~ 100% of the protein) but only approximately 5% of the normal isomerase activity (5% isomerase activity ~ 100% of the protein) (Fig. 5). Thus, the oxidase activity of PDI is needed at very nearly the levels that are present normally in the yeast ER, but only very low levels of its isomerase activity are needed. Clearly, the oxidase activity of PDI is what limits growth in these experiments.

For wild-type PDI, the level of protein required for viability, the ability to replicate and grow when plated back onto galactose medium, is less than ~60% of the amount normally present in a wild-type yeast strain. This represents an upper limit because strains expressing PDI from a fully suppressed GAL1-10 promoter are still viable, although they grow slowly. Strains supported by PDIa, however, lose viability as the level of expression falls below the normal level of wild-type PDI expression (Fig. 5). Because PDIa has only 5% of the isomerase activity of wild-type PDI *in vitro*, we can estimate that the minimum amount of isomerase activity needed to maintain viability is approximately 5% of that normally available from wild-type PDI. The oxidase activity of PDI is certainly needed at much higher levels than the isomerase activity for both growth and viability. Although high levels of isomerase activity are not required for growth or viability under laboratory conditions, they might provide a survival advantage in the natural environment; for example, the folding of some specific substrate proteins, which is expressed by the cell under adverse conditions, might require PDI isomerase function to fold efficiently.

Although the oxidase activity of PDI is what limits growth and viability, we cannot exclude the possibility that PDI contributes to isomerization in the ER or that other ER homologues of PDI1 (MPD1, MPD2, EUG1, and EPS1) (15) supplement PDI activity. Xiao et al. have found that PDI contributes both oxidase and isomerase activities to the yeast ER and that PDI exists in both oxidation states, consistent with its ability to catalyze both oxidation and isomerization.

Extrapolating the results of *in vitro* assays to *in vivo* situations is always associated with some uncertainty. A potentially different redox environment, the presence of additional ER folding assistants, and different growth rates at which the catalytic domains need to be assessed may lead to a more accurate understanding of the oxidase and isomerase activities of PDI.

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Extrapolating the results of *in vitro* assays to *in vivo* situations is always associated with some uncertainty. A potentially different redox environment, the presence of additional ER folding assistants, and a limited choice of substrates to test PDI catalytic activities limit our ability to make conclusions about how a specific construct will perform *in vivo*. Complementation with yeast PDI produces a profile of CPY maturation that is indistinguishable from wild-type cells; however, cells complemented with PDIa display CPY folding that is compromised and without a significant effect on the growth rate. Holst et al. (16) have pointed out that low CPY refolding rates do not correlate with growth and suggested that compromising the oxidase activity was responsible for the growth limitation. Cuozzo and Kaiser (17) have also suggested that the oxidase activity of PDI is important for maintaining high growth rates in *S. cerevisiae*. Ero1p donates oxidizing equivalents to PDI, which, in turn, delivers them to substrates in the yeast ER. Compromised oxidation of PDI by Ero1 provides a similar phenotype of limited growth. Kaiser and colleagues (18) suggested that PDI must play a role of oxidase based on their finding that PDI exists mostly in the oxidized form in the yeast ER.

For wild-type rat PDI, a significantly higher level of the protein, ~3 times the normal amount of yeast PDI, is needed to support growth and viability (Table II), despite the fact that both the oxidase and isomerase activities of the rat protein are

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2 R. Xiao, B. Wilkinson, A. Solovyov, A. Holmgren, J. Lundström-Ljung, and H. F. Gilbert, submitted for publication.
identical to those of the yeast enzyme in vitro (19). This difference could be caused by the diminished ability of rat PDI to integrate correctly into the redox transfer pathway in the yeast ER, possibly because of poor interaction of the heterologous rat protein with yeast Ero1p. Reduced PDI is needed to catalyze substrate isomerization so that compromising the ability of the yeast ER to transfer oxidizing equivalents into PDI will not diminish the isomerase activity, suggesting that the defect in CPY folding is because of inefficient oxidation rather than compromised isomerase activity. Recent experiments by Xiao et al. support this idea.

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