THERE IS EVIDENCE THAT YEAST SACCHAROMYCES CEREVISIAE PROTEIN DISULFIDE ISOMERASE IS TRANSPORTED FROM THE ER TO THE GOLGI APPARATUS

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Abstract

As disulfide bonds are added, newly generated membrane and secretory proteins in cells fold in the endoplasmic reticulum and acquire the proper three-dimensional shape. Disulfide bonds play a crucial role in protein folding. It has been hypothesised that a protein disulfide isomerase enzyme is primarily responsible for the production of protein disulfide bonds in eukaryotes. The endoplasmic reticulum (ER), a eukaryotic cellular organelle involved in protein synthesis, processing, and transport, has been thought to recycle proteins with the C-terminus of amino acids sequences containing His-Asp-Glu-Leu (HDEL) sequence in yeast. The analysis of a fusion protein with an HDEL tag led to the suggestion for this recycling mechanism. The investigation of protein disulfide isomerase's localization and oligosaccharide modification in yeast provided the first concrete proof that this intrinsic ER protein travels from ER to Golgi. According to the results, this native protein accumulates in the ER while being accessible to post-ER enzymes.

Keywords: Protein Localization, Oligosaccharide Modification, Golgi Apparatus, Protein Disulfide Isomerase

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1. Introduction

Escherichia coli is a bacteria that is frequently utilised in biotechnology. Because of its completely sequenced genetic gene, it is incredibly versatile. Prokaryotes may express recombinant genes and are simple to cultivate. Post-translational modification of proteins in prokaryote cells, in contrast to eukaryotes, is drastically altered, if not absent. The lack of physiologically active proteins or the precipitation of inclusion bodies in cells are frequent side effects of attempts to express human genes in E. coli (Kane & Hartley, 1988). Newly generated polypeptides were originally supposed to be folded in the endoplasmic reticulum to create a specific shape, however Escherichia coli lacks this process.

The primary cause of protein folding is the oxidation of cysteine residues' thiol groups (-SH) to produce (-S-S-). Proper disulfide bonds are necessary for the tertiary and quaternary structures of proteins as well as for mature proteins' biological function (Hwang et al., 2014). Having a lot of cysteine residues raises the chance that a bad disulfide bond may form. In the world of biotechnology, improving protein misfold has long been a problem. Enzymes that facilitate the proper repair of misfolded disulfide bonds are often present in living things. The endoplasmic reticulum (ER) contains refolding enzymes, one of which is protein disulfide isomerase (PDI) (Banach et al., 2016).

The carboxyterminal sequence (HDEL) of the yeast Saccharomyces cerevisiae has been hypothesised to serve as an ER retention signal. This signal permits proteins to be retrieved from subsequent compartments of the secretory pathway but does not prevent them from leaving the ER. By discovering that proteins with an ER retention signal can experience carbohydrate changes that take place only in early Golgi compartments (Banach et al., 2016), Pelham et al. have provided evidence for this recycling hypothesis. Also, they demonstrated the presence of the ERD2 gene's product, the receptor for recycling ER proteins, in yeast genetic investigations (Wilkinson & Gilbert, 2004). However, experiments conducted on the yeast Saccharomyces cerevisiae revealed that an artificial prepro -factor fusion protein, which has the HDEL sequence attached to its carboxyl terminus, accumulates intracellularly as a precursor that contains both ER- and Golgi-specific oligosaccharide modifications.

In earlier research, the gene sequence for the ER protein PDI from S. cerevisiae was identified. This enzyme is an N-glycosylated glycoprotein with roughly 5 core oligosaccharides, according to earlier findings (Mizunaga et al., 1990). This ability was used to investigate the possibility that yeast PDI underwent Golgi-specific glycosylation.

2. Materials and Methods

2.1. Yeast cells and the state of the culture

The yeast Saccharomyces cerevisiae cells were chosen as TM5 (MAT pho3 pho5 trp1 leu2 ura3 his3) cells. The multicopy plasmid Yep13 encoding the PDI1 gene was present in TM5 in order to express a significant quantity of PDI. After growing for 18 hours at 24 °C, the yeast cells were collected. Saccharomyces cerevisiae X2180-1A (MAT, gal 2, cup 1) strain and the temperature-sensitive mutant HMSF176 (MAT, sec 18-1) strain were utilised to radioisotope label yeast cells.

2.2. Fractionation of Extracts from Yeast Cells

Yeast cell extracts were separated on a sucrose density gradient in accordance with Ruohola et alinstructions .'s in order to look into the intracellular location of the PDI in yeast. (Dean & Pelham, 1990) Thereafter, yeast cells (100 OD600 units) were resuspended in 1 mL of the spheroplast medium, which contains zymolyase (1 mg/mL) and is composed of 1.4 M sorbitol, 50 mM potassium phosphate, 56 mM -mercaptoethanol, pH 7.5, for 1 hour at 38 °C. After centrifuging these sedimented spheroplast mixtures in sorbitol cushion (1.7 M sorbitol, 20 mM sodium phosphate, pH 7.5), purified spheroplasts were resuspended in 4 mL of the lysis buffer (0.3 M mannitol, 10 mM Mops, pH 7.0) containing N, N'-diphenyl-p-phenylene diamine (0.1 mg/mL) and aprotinin (100 U/mL). Ruohola et alinstructions .'s for homogenization were likewise followed, and 3 mL of homogenate in 60% (W/W) sucrose was the end result. The following sucrose solutions were added on top of two millilitres of homogenate at the bottom of an ultracentrifuge tube: 1 mL of sucrose at a rate of 55%, 1.5 mL at a rate of 50%, 45%, 40%, and 35%, and 2 mL at a rate of 30%. The gradient was fractionated in microtubes after being centrifuged at 4 °C for 12 hours at 170,000 g. A gradient tube fractionator was used to gather a total of 23 fractions, each approximately 500 L in volume. The density of sucrose in each fraction was then ascertained using a refractometer.

2.3. Using marker enzyme analysis to determine the distribution of yeast organelles

NADPH-cytochrome c reductase, Kex2, and -mannosidase were utilised as marker enzymes for the distribution. After changing the buffer of each fraction to 50 mM sodium phosphate buffer (pH

7.5) containing 50 mM EDTA, their tests were carried out exactly as stated (Dean & Pelham, 1990), and the PDI assay was carried out as described by (Semenza et al., 1990).

2.4. Saccharomyces cerevisiae strains X2180-1A (MAT, gal 2, cup 1) and temperature-sensitive mutant HMSF176 (MAT, sec 18-1) cultivated at 24 C were tagged with 35S (Trans 35S-label Metabolic Labeling Reagent, ICN) for 30 min at 24 C or 37 C and collected as reported in reference (Mizunaga et al., 1990). Radiolabeled cells were resuspended at 100OD600 units/mL in 50 mM Tris-HCl buffer (pH 7.5) containing l% SDS and 1 mM phenylmethylsulfonylfluoride and homogenised as reported in reference

after being washed once with 10 mM sodium azide. After boiling cell extracts for 5 minutes, debris was removed by centrifuging them at 6000 g for 5 minutes. After being diluted with 800 ml of ice cold, 1.25% TritonX-100 in 60 mM Tris-HCl buffer (containing 190 mM NaCl and 6 mM EDTA, pH 7.4), 200 L of the respective supernatants were immunoprecipitated for 12 hours at 4 C by adding 10 L of anti-PDI antibody(Castresana, 2000) and 50 L of 20% protein A-Sepharose suspension. For two hours, samples were rotated at room temperature.

After two washes with PBS, immunoprecipitates were washed twice with 1 mL RIPA buffer (0.5 M NaCl, 50 mM Tris-HCl [pH7.4], 0.5% NP-40, 0.5% deoxycholate, and 0.1% SDS). Each sample was now separated into portions for SDS-PAGE or reimmunoprecipitation using an anti—1,6 mannose antibody. Samples were then reconstituted in SDS sample buffer, boiled for five minutes, and subjected to SDS-PAGE analysis. Anti-PDI antibody reactive proteins were removed from the resin for the second immunoprecipitation by boiling it for five minutes with 50 L of 1% SDS in 50 mM Tris-HCl (pH 7.4). Eluted proteins were reprecipitated using the proper anti—1,6 mannose antibody before being subjected to the same SDS-PAGE analysis as previously mentioned. Amplify was applied to the electrophoresed gel before it was dried and subjected to an X-ray film.(Chan et al., 1983)

2. Result and Discussion

3.1. Yeast PDI Was Found in the ER

The outcome was displayed in Figure 1. The ER marker enzyme NADPH-cytochrome c reductase co-migrated with the activity of PDI. The Golgi marker enzyme Kex2 is eluted in the five to six fractions prior to the PDI peak fraction. As a vacuole marker enzyme, -mannosidase is distinctly distinct from the actions of PDI and NADPH-cytochrome c reductase.(Opheim, 1978)

3.2. Proof That Yeast PDI Was Transported to the Golgi Apparatus Together with It in the ER

Moreover, it has been shown that, like mammalian cells, yeast early Golgi apparatus is where insertion of -1,6 linked mannose residues happens(Ruohola & Ferro-Novick, 1987). We tested whether yeast PDI receives this Golgi-specific glycosylation and made an effort to show that PDI is recycled from the Golgi to the ER using the anti—1,6 mannose antibody.

As seen in Figure 2, the precipitation of material containing 1,6 mannose with anti-1,6 mannose antibody demonstrated that PDI isolated from X2180-1A cells radiolabeled at either 24 or 37 °C (lane 1 and 2) had acquired the addition of 1,6 mannose (lane 8 and 9).

The -1,6 mannose addition was also present in PDI recovered from secl8 mutant cells tagged at the permitted temperature (24 °C) (lane 3). (lane 6). On the other hand, anti—1,6 mannose antibody did not react with PDI recovered from sec18 cells when they were radiolabeled at the non-permissive temperature (37 C) (lane 4). (lane 7). All vesicular traffic is halted in the sec18 mutant at the nonpermissive temperature, and freshly produced proteins are unable to exit the ER (Kreibich et al., 1978). Based on these findings, it was possible that the luminal ER protein PDI had entered the early Golgi compartment and been added with 1,6 mannose.

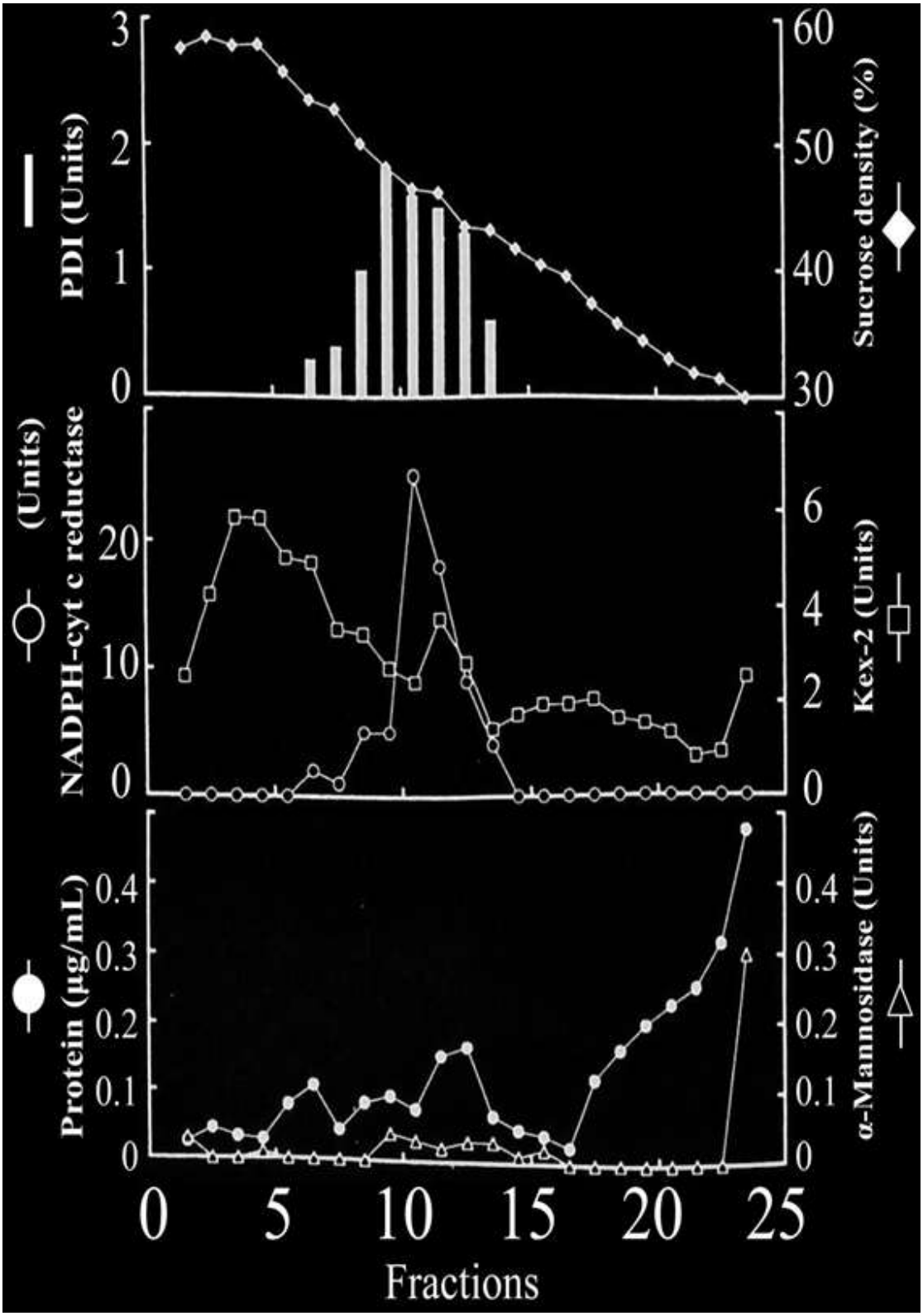

Figure 1: NADPH-cytochrome c reductase activity co-migrates with PDI. After fractionating S. cerevisiae TM5 cells that carried the multicopy plasmid Yep13 carrying the PDI1 gene, the activity of marker enzymes and PDI were assessed as stated in the text.

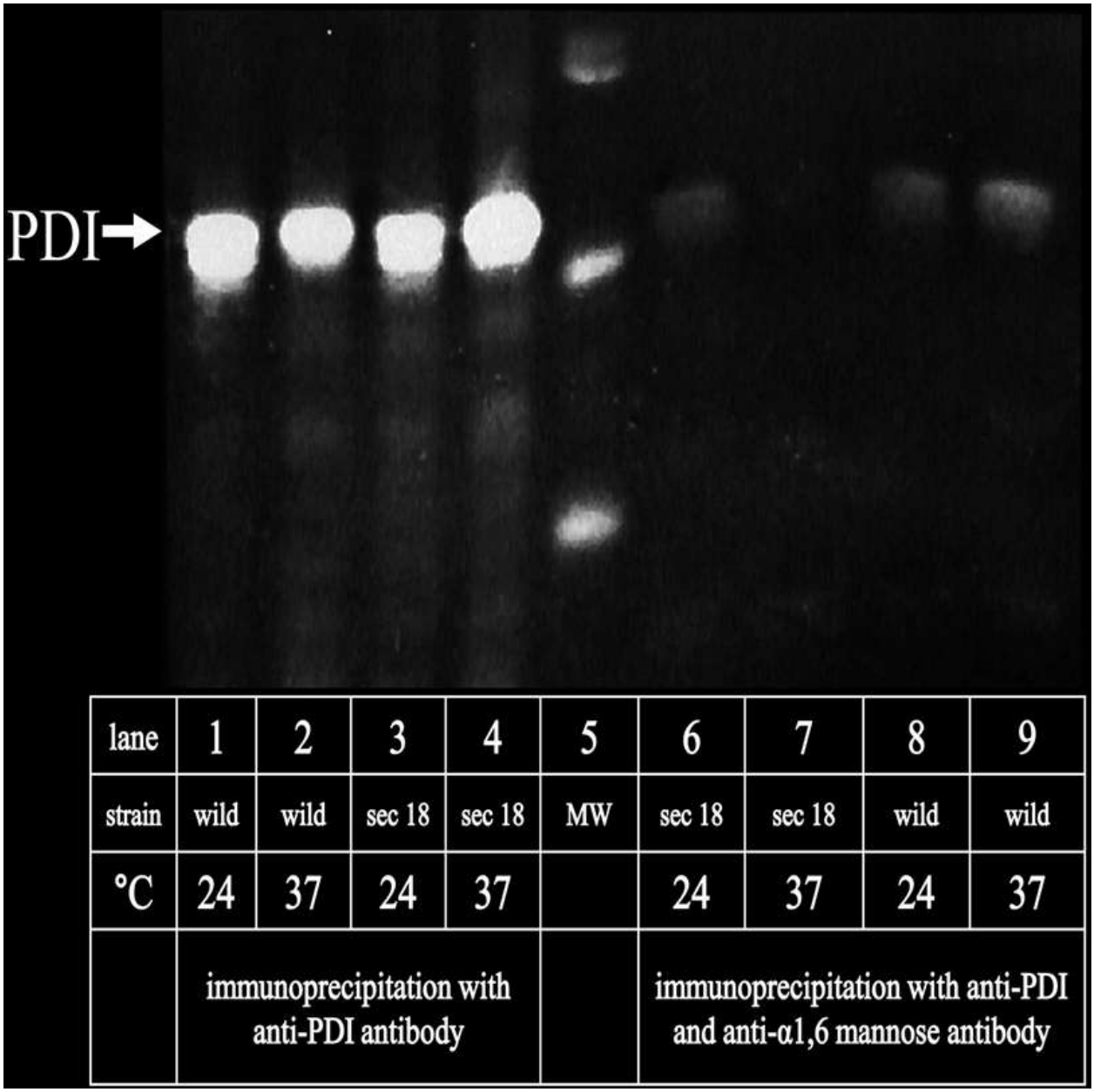

Figure 2: In vivo, PDI gets 1,6 linked mannose additions. Sec18 (HMSF176) and wild-type (X2180-1A) cells were both tagged for 30 min at either a permissive (24 C) or nonpermissive (37 C) temperature. Equivalent portions of the cell lysates were immunoprecipitated with either the anti-PDI antibody or the anti-PDI antibody, and then they underwent a second precipitation with the anti—1,6 mannose antibody. Autoradiography was used after SDS-PAGE electrophoresis to evaluate the immunoprecipitates. The molecular weight (MW) standards were ovalbumin (68,000), bovine serum albumin (68,000), and phosphorylase b (97,000). (43,000)

3. Conclusion

The luminal ER protein, PDI, was shown to be accessible to Golgi-specific modifying enzymes in this study, despite accumulating in the ER. The findings of this work provided the first concrete proof that a luminal ER protein, PDI, travels from the ER to the Golgi compartment using a natural yeast protein. They also significantly validated the recycling model of the luminal ER protein put forward by Pelham H.R. et al. The fact that PDI was not visible in the Golgi fraction in Figure 1 supports the idea that the majority of PDI is found in the ER because it swiftly returns to there after leaving the early Golgi compartment. By performing correct folding on nascent proteins while maintaining bioscientific activity, understanding the intracellular localization of PDI—which catalyses repairing of incomplete protein conformations—leads to promoting

increased efficiency in industrial application of biomaterials, such as artificial antibodies. To demonstrate the phenomenon that yeast PDI, which was originally carried from the ER to the Golgi, returns from the Golgi to the ER, more study is required.

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