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Review

Insights from Yeast on Oxidative Stress in Alzheimer's Disease, Focusing on Ahp1p/Prx5

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Abstract

The yeast, *Saccharomyces cerevisiae*, the model eukaryote, has provided much understanding of molecular and cellular biology, as well as insights into many human diseases. In this paper we review how yeast studies are contributing to knowledge about the role of oxidative damage to cell health, and how one of the key players in Alzheimer's disease, amyloid beta (Aβ) is linked to the reactive oxygen species response involving *AHP1*, which encodes an alkyl hydroperoxidase, Ahp1p, a protein involved in protection from lipid peroxidation.

Keywords

Alkyl hydroperoxidase; *Saccharomyces cerevisiae*; yeast model for Alzheimer's disease

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1. Introduction

There are many factors contributing to Alzheimer's Disease (AD) and oxidative stress is proposed as one such factor [1]. The production of moderate levels of reactive oxygen species (ROS) is an essential and natural process that plays a role in cell signalling. However, ROS is also considered to be a factor in aging [2]. Oxidation can cause damage to lipids, DNA and proteins, which can lead to loss of mitochondrial dysfunction and cause cell death [3]. However, the study of these effects in humans (and in animal models) can be very challenging and limited.

Yeast cells bear many similarities to human cells, including neuronal cells, and for many years yeast have been utilised as a model for Alzheimer's Disease and for studying oxidative stress [4]. One of the attributes of yeast that make it particularly suitable for this research is its ability to be grown by fermentative metabolism even with extensive mitochondrial damage [5]. The outcome of loss of mitochondrial function is that cells no longer have respiratory function and cannot be grown on carbon sources such as ethanol. However, they can still exhibit fermentative growth on fermentable carbon sources such as glucose. Furthermore, ROS accumulation has also been reported to be the major signalling molecules for activation of the genes for pro-inflammatory mediators like cytokines and Nuclear Factor kappa B [6]. It would be intriguing to see how yeast can provide support for studies pertaining to inflammation, specifically in neuroinflammatory diseases.

Many yeast genes that govern important cellular processes are conserved from yeast to humans. Similarities of neurons and yeast cells are not just limited to conserved fundamental processes. Molecules involved in cellular polarization in yeast (during budding and mating) and neurons (during growth of neurons) are structurally and functionally analogous and drive the phenotypic plasticity in these cells [7]. Conserved voltage gated ion channels and other molecules involved in cell-cell communication in yeast cells make them an excellent neuronal model. Formation of yeast flocculation also provides potential for exploring the multicellular dimension that is normal for higher eukaryotes [7].

2. Pro-Oxidant Chemicals that May be Involved in Alzheimer's Disease

We are exposed to many chemicals throughout our lives and the effects of the worst of these may be obvious because of their acute toxicity. Chemicals occur naturally in our foods and many are produced by us as well so many have not been subjected to rigorous investigation for their involvement in neurodegenerative disease. Cells make antioxidants such as glutathione and dihydroascorbate as part of their protection against oxidative damage, however, the levels may not always be high enough to provide full protection [8]. Dietary addition can be considered but levels may not reach those required for protection, especially when crossing the blood-brain barrier needs to be considered. Currently evidence that dietary antioxidants provide protective effects in human health is lacking.

In AD the involvement of Aβ [through this manuscript we are referring only to Aβ(1-42)] is strong and there are links between Aβ and ROS production [9]. In yeast, the constitutive expression of Aβ in the secretory pathway is also implicated in a ROS response: expression of the Aβ caused reduced growth and respiration and increase oxidative stress [2]. In a genome wide expression study of yeast producing cytosolic Aβ fused to GFP (see Figure 1), a stress response was

observed that indicative of the yeast heat shock response (HSR) [10]. This HSR was confirmed by co-transformation with a plasmid encoding a heat shock reporter: the plasmid contained *lacZ* downstream of heat shock elements (HSE). In the co-transformants the levels of the enzyme β-galactosidase were highly elevated by the presence of Aβ fused to GFP [11]. This HSR is likely to be due to the stress caused by ROS, but it should be noted that HSR in yeast can also be triggered by heat shock itself and when protein misfolding is detected.

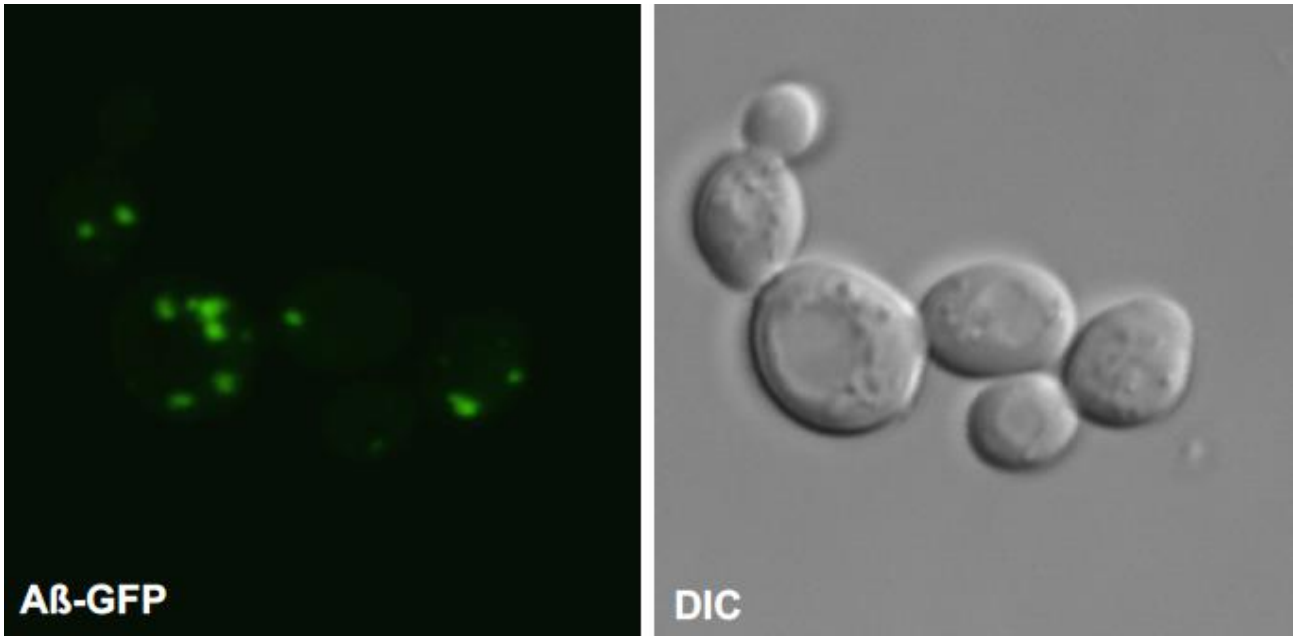

Figure 1 *Saccharomyces cerevisiae* transformants producing Aβ-GFP observed by confocal microscopy and differential interference contrast (DIC) microscopy. Courtesy of Sonia Sankovich, CSIRO.

More recently, using a new yeast reporter system with HSEs upstream of sequences encoding mCherry, red fluorescence was induced by the external addition of chemically-synthesised Aβ peptide. This indicates that extracellular Aβ also causes a HSR [12]. The design of this experiment indicates extracellular Aβ is causing a ROS response.

In addition to the general HSR observed in the genome wide expression analysis, cells producing GFP-Aβ expressed almost twice as much mRNA for *AHP1* than those expressing just GFP [10]. This suggested that the encoded alkyl hydroperoxidase is a major contributor used for protection against Aβ. Therefore, the use of yeast in screening systems to find chemo preventatives of Aβ-induced damage has utilised strains that are deleted for *AHP1* [4, 13]. The lack of Ahp1p (the protein encoded by *AHP1*), increases the sensitivity of these assays [13] so that compounds altering the levels and localisation of Aβ fused to GFP can be more readily ascertained.

The involvement of chemicals as causative agents of AD is currently unclear. We have begun to look for an association by focusing on the effects of biogenic amines that affect mitochondrial respiration. As a start we have looked for, but not found, a synergistic interaction between dopamine and Aβ (Dhakal and Macreadie, unpublished). The effects of other biogenic amines are currently being examined.

3. Proteins that May be Involved in Protection against Alzheimer's Disease

It is also obvious that our genes provide protection against life's stresses. In yeast this has been well studied and the information is readily available in the literature as well as in searches within the *Saccharomyces cerevisiae* genome database (https://www.yeastgenome.org). In this database the ~6000 genes of yeast are listed, along with the descriptions of their gene products. The descriptions are systematic, extensive and linked to the relevant literature. Systematic deletions of these genes have led to information about their function and guides the discernment of human orthologs. With regards to protection against ROS the number of genes is considerable. Table 1 provides a list of some yeast genes involved in various modes of protection against ROS and shows their human orthologs that can be identified by sequence similarity. As already mentioned the involvement of Ahp1p in protection in yeast is substantial. This leads to the suggestion that Aβ damage may be via lipid peroxidation. Indeed, this is consistent with the localisation of the 42 amino acid peptide Aβ: it is very hydrophobic and readily localises to membranes. Even the fusion to GFP results in its localisation to "punctate patches" which appear novel (Figure 1). Our current thoughts are inclined to the view that the Aβ/GFP fusion proteins are localised into membranes that have been disrupted from within the cell.

Table 1 Conserved yeast and human genes and encoded proteins conferring protection against Oxidative Damage.

Yeast genes	Encoded proteins	Human orthologs
GPX1, GPX2, GPX3/HYR1	Phospholipid hydroperoxide glutathione peroxidase	*GPX4:* isoform A, isoform B, isoform C, isoform D
TSA1, TSA2	Thioredoxin peroxidase	*PRX2*
PRX1 (Mitochondria) *AHP1* (Cytoplasm)	Thiol peroxiredoxin	*PRX6* *PRX5*
TRX1, TRX2 (Cytoplasm) *TRX3* (Mitochondria)	Thioredoxin	*TRX* isoform 1
SOD1 (Cytoplasm) *SOD2* (Mitochondria)	Superoxide dismutase	*SOD1* *SOD2*
GSH1, GSH2	Glutathione synthase	Gamma-glutamyl cysteine synthetase (GCS), GSH
GTO1 (Peroxisome) *GTO3* (Cytoplasm)	Omega Class glutathione transferase	*GST*
CTA1, CTT1	Catalase	*Catalase*
GLR1 (Mitochondria)	Glutathione reductase	*GSR* (Mitochondria)
GTT1 (ER stress) *GTT2* (DNA replication stress) *GTT3*	Glutathione *S* transferase	Glutathione *S* transferase

4. Ahp1p and its Human Ortholog Prx5: Significance in Neurons

Following its discovery in 1999, Ahp1p, a peroxiredoxin family protein, has been found to have a significant role in detoxifying the yeast cells from ROS, RNS and alkyl hydroperoxides.

The protein contains two catalytic cysteines (Peroxidatic Cysteine (C_P) at Cys62 and Resolving Cysteine (C_R) at Cys31), one of which gets oxidized forming cysteine sulfenic acid during the reduction of the oxidized molecules referred to as peroxidatic cysteine and the other one resolves the oxidized cysteine by forming a disulphide bond termed as resolving cysteine [14]. In most cases, cysteines are conserved from Ahp1p to Prx5 (human ortholog of Ahp1p), however some yeast may not have both residues in a monomeric form. *S. cerevisiae* Ahp1p, existing as a homodimer, requires another molecule of itself to form the cysteine disulphide bond during reduction of the lipid peroxides [14]. In the meantime, Prx5, acts as monomer, has both the residues (Cys47 as C_P and Cys151 as C_R) within itself and acts independently catalysing the reduction of oxidized lipids (Refer to Figures 2 and 3) [14, 15]. The reduced state of the original molecules is achieved through the help of the NADPH cofactor and enzymes like thioredoxin and thioredoxin reductase [16].

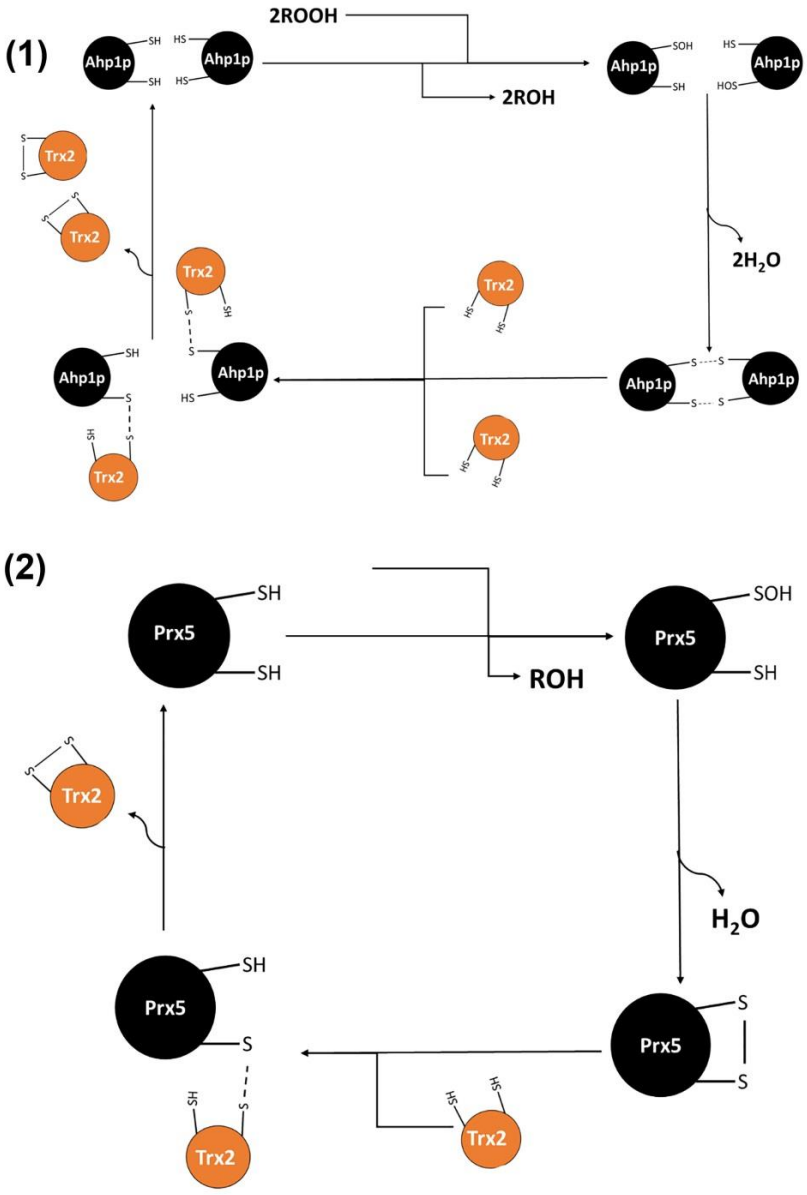

Figure 2 Schematic representation of mechanism of action of Ahp1p (1) and Prx5 (2) against lipid peroxides.

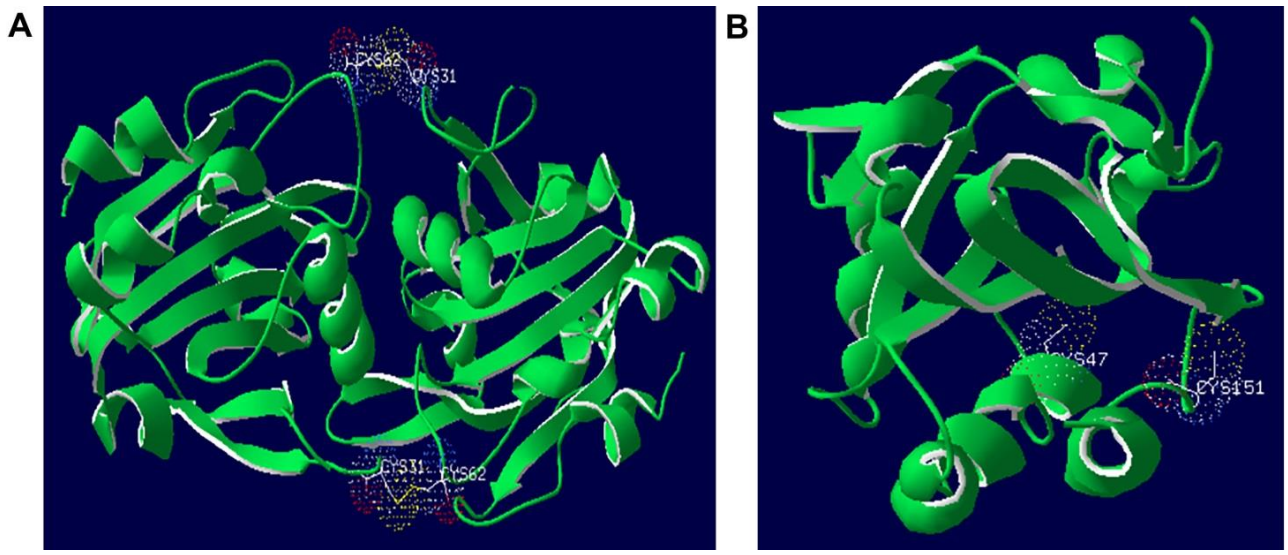

Figure 3 Three-dimensional model for *S. cerevisiae* Ahp1p (A) and human Prx5 (B) showing conserved cysteine residues.

Among the peroxiredoxin family of proteins in yeast, Ahp1p is a major contributor in providing protection against the metal associated oxidative stress [16]. As shown in Figure 4, the human ortholog of the yeast Ahp1p, peroxiredoxin 5 isoform S, exhibits 30% sequence identity and 52% sequence similarity with Ahp1p demonstrating the conserved nature of the Ahp1p. One of the intriguing aspects of human Prx5 is the presence of isoform L which contains a leader peptide (absent in isoform S) that directs it to the mitochondrion. Thus, one gene, through differential transcriptional initiation produced two transcripts. One encodes the L isoform which is a 214 amino acid peroxiredoxin protein that is directed to the mitochondria in humans while the other encodes the shorter 162 amino acid version is located in vacuoles and the cytoplasm.

Multiple functions have been assigned to the human ortholog of Ahp1p from various studies. Some of the important functions of the Prx5 could include down-regulation of the cyclin dependant kinase-5 (Cdk5), clearance of ROS and RNS, protection against oligomeric Aβ-associated mitochondrial fragmentation, endoplasmic stress and metal-induced toxicity including iron-induced toxicity represented in Figure 5 [17-20].

Cdk5-p25 complex activation has also been found to be causing hyperphosphorylation of tau that will lead to the formation of neurofibrillary tau tangles in neuronal cells [21]. Considering this evidence from the literature, the protection of the Prx5 could possibly be one of the crucial events in protecting cells from oxidative stress including in the ageing neuronal cells, which will otherwise deteriorate with accumulation of ROS and misfolded proteins. Studies in cancer cells also highlight the positive effect (to the cancer cells) of overexpression of Prx5 in proliferation and tumorigenicity [22]. This could possibly align with the neuronal cell cycle re-entry hypothesis, where the neuronal cells are triggered to enter the cell division cycle causing the death of the neuronal cells: normally neuronal cells are in a G_0 cell cycle and do not divide. Instead, entry into cell division cycle means proceeding towards the death of the cell causing the neuronal loss [23].

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  1 MGLAGVCALRRSAGYILVGGAGGQSAAAAARRYSEGEWASGGVRSFSRAA     50

  1   MSDL-VNKKFPAGDYKFQYIAISQSDADSESCKMPQTVEWSKLISENK     47
        |:.: |....||       :.:.:.:..::       |..::|. :.|
 51 AAMAPIKVGDAIPA-------VEVFEGEPGNK-------VNLAELF-KGK     85

 48 KVIITGAPAAFSPTCTVSHIPGYINYLDELVKEKEVDQVIVVTVDNPFAN     97
    |.::.|.|.||:|.|:.:|:||::... |.:|.|.|..|..::|::.|..
 86 KGVLFGVPGAFTPGCSKTHLPGFVEQA-EALKAKGVQVVACLSVNDAFVT    134

 98 QAWAKSLGVKDTTHIKFASDPGCAFTKSIGFELAVGD------GVYWSGR    141
    ..|.::  .|....:..:||..||.|..  :|.:.|      |.....|
135 GEWGRA--HKAEGKVRLLADPTGAFGKET--DLLLDDSLVSIFGNRRLKR    180

142 WAMVVENGIVTYAAKETNP-GTDVTVSSVESVLAHL*   177
    ::|||::|||  .|....| ||.:|.|...::::.|
181 FSMVVQDGIV--KALNVEPDGTGLTCSLAPNIISQL*   214
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Figure 4 Alignment of the Ahp1p protein (*S. cerevisiae* S288c) with the human peroxiredoxin 5, mitochondrial protein precursor protein, isoform L (NP_036226.2) of 214 amino acids. A second variant of the human peroxiredoxin 5, isoform S, (NM_001358516.1) results from internal transcription start site, producing a form that lacks the first 52 amino acids and is underlined above. Conserved cysteines are highlighted.

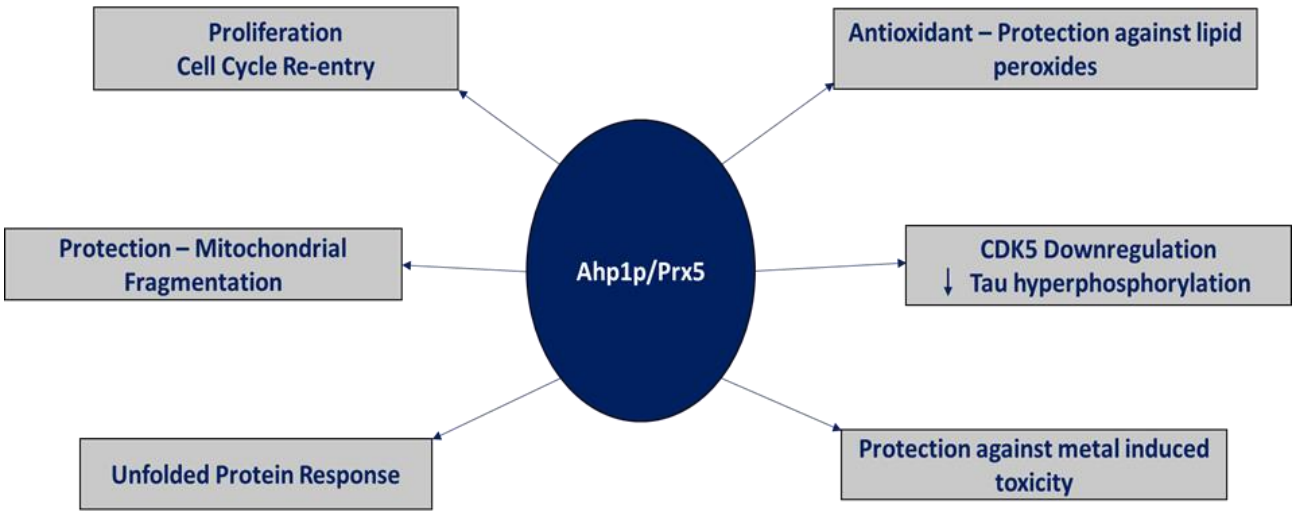

Figure 5 Representation of predicted roles of Ahp1p/Prx5 from various studies.

5. Conclusions

Yeast offers a convenient system to dissect the mechanisms in biology, including the understanding of neurodegenerative disease. Here we have presented an analysis of the yeast cellular response to the presence of Aβ, which demonstrates a lipid peroxide response. Further studies will address how Aβ causes this particular response and whether there may be other

factors, such as biogenic amines, drugs and food products, that exacerbate or ameliorate the effects of Aβ. The use of yeast enables this work to be performed with relative ease.

Author Contributions

IGM and SD contributed equally to the conception, research and writing of this article.

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Competing Interests

The authors have declared that no competing interests exist.

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