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Research Article

Identification of human ferritin, heavy polypeptide 1 (FTH1) and yeast *RGI1* (*YER067W*) as pro-survival sequences that counteract the effects of Bax and copper in *Saccharomyces cerevisiae* ☆

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ABSTRACT

Ferritin is a sub-family of iron binding proteins that form multi-subunit nanotype iron storage structures and prevent oxidative stress induced apoptosis. Here we describe the identification and characterization of human ferritin, heavy polypeptide 1 (FTH1) as a suppressor of the pro-apoptotic murine Bax sequence in yeast. In addition we demonstrate that FTH1 is a general pro-survival sequence since it also prevents the cell death inducing effects of copper when heterologously expressed in yeast. Although ferritins are phylogenetically widely distributed and are present in most species of Bacteria, Archaea and Eukarya, ferritin is conspicuously absent in most fungal species including *Saccharomyces cerevisiae*. An *in silico* analysis of the yeast proteome lead to the identification of the 161 residue *RGI1* (*YER067W*) encoded protein as a candidate for being a yeast ferritin. In addition to sharing 20% sequence identity with the 183 residue FTH1, *RGI1* also has similar pro-survival properties as ferritin when overexpressed in yeast. Analysis of recombinant protein by SDS-PAGE and by electron microscopy revealed the expected formation of higher-order structures for FTH1 that was not observed with *Rgi1p*. Further analysis revealed that cells overexpressing *RGI1* do not show increased resistance to iron toxicity and do not have enhanced capacity to store iron. In contrast, cells lacking *RGI1* were found to be hypersensitive to the toxic effects of iron. Overall, our results suggest that *Rgi1p* is a novel pro-survival protein whose function is not related to ferritin but nevertheless it may have a role in regulating yeast sensitivity to iron stress.

1. Introduction

Iron is an essential co-factor for numerous proteins that are involved in a large variety of different biological functions that range from oxygen and electron transport to DNA synthesis and maintenance [1]. The ferritin-like super-family of proteins is one of the largest groups of iron binding proteins [2,3]. Members of these 12 sub-families contain a structurally and evolutionary related iron binding domain that is present in a number of different functional proteins. The ferritin sub-family consists of three distinct members including the ferritins, the bacterioferritins and the DPS (DNA-binding protein from starved cells) [2]. These proteins are capable of binding iron and they are also capable of acting as storage molecules. These proteins carry out this function by forming multi-subunit nano-cage like structures that can store as much as 4000 iron atoms in the large multi-subunit structure formed by ferritin [4]. In addition, the ferritin proteins can protect cells from the toxic effects of iron as well as other free radical producing stresses such as hydrogen peroxide (H_2O_2) [2,4–6].

The unique chemistry of metals such as iron, which has the ability to switch oxidative states ($Fe^{3+} + \dot{e} \leftrightarrow Fe^{2+} - \dot{e}$), makes these atoms indispensable for biological systems for such functions as a carrier of electrons [1,7]. There are, however, numerous problems associated with the use of iron including the fact that although iron is very abundant, most forms are insoluble and are thus not easily accessible biologically [7]. Of further importance is the toxicity associated with iron *in vivo*. In a series of different reactions, including the Fenton's reactions, both Fe^{3+} and Fe^{2+} can react with other cellular molecules including H_2O_2 to produce destructive free radicals including $\cdot OH$ [1,4,7]. Cells have developed a large repertoire of mechanisms to deal with free radicals

☆Pro-survival functions of human H-ferritin and the yeast *RGI1*.

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generated by iron or other cellular stresses [8]. In addition, specific regulatory mechanisms have evolved to allow cells to grow and survive in environments with either excess or limiting levels of iron [9,10]. One is to coordinately increase and decrease the expression of genes that code for proteins such as iron transporters that alter iron uptake [11]. Another mechanism of importance involves using ferritin cages to safely store excess iron and serve as a reservoir in times of iron limitation [4,12]. Although the ferritin sub-family may be one of the most well studied iron binding proteins many unanswered questions remain including understanding the mechanism responsible for the protective effects of ferritins against ROS [2,3,13–15]. The yeast *Saccharomyces cerevisiae* is an excellent model to study iron metabolism given that there are a great number of similarities between yeast and humans [9]. One notable difference is the absence of the ferritin iron storage proteins in many fungi including yeast [15]. Hence iron storage is thought to involve the vacuole in yeast while its mammalian counterpart, the lysosome, appears to play a less prominent role than ferritin in iron storage [10,15].

Here we report that human ferritin, heavy polypeptide 1 (FTH1) was identified in a previously described screen for sequences that prevent cell death in yeast in response to the heterologous expression of the murine apoptogenic Bax [16]. In addition, we report that we identified a 20% sequence similarity between the protein encoded by the functionally uncharacterized *RGI1* (*YER067W*) gene in yeast and FTH1. Other similarities and links between ferritin and *RGI1* include the previously reported observation that *RGI1* expression is responsive to iron and to the heterologous expression of human ferritin [17,18]. Given that yeast has no reported ferritin and the importance of ferritin for iron metabolism, we carried out a detailed functional analysis of *RGI1* to examine its potential role in iron metabolism as well as its ability to function as a negative regulator of Programmed Cell Death (PCD).

2. Results

2.1. Both human ferritin, heavy polypeptide 1 (FTH1) and yeast RGI1 are Bax suppressors in yeast

In a previous screen of a human cardiac cDNA expression library for suppressors of the pro-apoptotic Bax, we isolated an 874 nt cDNA with a 3′ poly A tail that has a predicted ORF between nt 138–687. Analysis of the sequence of the predicted183 aa residue protein revealed that it represents human ferritin, heavy polypeptide 1 (FTH1) (GenBank BC104643). To confirm the results of the screen, the FTH1 cDNA was re-transformed into naïve yeast cells with the Bax expressing cDNA. The transformants were grown in liquid glucose media, serially diluted and aliquots were spotted onto nutrient agar media with glucose or galactose. The Bax and FTH1 sequences are expressed under the control of the yeast *GAL1* promoter and are thus induced in media that contains galactose. With glucose, all strains grew equally well but cells harbouring Bax alone showed, as expected, a significant inhibition of growth when plated on media containing galactose (Fig. 1A). In contrast, cells that co-express FTH1 or human 14-3-3β/α, a previously characterized Bax suppressor [19], show significant growth on galactose. This confirms that FTH1 is a Bax suppressor and given that ferritin is a known pro-survival protein, FTH1 thus appears to be a functional protein in yeast [18,20].

The widespread distribution of ferritins in all species except yeast suggests that ferritins were lost or systematically eliminated from fungal genomes [3,15]. Alternatively it is possible that yeast have ferritins but that their sequences have diverged to such an extent that they are not identifiable by routine Blast searches. The latter is supported by studies reporting a ferritin-like iron binding protein is present in the extracts of *S. cerevisiae* [21]. Although many yeast proteins retain high sequence identity with their human counterparts many others show more limited identity in the 20% range [22–24]. In our search of the yeast proteome in the *Saccharomyces* Genome Database (SGD; http://www.yeastgenome.org/), we identified the 161 residue protein encoded by *YER067W*

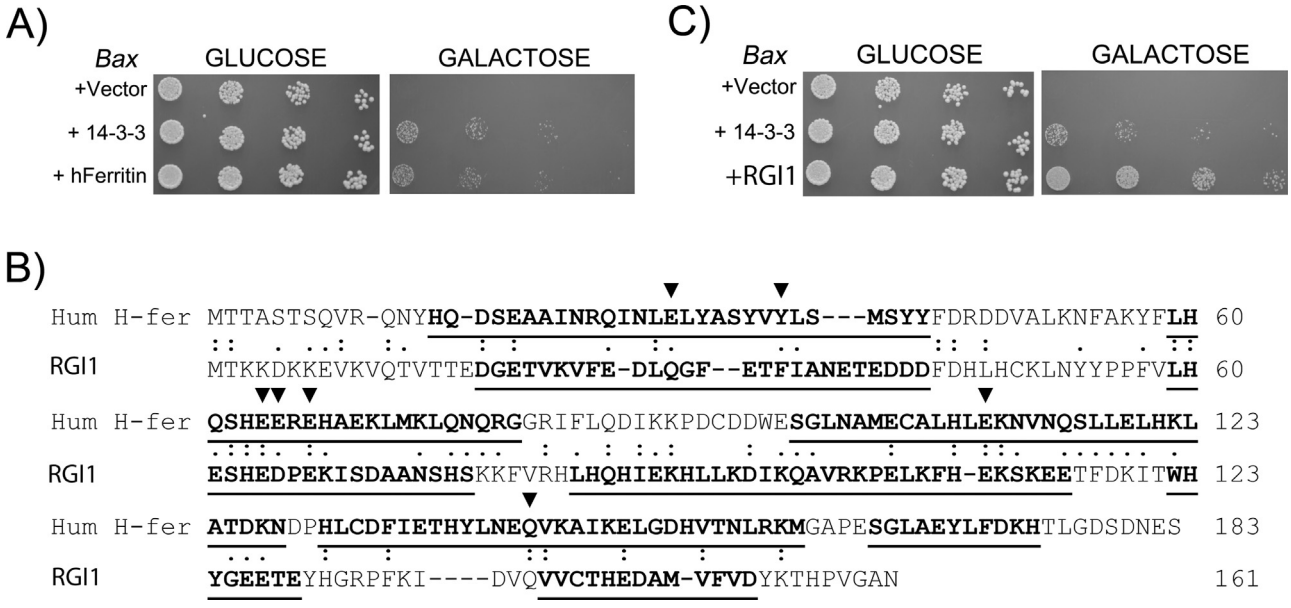

Fig. 1. Identification of the yeast *RGI1* gene as a Bax suppressor. (A) Spot assay of cells expressing Bax alone and Bax with human ferritin. Fresh cultures of glucose grown yeast cells harbouring Bax or Bax and human H-ferritin (hFerritin) were serially diluted and aliquots were spotted on nutrient agar plates with glucose or the *GAL1* promoter inducing galactose media. (B) The 183 amino acid sequence of human (h) H-ferritin is shown aligned to the deduced 161 residue amino acid sequence of *RGI1* (*YER067W*). The sequences were aligned using the Blast program and manually adjusted to maximize identity. A colon (:) indicates a conserved residue, a point (.) represents a conserved residue and gaps indicate no residue (-). Regions of α-helices deduced by an algorithm using the method of Chou and Fasman (http://web.expasy.org/protscale/) are shown in bold and underlined. Inverted triangles (▾) denote residues that are important for the ferroxidase activity of H-ferritin. (C) The spot assay was used to assess the growth of cells expressing Bax alone or Bax with *RGI1*. In (A) and (C), 14-3-3 is a positive control that refers to the previously characterised Bax suppressor human 14-3-3β/α.