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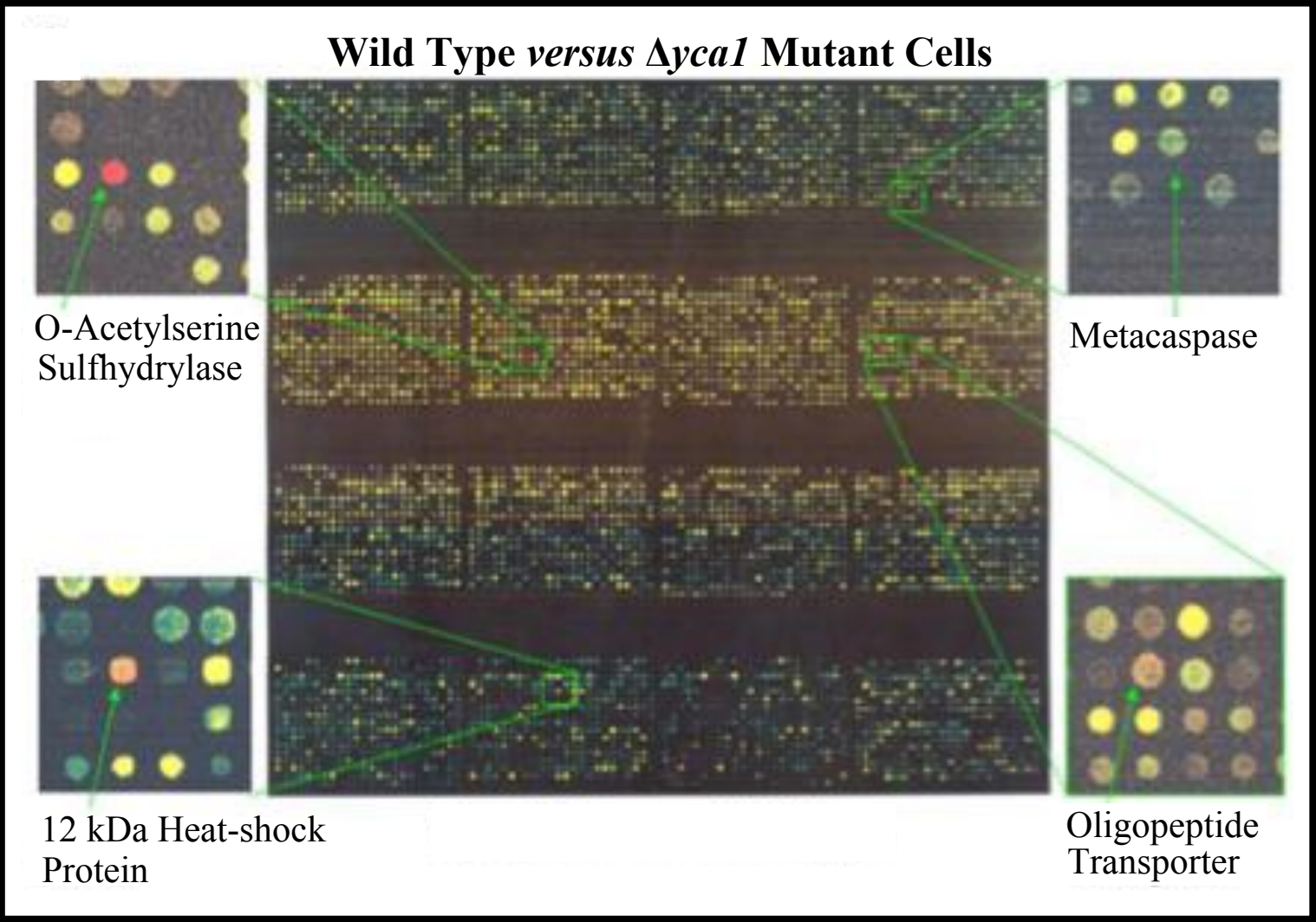

Yeast Mutant Whole Genome Microarray

Elimination of Apoptotic Gene, *yca1*, Prompts Upregulation of O-Acetylserine Sulfhydrylase in Mutant *Saccharomyces cerevisiae*

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Abstract: Caspases are not only integral part of an apoptotic process, but also participate in non-apoptotic processes. Deletion of caspase can modulate transcriptomics. To determine the gene regulation after metacaspase (*yca1)* deletion from genome in *Saccharomyces cerevisiae*, we used microarray-based technique to compare wild type (WT) and Δ*yca1* mutant cells with and without hydrogen peroxide treatment. Analysis of transcriptomics showed increased expression of O-acetylserine sulfhydrylase (OASS), heat-shock protein and oligopeptide transporter in Δ*yca1* mutant cells. The findings suggest a potential link between *YCA1* (cysteine protease) and OASS (responsible for cysteine biosynthesis) during oxidative stress and apoptosis.

Keywords; Hydrogen peroxide, metacaspase, microarray, O-acetylserine sulfhydrylase, Δ*yca1* mutant.

Introduction: Caspases belong to a family of cysteine-dependent aspartyl-specific endoproteases. Several caspases are present in eukaryotic cells that participate in program cell death or apoptosis.[1] Caspases also regulate a variety of cell behaviors such as differentiation, proliferation and growth control.[2] Yeast cells contain a homologue of mammalian cells and termed as "*YCA1* or Metacaspase".[3] Analysis of Proteomics, transcriptomics and metabolomics has added a new dimension to the non-apoptotic function of mutants cells in yeast.[4-7] Deletion of *yca1* in *Saccharomyces cerevisiae* also affect non-apoptotic events such as post-translational modification of proteins, protein biosynthesis, folding and transport, and proteostasis.[4,8] Δ*yca1* mutant cells have also been shown to alter stress response proteins in the absence of induced stress. Mutation of cysteine to alanine residue in catalytic domain of YCA1 (YCA1-C297A) render yeast cells to be resistant to apoptogenic insults.[9] O-acetylserine sulfhydrylase (OASS) catalyzes the final reaction in cysteine biosynthesis, whereas heat-shock protein and oligopeptide transporter participate in protein folding and transportation, respectively, in yeast. We hypothesize that the deletion of cysteine-dependent *yca1* triggers the up-regulation of genes responsible in cysteine biosynthesis, protein folding and peptide transporter protein. The upregulation of these proteins augments under oxidative stress after H_2O_2 treatment.

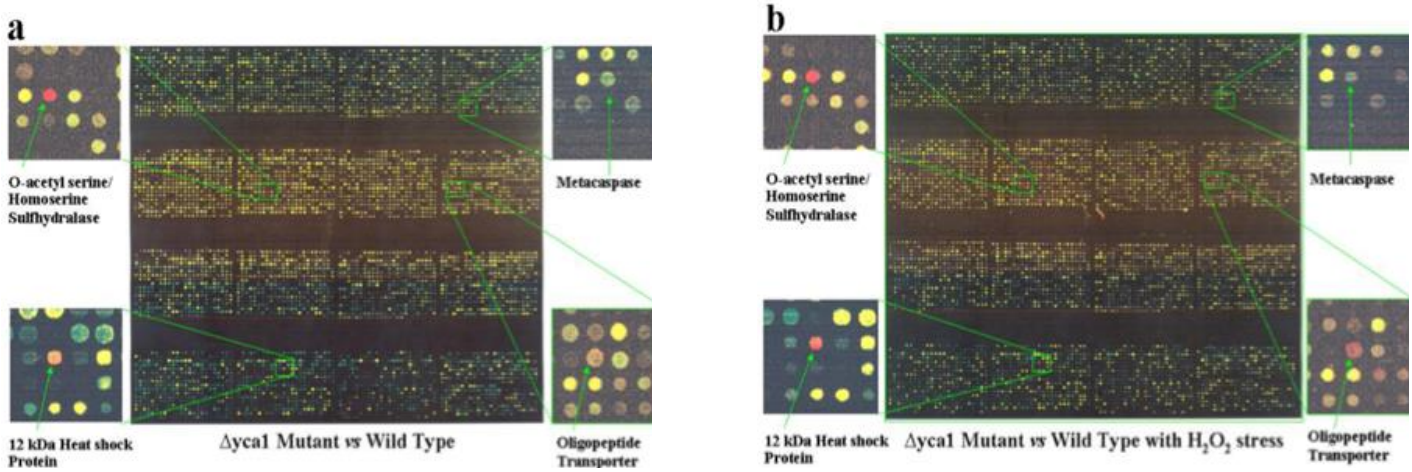

Fig. 1. Two color fluorescent microarray analysis and gene expression profile in *S. cerevisiae*. Comparison of differential gene expression in wild type *versus* Δ*yca1* mutant cells (a) and wild type *versus* Δ*yca1* mutant cells treated with 0.5 mM H_2O_2 (b). Each dot represents a single gene. Highly up-regulated genes after *yca1* deletion are shown in insets.

Methods: WT yeast, *Saccharomyces cerevisiae,* (BY 4743**)** and the Δ*yca1* mutant cells, (Open Biosystems, CO), were grown to early stationary phase (OD600 of 2.0) in 100 ml of YPD medium at 30 °C for 4 h. Both WT and Δ*yca1* mutant cells were treated with (0.5 mM) or without (0 mM) H_2O_2. Total RNA was isolated using Qiagen's RNeasy Kit (Valencia, CA). Spotted oligonucleotide microarrays, ID YO06N, (Duke University DNA Microarray Center) was used for two-color hybridization. Probe preparation and hybridization were performed according to method described elsewhere.[10] Microarray slides were scanned in both Cy3 (532 nm) and Cy5 (635 nm) channels using Axon GenePix 4000B scanner (Axon Instruments Inc., Foster City, CA) with GenePix pro 3.0 software to locate the signal arising from the spots. Data analysis was performed using Gene Spring GX version 7.3 software tool from Duke University DNA Microarray Center.

Table 1. Upregulation of genes in Δ*yca1* mutant cells with and without H_2O_2 treatment.

Systemic Name	Alias	Description	WT vs Δyca1	WT vs Δyca1 + H_2O_2	Biological Function
			Fold Increase*		
Oxidative Stress					
YFL014W	HSP12 GLP1/HOR5	Heat shock protein	5.6	11.2	Response to oxidative stress
YOR028C	CIN5 HAL6/YAP4	Basic leucine zipper transcriptional factor	4.4	4.1	Regulation of transcription from RNA polymerase II promotoer
YGR088W	CTT1	Catalase	2.1	2.9	Protects from oxidative stress
YIR038C	GTT1	Glutathione S transferase	1.5	2.3	Glutathione metabolism
YMR250W	GAD1	Glutamate catabolism	1.3	4.6	Response to oxidative stress
Amino Acid Biosynthesis					
YLR169C	MET17 MET15/MET25	O-acetyl homoserine sulfhydrylase	**52.3**	**84.7**	Sulphur amino acid biosynthesis
YIL169C	---	Serine/Threonie rich protein	7.5	8.2	Molecular function unknown
YLR058C	SHM2/SHMT2	Serine hydroxymethyl transferase	1.7	4.5	Glycine biosynthesis from serine
YNL277W	MET2	Homoserine transacetylase	1.4	2.6	Sulphur amino acid biosynthesis
YMR189W	GCV2/GSD2	P subunit-glycine decarboxylase	1.2	6.5	Glycine metabolism
YKL218C	SRY1	3-hydroxyaspartate dehydratase	1.0	2.1	Amino acid derivative catabolism
YDR019C	GCV1/GSD1	Glycine cleavage T protein	0.9	4.6	Glycine Metabolism
Protein Kinases					
YGL158W	RCK1	Protein kinase	4.8	6.1	Serine/Threonine kinase activity & Regulation of meiosis
YGR052W	FMP48	Mitochondrial protein	2.7	3.0	Kinase activity
YJL107C		Uncharacterized	2.8	2.4	MAPK signaling pathway
Peptide Transporters					
YKR093W	PTR2	Peptide transporter	2.2	3.3	Peptide transport
YPR194C	OPT2	Oligopeptide transporter	1.4	8.9	Oligopeptide transport
Uncharacterized Proteins					
YOL155C			6.8	7.5	Cell wall organization, biogenesis
YHR214C		Putative membrane protein	5.9	6.6	Molecular function unknown
YAR068W		Putative membrane protein	5.3	5.9	Molecular function unknown
YNL164C	IBD2	Component of checkpoint pathway	2.3	4.2	Mitotic spindle checkpoint

*Fold increase compared to wild type (WT). Δ*yca1*, yeast metacaspase.

Results: Microarray data of transcriptomics (microarray) revealed that OASS, 12 kDa heat-shock protein and oligopeptide transporter genes were up-regulated more than 52.3, 5.6 and 1.4 fold in Δ*yca1* mutant cells. However, the upregulation of these genes were further increased to 84.7, 11.2 and 8.9 fold in Δ*yca1* mutant cells after H_2O_2 (0.5 mM) treatment compared to wild type (WT) cells or WT cells treated

with 0.5 mM H_2O_2, respectively, Figs. 1a & b). The upregulation of genes in WT vs Δ*yca1* mutant cells with or without H_2O_2 is shown in Table 1.

Discussion: The salient findings of this study are that *S. cerevisiae* deficient in *yca1* increases OASS (responsible to execute the final reaction in cysteine biosynthesis), low molecular weight heat-shock protein (act as chaperones during protein folding) and oligopeptide transporter (translocate their substrates). The up-regulation of OASS, heat-shock protein and oligopeptide transporter gene products are suggestive of perturbation in cysteine biosynthesis, protein folding and transportation due to *yca1gene deletion*. The superimposition of oxidative stress by H_2O_2 in Δ*yca1* mutant cells was further up-regulated these gene products.

Interestingly, mammals have several caspases but do not have OASS, because the mammals synthesize cysteine from methionine.[11] It has been shown that H_2O_2 treatment of Δ*yca1* mutant cells with the catalytic inactivation of cysteine, substituting cysteine to alanine (C297A), demonstrate lower apoptotic response.[9] A genome wide analysis show that Δ*yca1* mutant cells are enriched for the high molecular weight chaperones (e.g., Hsp70 and 104), which involve in disaggregation of insoluble protein aggregates.[12] Small heat-shock proteins with a molecular mass of 12 kDa form only dimers and tetramers and possess small chaperone activity.[13] It is intriguing to pursue whether the mRNA and protein levels of OASS, heat-shock protein and oligopeptide transporter also consistently increase reflecting upregulation of these genes, and the their mRNAs could be translated to functional proteins. We and others showed that the disruption of caspases in mammalian as well as in yeast cells not only participate in apoptotic process, but also in non-apoptotic physiological and metabolic processes such as amino acid biosynthesis, protein folding and transport.[1-9,12,13]

Conclusion

Overall, our findings suggest that deletion of *yca1* gene in *S. cerevisiae* is responsible for the up-regulation/accumulation of OASS, heat-shock protein and oligopeptide transporter gene products and could affect cysteine biosynthesis, protein folding, and protein transportation, respectively, transcription and/or translation levels. The establishment of link especially between YCA1 and OASS could open an avenue to understand the molecular mechanisms in *de novo* biosynthesis of cysteine under *yca1* regulation.

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