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Mini Review

Humanized Yeast Models of Misfolding Diseases: A Comparative Study of Experimental Advantages

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Abstract

Saccharomyces cerevisiae, is a multifaceted experimental system for investigating complex biological processes (i.e. functional genomics and systems biology) and to study crucial questions concerning the pathogenic role of human proteins in neurodegenerative diseases The yeast cell, this humble servant of mankind, has been upgraded to the status of the first eukaryotic cell from which informatic boolean networks have compiled all molecular interactions between genes and gene products and all metabolic fluxes in hundreds of different physiological conditions, thus that *Saccharomyces cerevisiae* has been a pioneering model for studying the regulation of eukaryotic metabolism [1]. The yeast, *Saccharomyces cerevisiae*, is the best-studied eukaryotic cell, at both genetic and physiological levels. Thus, through a worldwide collaboration it is was the first eukaryote organism to be fully sequenced in 1996 [2]. The sequence of 12,068 kilobases defines 5885 potential protein-encoding genes, approximately 140 genes specifying ribosomal RNA, 40 genes for small nuclear RNA molecules, and 275 transfer RNA genes. Moreover, yeast has became an important resource as a test tube to enhance and accelerate the human genome project.

Keywords: *Humanized Yeast Models; Misfolding Diseases; Saccharomyces cerevisiae*

High throughput data obtained from functional genomics approaches, such as transcriptomics, proteomics, metabolomics, interactomics (protein–protein interactions) and locasomics (protein subcellular localization), are well organized and permanently actualized in yeast public databases. Notably, information about predicted orthologs in humans is also organized and available for each yeast gene (see table 1 and 2) as reported in Tenreiro S and Outeiro 2010 [3].

	Chromosome	Length (in kb)	Coordinator	Public availability	Reference*
S. cerevisiae	III	315	S. Oliver (UK)	March 1992	1
S. cerevisiae	XI	666	B. Dujon (France)	June 1994	2
S. cerevisiae	VIII	589+CUP1	M. Johnston (USA)	September 1994	3
S. cerevisiae	II	813	H. Feldmann (Germany	December 1994	4
S. cerevisiae	I	230	H. Bussey (Canada)	April 1995	5
S. cerevisiae	VI	271	Y. Murakami (Japan)	July 1995	6
H. influenzae		1850	R. Fleischmann, C. Venter (USA)	July 1995	7
M. genitalium		471	C. Fraser, C. Venter (USA)	October 1995	8
S. cerevisiae	Total genome (web)	13 478	W. Mewes, J. Sgouros, K. Kleine, J. Hani, A. Zollner (MIPS, Germany), M. Cherry (SGD, USA)	April 1996	9
S. cerevisiae	X	745	F. Galibert (France)	June 1996	10
M. jannaschii		1660	C. Bult, C. Venter (USA)	August 1996	11

S. cerevisiae	Total genome (paper)	13 478	A. Goffeau (Belgium)	October 1996	12
M. pneumoniae		816	R. Himmelreich (Germany)	November 1996	13
Synechocystis		3573	T. Kaneko, S. Tabata (Japan)	November 1996	14
S. cerevisiae	Genome directory		A. Goffeau and 641 others (Europe, USA, Japan, Canada)	May 1997	15
S. cerevisiae	IV	1532+PMR2	C. Jacq (France)	May 1997	16
S. cerevisiae	V	577	F. Dietrich (USA)	May 1997	17
S. cerevisiae	VII	1091	H. Tettelin (Belgium)	May 1997	18
S. cerevisiae	IX	440	B. Barrell (UK)	May 1997	19
S. cerevisiae	XII	1078 + 1 Mb rDNA	M. Johnston (USA), J. Hoheisel (Germany)	May 1997	20
S. cerevisiae	XIII	924	B. Barrell (UK)	May 1997	21
S. cerevisiae	XIV	784	P. Philippsen (Switzerland)	May 1997	22
S. cerevisiae	XV	1091	B. Dujon (France)	May 1997	23
S. cerevisiae	XVI	948	H. Bussey (Canada), B. Barrell (UK), K. Davis (USA), M. Johnston (USA), A. Goffeau (Belgium)	May 1997	24
S. cerevisiae	mtDNA	86	F. Foury (Belgium)	December 1998	25

Table 1: *The first yeast and bacterial chromosome sequences (from Four years of post-genomic life with 6000 yeast genes, André Goffeau. https://doi.org/10.1016/S0014-5793(00)01775-0 and * references therein from 1 to 25.*

	Database	Type	Website
1	*Saccharomyces* genome database (SGD)	Wide-range infos	http://www.yeastgenome.org/
2.	MIPS comprehensive yeast genome database (CYGD)	Wide-range infos	http://mips.gsf.de/genre/proj/yeast/
3	European *Saccharomyces cerevisiae* archives for functional analysis (EUROSCARF)	Strain and plasmid collections	http://web.uni-frankfurt.de/fb15/mikro/euroscarf/
4	Yeast microarray global viewer (yGMV)	Microarrays data	http://transcriptome.ens.fr/ymgv/
5	Database yeast search for transcriptional regulators and consensus tracking (YEASTRACT)	Transcription regulatory associations	http://www.yeastract.com/
6	Profiling of phenotypic characteristics in yeast (PROPHECY)	Phenotypes of deletion strains	http://prophecy.lundberg.gu.se/
7	Mitochondrial proteome (MitoP)	Mitochondria-related genes, proteins and diseases	http://www.mitop.de:8080/mitop2/
8	Eukaryotic orthology (YOGY)	Orthologous proteins from eukaryotic orgranisms	http://www.bahlerlab.info/YOGY/
9	Princeton protein orthology database (P-POD)	Orthologous proteins;	http://ppod.princeton.edu/
10	Yeast protein localization database (YPL.db)	Subcellular localization of yeast proteins	http://ypl.uni-graz.at/pages/home.html
11	BioGRID	General repository for interaction datasets	http://www.thebiogrid.org/

Table 2: *Saccharomyces cerevisiae databases employable on the WEB (in the list are reported Database, Type and Website).*

Thus, yeast is approved to be a robust system for studying the mechanisms underlying several cellular pathways, with strong applicability to human disease and for the discovery of novel drug targets for therapeutic activities. One of the paramount research goals in the field is the elucidation of mechanisms causing proteins to misfold and aggregate, as well as to understand their function in normal biology. Protein folding is critically important for all of life, from microbes to man [2].

In fact yeast cells possess strong similarities to human cells. Around 60% of the yeast genes show sequence homology to a human orthologue [4] and of the human disease-related genes, over 25% have a close homologue in yeast [5]. The basic biomolecular events involved in neurodegenerative processes such as mitochondrial dysfunction, transcriptional dysregulation, trafficking defects and proteasomal impairment can be studied in simple organisms such as yeast [6] given that are highly conserved between yeast and human species.

As world's population continues to age, neurodegeneration will increase in prevalence and thus pose a daunting challenge to public health worldwide. Neurodegenerative disorders, such as Parkinson's Disease (PD) and Alzheimer's diseases (AD), belong to the wide superfamily of pathologies known as protein misfolding disorders [7]. The manner in which a newly synthesized chain of amino acids transforms itself into a perfectly folded protein depends both on the intrinsic properties of the amino-acid sequence and on multiple contributing influences from the crowded cellular milieu. Folding and unfolding are crucial ways of regulating biological activity and targeting proteins to different cellular locations. Aggregation of misfolded proteins that escape the cellular quality-control mechanisms is a common feature of a wide range of highly debilitating and increasingly prevalent diseases [8]. The common hallmark of these disorders is the folding of particular proteins into an abnormal three-dimensional conformation, which makes these proteins more prone to aggregate and form amyloid-like b-sheet structures.

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of unknown aetiology, characterized by irreversible cognitive and physical deterioration. It is a major cause of morbidity and death in the elderly and a growing public health problem as life expectancy in the general population increases. AD is both genetically and phenotypically a heterogeneous disorder.

The neuropathological hallmarks of AD are senile (neuritic) plaques (SPs) and neurofibrillary tangles. Several types of SPs can be distinguished, but all plaques contain extracellular deposits of amyloid- b peptide (Ab) that include abundant amyloid® brils with non® brillarforms. A b is generated during proteolytic processing of amyloid precursor protein (APP).

Parkinson's disease is a chronic, progressive neurodegenerative disorder involving the dopaminergic neurons in the substantia nigra pars compacta of the brain. Oxidant-generating enzymes, such as monoamine oxidase and tyrosine hydroxylase are contained in substantia nigra dopaminergic neurons Thus oxidative stress is a hallmark in the degenerative process of PD [10].

Further, the proposed elements that potentially cause oxidative stress in PD are the neurotransmitter dopamine metabolism, mitochondrial dysfunction, and neuroinflammation.

Neuronal ATP production depends on mitochondrial aerobic respiration, which typically provides H_2O_2 and superoxide radicals as byproducts in the course of oxidative phosphorylation in mitochondria. Mitochondrial dysfunction can produce a powerful increment in reactive oxidant species (ROS) overcoming the cellular antioxidant mechanisms. Moreover, environmental factors, such as neurotoxins, pesticides, insecticides, dopamine metabolism, and genetic mutations in PD-associated proteins contribute to mitochondrial dysfunction [3]. Indeed, α-synuclein seems to inhibit mitochondrial Complex I, and dopamine quinone species target Complex I and Complex III of electron transport chain. The increment in ROS formation is comparative to the extent of complex I inhibition. Consequential to it, aconitase, a Kreb's Cycle enzyme, is inactivated due to oxidation of its iron-sulfur clusters, further to the increased peroxidation of the mitochondrial phospholipid cardiolipin releasing cytochrome c, and finally triggering apoptosis. Collectively, mitochondrial dysfunction leads to increased mitochondrial ROS contributing to PD pathogenesis. Neurodegeneration in PD is related with chronic neuroinflammation

restrained permanently by activated microglia. Yeast cells in stationary phase must cope with the same challenges that the neurons in our brain face over a lifetime: the accumulation of damaged proteins, oxidative stress, proteasome impairment and the buildup of mutations, without the ability to dilute out these cellular insults by dividing. It was been important to test the effect of stationary phase aging on existing as well as yeast models of neurodegenerative disease [11]. It has already been from 2003 since yeast was used for the first time as a model to study α-synuclein toxicity [12]. More recently, a yeast model was also designed to study the presumed pathobiology of the α-synuclein interaction partner synphilin-1 after that in the past fifty years, different investigators had developed great expertise in uncovering the cellular aspects of α-synuclein toxicity using humanized yeast models Despite its limitations as a unicellular eukaryote, yeast can faithfully reproduce key features of PD pathology. Moving on from studying mere protein aggregation and growth inhibition, these models have started to provide a tool to study new features of the α-synuclein induced-cellular toxicity. One of the new advances that has been studied in yeast addresses the role of an intracellular Ca^{2+} buildup upon α-synuclein expression, mediated by the plasma membrane-related Ca^{2+} ATPase. This Pmr1- induced Ca^{2+} increase appears to be essential for α-synuclein toxicity from yeast to flies and nematodes. Furthermore, the yeast polo-like kinase 2, Cdc5, which was thought to lead to α-synuclein toxicity by phosphorylating Ser129, seems to be inhibited itself by α-synuclein, inducing to diminished cell wall integrity signaling. Furthermore, an α-synuclein-induced diminution of PLK2 signaling, originating in inhibition of MAPK signaling, was also demonstrated to increase stress sensitivity in mammalian cells. Lastly, yeast has disposed some proofs that α-synuclein- induced toxicity is subordinate on the mechanism of mitophagy, which has currently also been involved in the PD pathology mediated by human PINK and Parkin-1. These issues try the advantage of humanized yeast models in bringing to light to new biomolecular and cellular functions of α-synuclein and synphilin-1 toxicity [12].

Saccharomyces cerevisiae cultures display a fast growth, with a doubling time of 1,5 to 3 hours. This consents as a rapid and easy scale-up, which is beneficial for high-throughput genetic and small-biomolecule screens. Yeast is easily responsible to several distinct genetic changes. Accessible DNA transformation and the convenience of a host of selectable markers allow the introduction of multiple self-replicating plasmids. Further, stable and highly specific introduction of genes, modifications, and markers in the genome, through introduction of DNA sequences by homologous recombination, is deeply competent in yeast. Finally, what makes yeast especially attractive is an extensive set of high-throughput tools that lend themselves to a systematic and genome-wide analysis of particular cellular processes or screenable phenotypes. These include not only comprehensive collections of yeast mutants with gene deletions, hypomorphic alleles, and conditionally repressible promoters, but also plasmid libraries that allow the study of gene overexpression or systematic localization studies using the yeast GFP-fusion collection [5].

Thus, *Saccharomyces cerevisiae* that is among the best-studied experimental organisms thus the passage from model organism to the simplest eukaryotic model for studying human diseases happened naturally. The experimental advantages of the budding yeast *Saccharomyces cerevisiae* are increasingly being exploited to elucidate the function of genes mutated in human disease states. Classical and recombinant genetics approaches have, for example, allowed researchers to isolate second-site revertants for the most common mutation in the human CFTR gene associated with cystic fibrosis1 thus its readily manipulable system provides an opportunity to dissect the molecular pathways underlying normal and the pathogenic consequences of misfolding [13]. A lot of proteins can start to fold and start to assemble their quaternary structure at the same time as the biosynthesis on the ribosome. Co-translational folding is a nonequilibrium process, the outcome of which is dependent on the interplay between the rate of protein folding and the rate of translation by the ribosome. Finally, a profoundly comprehension of these mechanisms may influenced to enhanced protein expression for biotechnological employments, deeper competency to figure out and treat the diverse diseases originating from protein folding deficiencies, protein misfolding, and aggregation; and the promise of discriminating targeting polypeptide translation for therapeutic functions by means of small molecules.

Protein biosynthesis and quality control are energy-intensive processes that take place within the context of limited cellular resources, and efficient protein folding has therefore been under strong selective pressure since the earliest stages of the emergence of life. A wide range of mechanisms have been identified that help to ensure that protein biosynthesis within the cell occurs both correctly and efficiently.

These mechanisms include passive strategies, such as the evolutionary optimisation of co-translational free energy landscapes and site-specific variations in translation kinetics, and the holdase functionality of the ribosome surface, and active ones, including the intervention of chaperone systems such as TF and ATP-dependent chaperones such as Hsp70, and ultimately the proteasome. The ribosome is a central hub within this quality control network, ultimately providing effective and energy efficient defences against potentially lethal misfolding and aggregation processes. Describing the coupling between folding and translation requires the development and application of the appropriate theoretical and experimental tools. The immediate experimental challenges are the development of methods to expand our understanding of the mechanisms by which free energy landscapes, and the kinetics of kinetics may be modulated or regulated. In particular, a fascinating aspect of protein biosynthesis that has only recently become possible to explore is the development of a molecular understanding of the feedback and interplay of these various mechanisms on co-translational folding processes. The discovery of the coupling between folding and translation processes undoubtedly indicates fertile ground for future research. The further elucidation of these processes, both in prokaryotes and eukaryotes, presents an exciting challenge for the years ahead in the quest to define in molecular detail the way in which information encoded in the genome is converted into biological activity [14].

Conclusion

Yeast is still the most facile eukaryotic cell for analyzing the relationship of genotype to phenotype in eukaryotic cells. Much is known about the transmission of the traditional carriers of information, DNA and RNA, during mitosis and meiosis, but little is known about the inheritance of organelles, macromolecules such as polysaccharides and lipids, and the myriad small molecules that populate the cells of living organisms [13]. An essential pending issue is the subcellular transport of proteins and metabolites in the cell cycle. Yeast, more than any other organism, has led the way to another, potentially more important frontier beyond the functions of single genes and proteins: the "systems level" [13]. The purpose is knowing the functions of organization of genes and proteins as they act to sustain metabolism and cellular homeostasis under a huge variety of environmental surroundings and to supply for the regulation and organization of reproduction, cellular growth, and development. Thus, for the expected time to come, the experimental benefit provided by yeast will help to manage this model organism at the cutting edge of this topical frontier in order to draw more attention on this valuable model system for neurodegenerative diseases and improve health status of an increasing number of patients.

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