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An Overview of Yeast-Based Toxicology: Unraveling Molecular Mechanisms, Advancing Drug Discovery, and Addressing Translational Complexities

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Abstract

Saccharomyces cerevisiae, commonly referred to as budding yeast, has become widely recognized as an invaluable model organism for investigating eukaryotic cells. Yeast cells serve as an apt model for studying toxicity due to their convenient genetic manipulability, short life cycle, and easy access to genomic sequences, facilitating straightforward cultivation. Sensitivity to toxic materials is influenced by cellular components. Genes play a role in the transfer, partition, or sequestration of metallic or other toxic substances. These features, along with relevant pathological conditions, have been thoroughly explored and confirmed in the genomic sequence of Saccharomyces cerevisiae. Additionally, this model is effective for investigating the molecular mechanisms that regulate the toxic potential of metals or other harmful substances. Although still in the early stages, these technologies exhibit promise as inventive and effective methods for the development of target-specific therapeutic approaches.

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1. Introduction

Toxicity is characterized as "the extent to which a substance (toxin or poison) can cause harm to humans or animals." Various toxicity models have been devised to evaluate the detrimental effects of substances on the human body.[1] However, it is essential to acknowledge that insights derived from animal studies may not necessarily translate directly to humans. As awareness grows regarding potential risks associated with this alternative, there is a shifting perspective on relying solely on this approach.[2]

The gold standard for toxicity testing has traditionally involved assessments in animals. However, there has been a growing encouragement for the use of in vitro experiments as an alternative to animal experimentation in recent years. In vitro testing offers advantages such as speed, cost-effectiveness, and the avoidance of ethical concerns.[3] In summary, in vitro assays are crucial alternatives in the context of toxicogenomics (TGx) and play a significant role in the evolving risk assessment paradigm. They hold substantial potential for advancing non-animal testing within TGx systems. Both in vivo and in vitro experimental systems are pivotal for exploring functional performance in drug discovery, understanding vital processes in living organisms, and conducting toxicological research. However, the higher costs associated with in vivo experiments pose a challenge, making it more challenging to integrate them into large-scale projects.[4]

To address this challenge, a comprehensive understanding of the mode of action and mechanisms driving toxic responses

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in humans is essential. This understanding instills confidence in transitioning from animal experiments to in vitro human cell-based toxicology, thereby enhancing human protection. Despite the promise of in vitro models, they currently exhibit limitations, such as reduced metabolic competence and the use of cancer cells. Future research and development endeavors need to overcome these limitations, ensuring that the cell-based models accurately reflect the mechanisms required for toxicity in vivo. This is crucial to avoid false negative or false positive responses, where models may either fail to express essential in vivo mechanisms or express mechanisms that are not active in a healthy individual.[5]

Yeast, notably Saccharomyces cerevisiae, is extensively studied, with over 6,000 well-annotated protein-coding genes. The genome, fully sequenced in 1996, has been a foundational resource for systematic studies at both the genome and proteome levels, solidifying (Fig. 1) yeast as a leading model organism in scientific research.[6]

Yoshinori Ohsumi received the 2016 Nobel Prize in Physiology or Medicine for his pioneering research in autophagy, primarily conducted in yeast. Significantly, this accolade represents the fifth Nobel Prize awarded in the 21st century for research accomplished using yeast, emphasizing its crucial contribution to scientific advancements.[7]

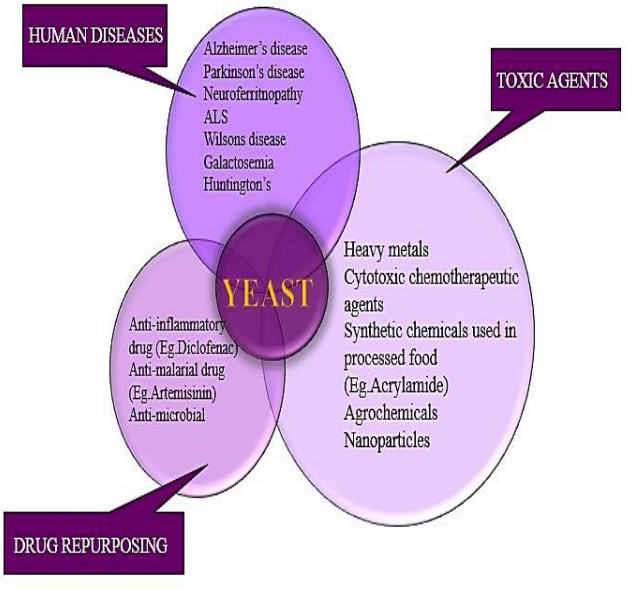

Fig. 1 Yeast as a model for different studies.

2. *In Vitro* assays for toxicity testing

A wide array of *in vitro* assays leverages yeast, bacterial, mammalian, or human cell cultures, as well as tissue slices and subcultures, to assess diverse toxic endpoints. These endpoints encompass ocular irritation, skin corrosion/irritation, carcinogenicity, and reproductive and developmental toxicity testing, among others. The trajectory of *in vitro* toxicology, as an alternative to animal testing, is marked by the application of molecular biological techniques to cell lines, aiming to enhance their resemblance to human responses.[8] In vitro systems prove particularly advantageous for probing into the molecular, cellular, and physiological mechanisms of chemically induced toxicity. This is especially pertinent for studying known target organ and target species toxicity, addressing specific questions about toxic effects that may not be easily investigated in vivo. The future of in vitro toxicology pivots on refining these systems to closely mimic human responses, thereby strengthening their effectiveness as alternatives to traditional animal toxicity studies. This evolution is crucial for advancing our understanding of toxicological mechanisms and ensuring the reliability of in vitro assays across various toxic endpoints.[8]

2.1 Key factors steering the advancement of in vitro toxicology

The progress of *in vitro* toxicology is guided by several crucial factors, each contributing to the growing importance of alternative testing methods: The necessity for cost-effective and efficient testing systems to evaluate a large number of chemicals is driving the evolution of *in vitro* methods. These approaches offer a reorganized way to estimate toxicity without the difficulties and expenses associated with traditional animal testing. Increasing public awareness and legislative pressures emphasize the reduction of animal experimentation. In response to ethical concerns and evolving regulations, the scientific community is increasingly adopting *in vitro* models as humane alternatives, aligning with societal priorities for animal welfare. There is a growing need for a deeper understanding of the mechanisms behind chemical-induced toxicity. *In vitro* approaches empower researchers to explore intricate details of toxicological mechanisms, contributing to the refinement of existing risk assessment procedures (Fig. 2).

In vitro testing offers researchers greater control over variables compared to whole-animal testing. This heightened control enhances the precision and reproducibility of experiments, enabling a more detailed exploration of toxicological mechanisms. The convergence of these factors underscores the pivotal role of *in vitro* toxicology in addressing contemporary challenges and shaping the future landscape of toxicity testing methodologies.[9]

2.2 Enhancing cellular toxicity assessments: unravelling molecular complexity

In the realm of cellular toxicity assessments, numerous

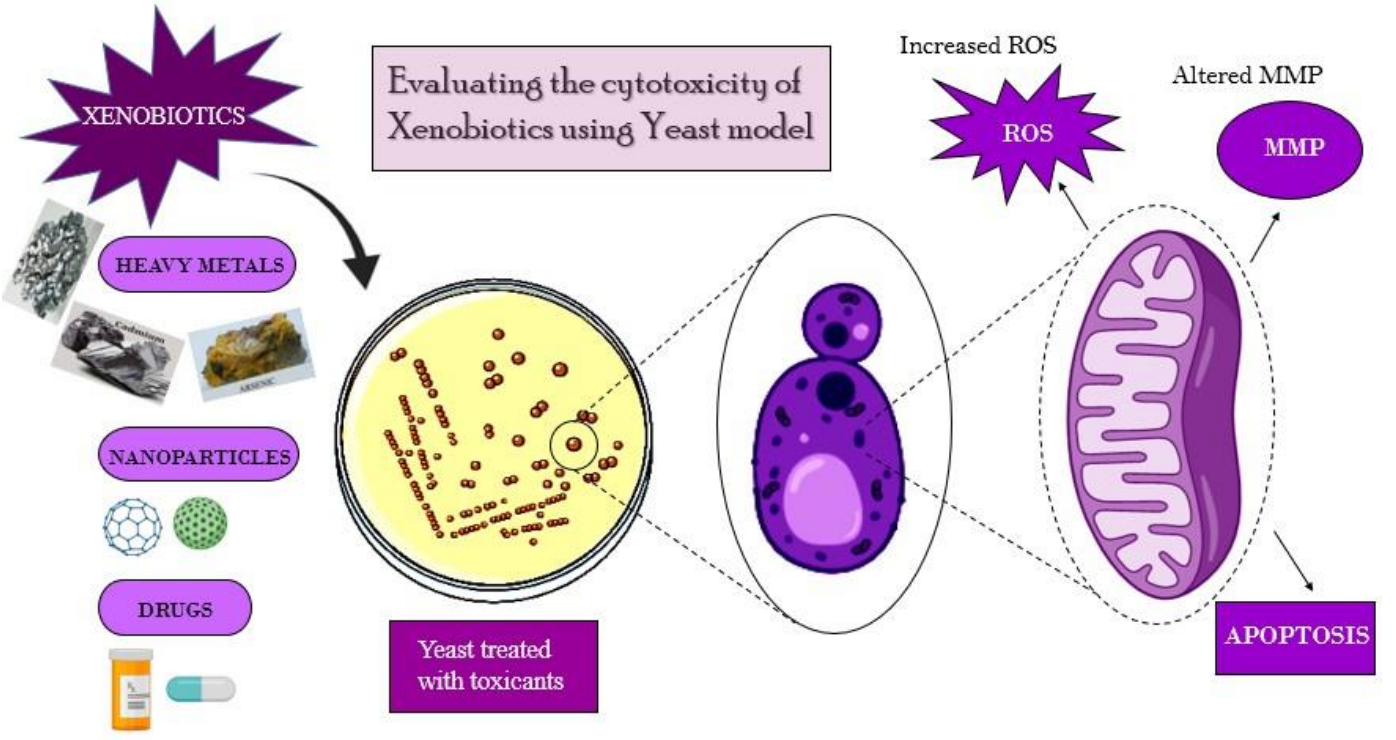

Fig. 2 Illustrations of xenobiotics and potential use of yeast as a testing model.

pathologies observed in both animal and human studies remain inadequately understood at the molecular and biochemical levels. The term "toxicity" encompasses a wide array of pre-apoptotic cellular events that necessitate models authentically representing *in vivo* cellular mechanisms. Oversimplified mimics often fall short in reliably testing these intricate events. Furthermore, uncovering idiosyncratic toxicities or cellular responses to novel drug candidates, especially those lacking extensive structure-toxicity information, relies heavily on cultured cellular models. These models should exhibit dependable, well-known, and intact biochemical pathways and structural elements, ensuring the detection of toxicity signals with higher reliability.

The importance of such capabilities extends beyond traditional applications. It opens new avenues for the utilization of in vitro cell-based research by facilitating integrated data utilization, enhancing drug screening outcomes, and elevating the quality of pharmacokinetic assessments. This approach holds promise for a more comprehensive and nuanced understanding of toxicity, enabling more precise evaluation and prediction of cellular responses to various compounds.

Despite the challenges associated with assessing functional conservation between yeast and human genes, yeast provides invaluable insights into understanding gene functions associated with human diseases. Unlike humans, *S.cerevisiae* genes can be readily manipulated, allowing for swift deletion, mutation, reintroduction, overexpression, tagging, and in-depth study. This expeditious process yields a substantial amount of information crucial for unraveling the molecular basis of diseases, showcasing the utility of yeast as a model organism in advancing our understanding of cellular toxicity.[10,11]

There is significant homology between the functional biochemical pathways of yeast and humans. Key pathways involved in cell cycle, metabolism, programmed cell death, protein folding, quality control and degradation, vesicular transport are found to be conserved in yeast. Sharing more than 2000 genes with humans, yeast serves as a good model for human disorders.[12]

To give an example, mechanisms and pathways such as mitochondrial dysfunction, dysregulation in transcription and proteosomal impairment, involved in neurodegenerative disorders such as Parkinsons and Huntington's, are highly conserved in yeast and human.[13] Hence studying the fundamental events at the molecular level involved in pathologic progressions is possible in yeast. On the other hand, if the yeast lacks the disease associated genes, cloning the human cDNA heterologously in yeast expression vectors has led to a more humanized yeast models.[14]

Co-staining with annexin-V (AnnV) and propidium iodide (PI) enables the differentiation of early apoptotic cells, characterized by phosphatidylserine (PS) externalization (AnnV+, PI−), cells undergoing primary necrosis with a

ruptured plasma membrane (AnnV−, PI+), and late apoptotic/secondary necrotic cells displaying both PS exposure and membrane permeability (AnnV+, PI+). The TUNEL test is employed for assessing apoptotic DNA fragmentation, while dihydroethidium (DHE) is commonly used to measure the generation of reactive oxygen species (ROS) (Fig. 3). In the DHE assay, the conversion of non-fluorescent DHE into fluorescent ethidium (DHE → Eth) driven by superoxide can be monitored automatically. Visualization of stained cells through microscopy and quantification via cytofluorometry are standard practices. Nuclear fragmentation and chromatin condensation can be observed with DAPI staining. Notably, yeast offers the advantage of easily evaluating actual cell death rates using clonogenic assays. In these assays, a fixed quantity of cells is plated, and the ability to form a colony is assessed, providing precise quantification of dead versus living cells since only viable cells can form colonies.[15]

3. Exploring the potential of S. cerevisiae in drug discovery and toxicology

S. cerevisiae, a unicellular eukaryote, stands out as an excellent model system for drug discovery due to its cost-effectiveness, ease of maintenance, and GRAS (generally recognized as safe) classification. The fully sequenced genome of *S. cerevisiae,* coupled with the conservation of major signalling pathways and cellular processes between yeast and mammalian cells, makes it a compelling choice for high-throughput studies in drug discovery.

While the preference for whole-cell screens within human cells remains, yeast-based functional genomic and proteomic technologies offer an alternative that is inexpensive, robust, and conducive to identifying novel drug targets and chemicals influencing their biological functions. Conserved pathways are observed in eukaryotic cells, specifically in yeast and other mammal cells, which can be used as a model for drug delivery. Additionally, yeast toxicogenomic approaches have emerged as valuable tools for defining and predicting new toxicological outcomes and mechanisms, particularly in response to commercial pesticide formulations. These efforts aim to identify potential indicators of damage in more complex eukaryotes, highlighting the broader impact of *S. cerevisiae* in advancing toxicology research.[16,17]

The yeast *S. cerevisiae* serves as a convenient model organism for assessing toxic effects on human cells and tissues. It is frequently utilized in studies examining the genotoxicity of various chemicals.[18] Nweke explored the impact of metal toxicity on S. cerevisiae using the TTC-dehydrogenase activity inhibition test. This assay affirmed the toxic effects of metals on S. cerevisiae, with a testing duration exceeding 24 hours.[19] The toxicity mechanisms of silver nanoparticles were investigated. Evaluation of toxicity involved assessing growth inhibition in rich YPD medium and cell viability in deionized water.[20] Hosiner *et al*. determined the lowest observable effect level and EC50 values for 10 metal ions affecting yeast growth. Optical density was used for monitoring, and the results were obtained after more than 12 hours.[21]

The pivotal aspect of our study focused on assessing the impact of toxicants on yeast fermentation activity. A s conducted significant research in this area, observing changes in the fermentation activity of *S. cerevisiae*. Their methodology involved measuring CO_2 production in yeast

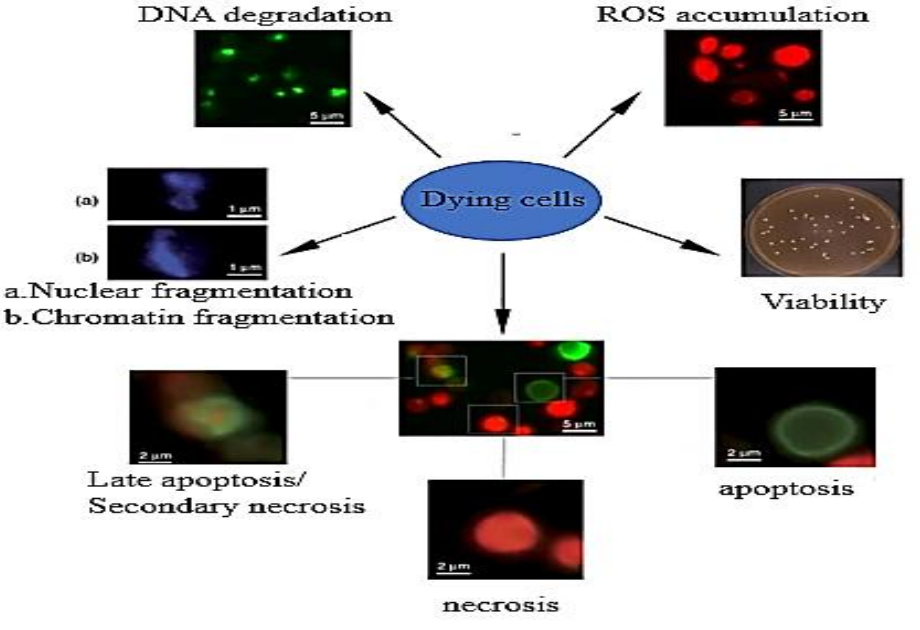

Fig. 3 Programmed cell death in yeast. Reproduced with the permission from [15], Copyright 2010 Macmillan Publishers Limited.

cell suspensions exposed to various toxic substances, including organic compounds, inorganic salts (particularly heavy metals), surfactants, and plant pesticides. In comparing their results with Tetrahymena pyriformis assays, the authors found a 90% congruence in the sensitivity of both tests.[22]

A study conducted on assessment of a yeast toxicity test, focusing on the inhibition of saccharose fermentation activity in *S. cerevisiae*. Standard toxicants, including copper sulfate, formaldehyde, sodium nitrite, sodium sulfite, phenol, and zinc sulfate, were employed in the study. It was observed that the toxicity of wastewater from the pharmaceutical industry, as determined by the yeast test, exhibited a satisfactory correspondence with results obtained through standard methods, such as the inhibition of V. fischeri bioluminescence and the inhibition of dehydrogenase activity.[23]

4. Autophagy in yeast

Autophagy, a highly regulated cellular process observed in various eukaryotes, ranging from yeast to more complex organisms, is integral for the degradation and recycling of cellular components. Unlike the proteasome, which primarily degrades short-lived individual proteins, autophagy plays a crucial role in breaking down and recycling long-lived proteins, large protein complexes, and organelles. The process involves delivering cytoplasmic cargo to the vacuole, where it undergoes ultimate degradation. There are three primary types of autophagy: microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA), with CMA notably absent in fungi.[24]

First identified in yeast, the autophagy process encompasses more than 40 genes organized under the autophagy-related (ATG) classification.[25]

Autophagy initiation starts at a specific site called the phagophore assembly site (PAS), positioned close to the vacuole. This crucial step is rigorously regulated by the Atg1 protein complex, which includes the Atg1 kinase, Atg13, and the ternary subcomplex Atg17-Atg31-Atg29.[26]

5. Multidrug resistance and open reading frames

Yeast may not naturally encounter many cytotoxic compounds found in its environment, the fundamental mechanisms governing adaptation and resistance to various stresses, including chemicals, appear to be conserved across phylogenetically distant organisms. This emphasizes the utility of yeast as a model system, offering in-depth insights into molecular mechanisms that might be challenging to unravel in more complex eukaryotes. The knowledge gained from studying multidrug resistance (MDR) and its regulation in *S. cerevisiae* extends beyond yeast itself. It holds the potential to inform the understanding of pathogenic yeasts, such as Candida species, paving the way for the development of innovative prophylactic, diagnostic, and therapeutic strategies to address the growing challenge of drug-resistant fungal infections.[27]

A notable achievement in utilizing the eukaryotic model *S. cerevisiae* is its successful application in identifying novel molecular mechanisms of action and targets for the anticancer drug imatinib. This milestone underscores the versatility of yeast in contributing to our understanding of drug actions and mechanisms.[28] The foundation of this profiling relies on the functional information derived from genes and gene groups. Notably, yeast has been extensively studied in the analysis of gene function. Conversely, many Open Reading Frames (ORFs) in the genomes of various organisms, including plants and animals like humans, remain annotated as "function unknown." The transcriptional response data for ORFs with unknown functions are limited and less informative. It has been reported that approximately 31% of proteins encoded by the yeast genome have homologs in humans. Conversely, around 50% of human genes associated with heritable diseases have homologs in yeast. This highlights the utility of the yeast system as a model for toxicogenomics.[29]

The insights gained from yeast-based studies and the identification of homologous systems in humans are expected to play a crucial role in advancing the diagnosis and treatment of diseases related to transition metals. Additionally, these studies contribute to the broader understanding of how cells maintain the balance of metals as essential nutrients versus toxic entities.[30] In cases where disease-associated gene(s) in humans lack close orthologs in yeast, the approach of heterologous expression of the human disease gene in yeast, often termed "humanized yeast," becomes valuable. This method enables the exploration of conserved protein interactions and their context in yeast, offering insights into the molecular mechanisms underlying disease development and progression.[31]

The Saccharomyces genome has undergone significant annotation, with approximately 85% of its genes having a rudimentary understanding of their biological roles. This percentage is notably higher for yeast compared to other eukaryotes. However, around 15% of Saccharomyces genes still lack complete annotation. While annotating the last 15% may be a challenging task, the ultimate goal is to comprehend the functions of gene and protein ensembles as they contribute to maintaining metabolism, cellular homeostasis, and the regulation of reproduction, cellular growth, and development. The experimental advantages offered by yeast position it as a key model organism at the forefront of advancing our

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The incorporation of omics has been of utmost importance in recent years in understanding the big picture of biological systems. Recently, systematic, and comparative omics was used to discover MTP family members in potatoes for the first time. Specific genes StMTP 8/9 were found to greatly influence cadmium content in yeast cells. The findings provide a reference for studying the molecular mechanisms of toxic metal accumulation in potatoes and also for environmental management in places of high Cd accumulation.[33,34] Recently, utilized 2DE and 1H NMR based proteomics and metabolomics to study the effect of Zinc oxide nanoparticles, with yeast as their experimental model. They found that exposure to ZnO-NPs downregulated the protein expression by 40% altering physiological and metabolic processes. They also performed metabolomics which revealed a vast range of important metabolites involved in central carbon metabolism, nucleoside and nucleotide synthesis pathways and amino acid and fatty acid synthesis pathways, were repressed. This suggested their approach of using different omics studies provided potential information about the molecular mechanisms of ZnO toxicity.[35]

Utomo et al summarise the usage of CRISPR Cas9 system as a solid approach to reconstruct complex metabolic pathways by perform multiplex gene editing. Synthesis of plant metabolites has become a vital part of the pharmaceutical industry. In order to elucidate the complex pathways and genes involved in, omics and genome editing has proven to be revolutionary technologies. Yeast as a model provides the perfect platform to investigate the metabolic pathways (Fig. 4) especially complex plant biosynthetic pathways. Gene integration, gene downregulation and upregulation are implemented using various RNA cleaving mechanisms including HDV, tRNA, Csy4, and HI-CRISPR as they are simple and efficient.[37]

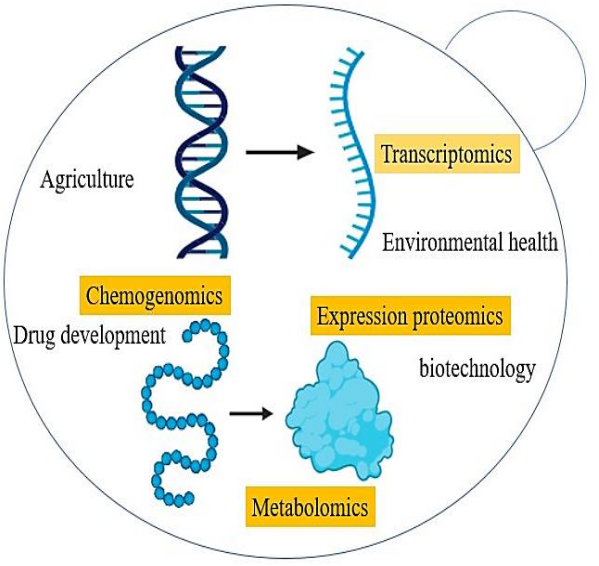

Fig. 4 Predicted contribution of Omics approaches applied in the yeast Saccharomyces cerevisiae to obtain toxicological mechanistic insights with application in environmental health, agriculture, drug development, and biotechnology. Reproduced with the permission from [36].

CRISPR Cas9 has been found to be a resourceful tool for bioremediation of Xenobiotic contamination. Understanding xenobiotic metabolism and therefore usage of gene editing to upregulate relevant degradative enzymes has been a promising exploration. Yeast as a model has been used to study major enzyme families, including cytochrome P450, which was found to degrade polycyclic aromatic hydrocarbons.[38] Understanding the rate of metabolism of xenobiotics when the enzyme is upregulated would be an interesting experiment as it could help proceed in using Gene editing as a tool for bioremediation.[39]

6. Screening platform for drug discovery

Modeling human diseases in yeast provides a crucial advantage through the potential for time- and cost-efficient high-throughput screens. In addition to the capability for uncovering mechanistic insights via genetic screens, yeast holds significant promise for drug discovery, including efforts targeting neurodegenerative diseases.[40-42] Research in this unicellular eukaryote has, for instance, contributed to the identification of anti-aging agents like spermidine[43] and resveratrol.[44]

Various sources and modulatory pathways contributing to the toxicity of mutant huntingtin (mHTT) have been pinpointed in diverse Huntington's disease (HD) models in yeast. These include dysfunctional mitochondria, protein aggregation, altered stress responses, involvement of the kynurenine pathway, and more. The combined impact of these toxic effects results in increased cell death. The microscopic inset depicts characteristic aggregations of GFP-tagged exon 1-poly(Q) constructs in a well-established *S. cerevisiae* model of HD.[45] The images were captured 24 hours after galactose induction of chronologically aged yeast cells.

S. cerevisiae possesses the necessary attributes and methodologies essential for a reliable model organism. To date, no other yeast species has achieved a comparable level of research utility. Nevertheless, in the future, other yeast-based models (Fig. 5), particularly Schizosaccharomyces pombe and Pichia pastoris, may gain increasing significance in Huntington's disease (HD) research. While *S. cerevisiae* has traditionally been the predominant yeast model due to its adaptability and proven efficacy in various neurodegenerative diseases, the landscape may evolve with emerging alternatives. Notably, *S. cerevisiae* remains a robust in vivo tool for conducting high-throughput screenings of drugs and mechanisms.[45]

7. Advantages and limitations

Yeast possesses the combined advantages of a microorganism, including rapid growth and straightforward genetics, along with the characteristics of a eukaryotic cell. Notably, mitochondrial disease genes are highly conserved among eukaryotes, and yeast genetics have proven instrumental in studying the mechanisms of mitochondrial toxicity induced by xenobiotics such as paraquat and diclofenac.

However, yeast does have its limitations in toxicology studies. One drawback is the often high concentration of compounds required to produce a toxic effect, potentially attributed to the cell wall barrier and various active efflux pumps. Furthermore, being a unicellular organism, yeast lacks the diverse cell environments and structures found in mammalian organs, including multicellular properties such as gap junctions. At the cellular level, yeast does not possess all the mammalian features that could impact toxicity, such as a limited number of biotransformation enzymes and the absence of the multi-subunit mitochondrial respiratory complex I, which may affect the detection of mitochondrial toxicants.[46] Yeast DNA microarrays have provided valuable toxicity assessment data. Upon exposure to toxicants, yeast cells undergo a reprogramming of RNA expression to adapt to new environmental conditions, resulting in a characteristic gene expression pattern or "signature" for the compound. These transcriptional signatures, shared by compounds with similar modes of action, can elucidate mechanisms of action and predict toxicological outcomes of uncharacterized toxicants. Yeast toxicogenomics data can also be integrated with studies

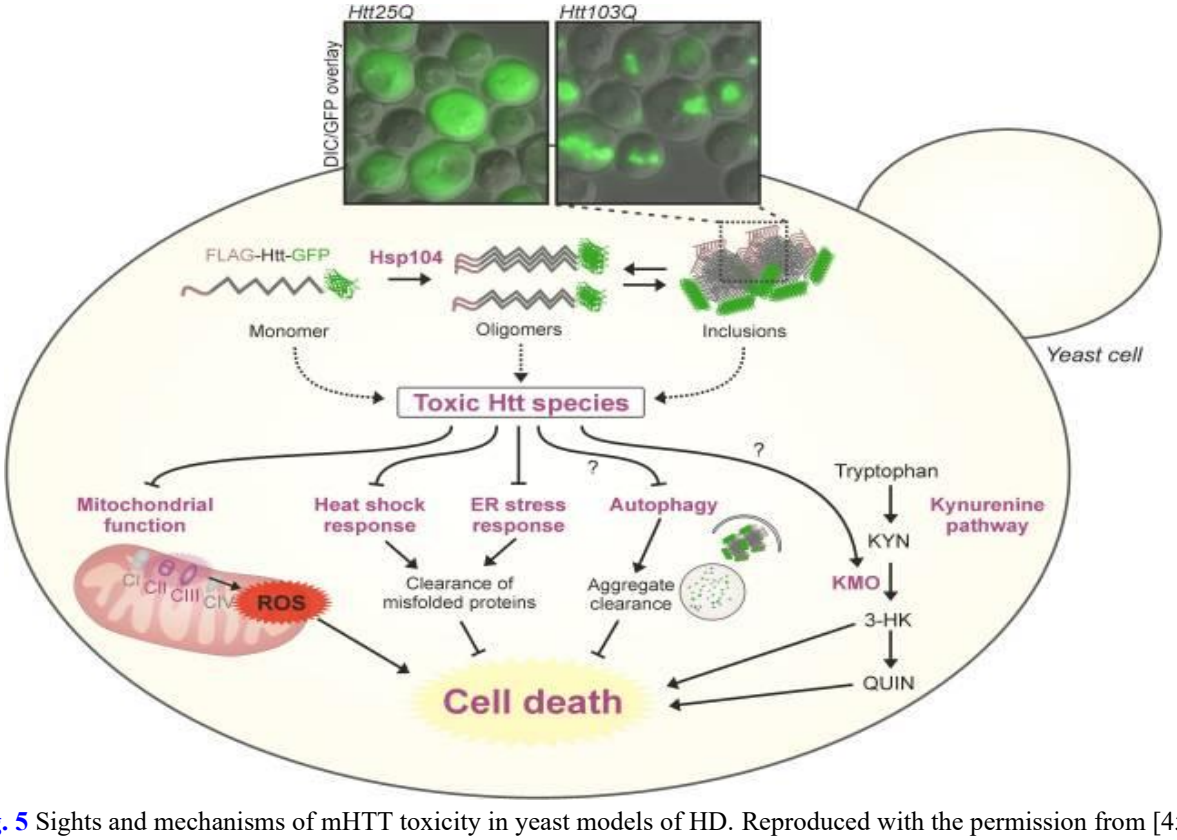

Fig. 5 Sights and mechanisms of mHTT toxicity in yeast models of HD. Reproduced with the permission from [45].

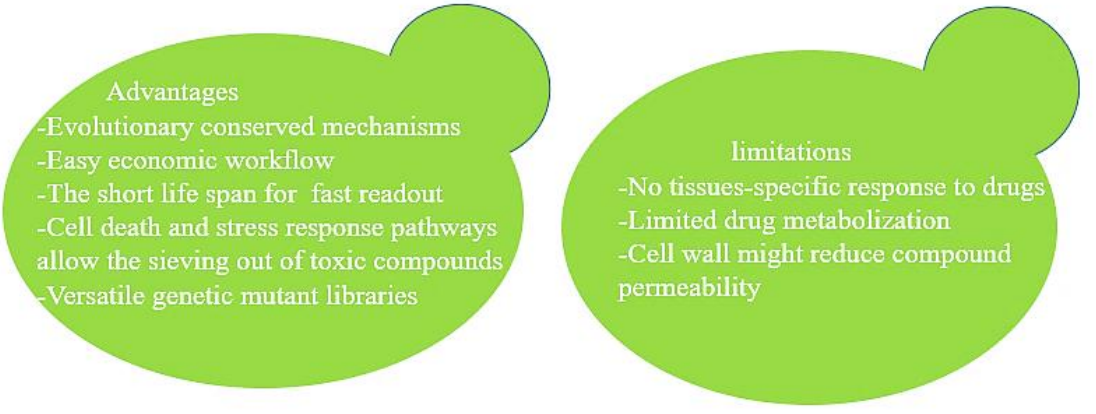

Fig. 6 Advantages and limitations of yeast for drug discovery. Reproduced with the permission from [47], Copyright 2018 FEMS.

from other sources, providing a comprehensive view of a compound's toxicity at the systems level.

Exploring both the strengths and constraints of utilizing yeast in the quest for anti-aging drug discovery, the budding yeast, *S. cerevisiae*, presents a genetically well-defined cellular setting coupled with a rapid and cost-effective experimental process. This includes the availability of diverse mutant libraries crucial for identifying potential drug targets. However, its unicellular nature imposes limitations on investigating responses specific to tissues (Fig. 6). Further restrictions encompass constrained drug metabolization capabilities and the suboptimal permeability of the fungal cell wall to xenobiotics. For a comprehensive understanding, refer to the main text for additional details[47]

Yeast has been extensively studied for its genome-wide response to toxic concentrations of metal ions, revealing insights into various functional groups involved in the yeast response to different metal ions. It is currently the only system capable of assessing all targets in the cell simultaneously and *in vivo*. Yeast has also served as a model for mechanistic studies with antimalarial drugs.

Yeast toxicogenomics offers a rapid and reproducible means of assessing the mechanisms of toxicity and resistance to various chemicals. This approach requires small amounts of growth medium and the compound under testing, leading to reduced costs and minimized toxic waste, aligning with the principles of reducing, refining, and replacing (3R) the use of animals in toxicological testing of pesticides.

The simplicity of yeast, stemming from its unicellularity, poses limitations in studying complex phenomena and hinders the acquisition of data on organ or tissue-specific toxicity. *S. cerevisiae*'s tolerance to high doses of toxicants, which exceeds that of higher eukaryotic cells, makes it challenging to determine accurate toxic doses. This heightened tolerance is attributed to the cell wall barrier, numerous active efflux pumps, and detoxification mechanisms abundant in yeast cells, making it difficult to ascertain the actual concentration acting on toxicant targets. Additionally, yeast may lack adequate molecular targets for certain cytotoxic compounds, as physiological mechanisms targeted by these compounds in their host organisms may not exist in yeast.

Furthermore, finding homology between yeast and human genes doesn't guarantee orthology, necessitating experimental verification of true homologs. Despite these limitations, yeast has proven valuable as an initial platform for screening and predicting the toxicological outcomes of new or unstudied drugs and chemicals, as well as for studying toxicity mechanisms.[38] Regarding metal and metalloid influx in cells, yeast, like other organisms, employs molecular mimicry through plasma membrane permeases and channels evolved for essential nutrient uptake. This includes metals such as Fe, Mn, Zn, and others. However, organisms, including yeast, have developed mechanisms to reduce influx by downregulating relevant transporters at transcriptional and post-transcriptional levels or inhibiting their transport activities (Fig. 7).

Fps1p is identified as the primary entry pathway for As(III) and Sb(III), regulated both at the transcriptional and post-transcriptional levels. Exposure to As(III) can result in both a decline and an increase in FPS1 transcription, depending on the duration of exposure. Fps1p exhibits transport activity for both the influx and efflux of As(III). Cadmium (Cd) enters cells through proteins involved in essential cation uptake, such as Zn, Mn, Fe, and Ca transporters.[48] The toxicity assessment of commercial polycarboxylate functionalized graphene nanoplatelets using the model fungus *S. cerevisiae* has revealed the potential impact of this nanomaterial in rapidly altering the physiological state of yeast (Fig. 8).

These findings contribute to understanding the molecular mechanisms underlying interactions between yeast and

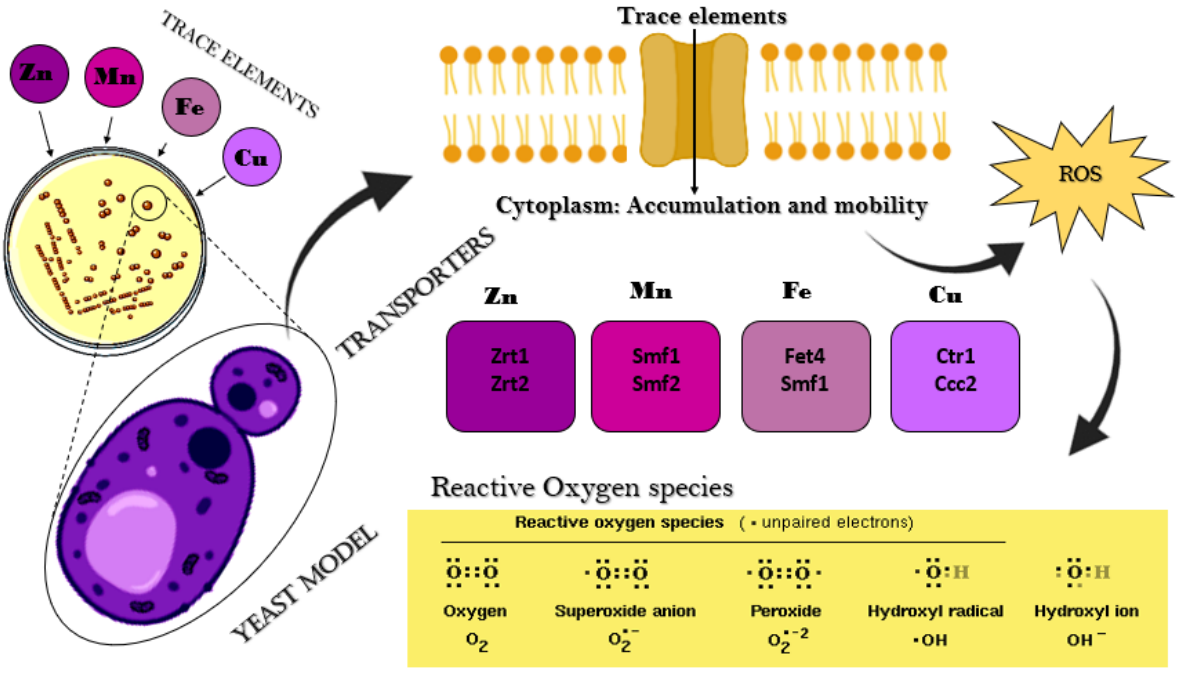

Fig. 7 Trace elements induce toxicity trough accumulation ROS is the system.

graphene, shedding light on how this nanomaterial might influence applications involving the interfacing of cells with graphene. Additionally, the results provide insight into the exposure risk for unicellular eukaryotic organisms, emphasizing the intricate nature of microbial systems-graphene interactions.[49]

Zinc serves as an essential cofactor for transcription factors and enzymes in all eukaryotic cells, playing a crucial role in cellular processes. However, excess zinc can be toxic to cell growth, leading to the generation of reactive hydroxyl radicals and disruptions in cellular redox potential. Moreover, excess zinc competes for binding sites in functional proteins with other metals. Although zinc possesses antioxidant properties, exposure to high zinc levels in yeast cells results in the generation of reactive oxygen species. *Saccharomyces cerevisiae,* a model organism, is frequently employed to study fundamental cellular processes, including zinc transport and homeostasis. In budding yeast, zinc homeostasis is rigorously

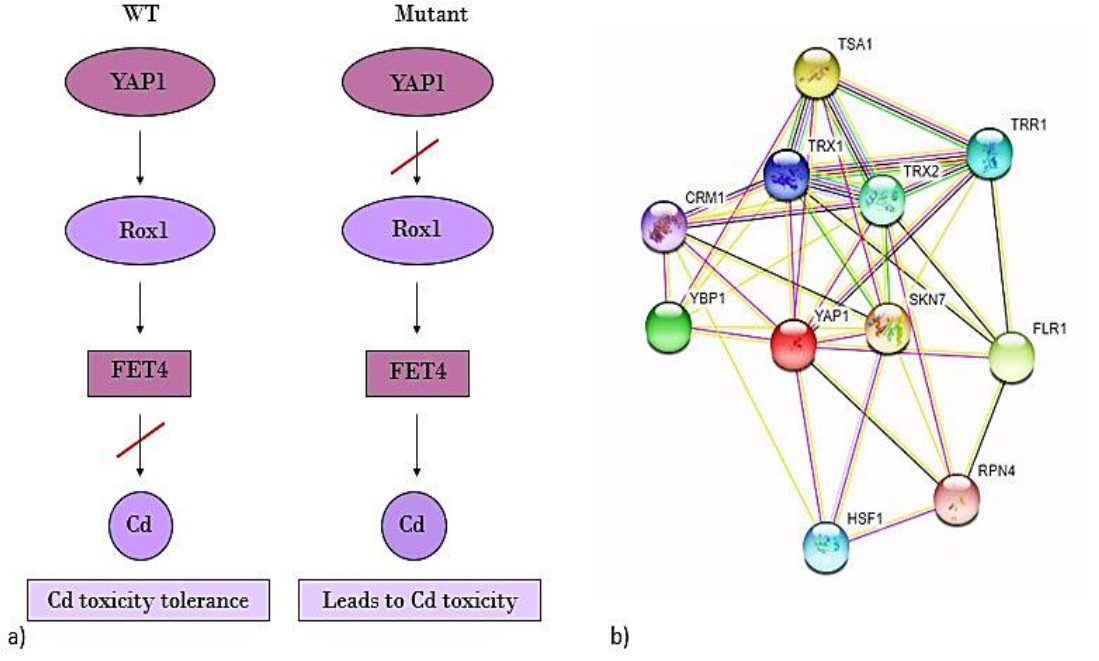

Fig. 8 Schematic representation of influence of YAP on Cadmium accumulation- YAP1 represses Rox1 transcription factor b) YAP1 interaction with other cellular proteins.

maintained through various transporters that assimilate extracellular zinc via high and low-affinity mechanisms at the plasma membrane.[50]

The predicted model for zinc homeostasis and its toxicity mechanisms in yeast cells involves the uptake of extracellular zinc through plasma membrane transporters such as Zrt1, Zrt2, Fet4, and Pho84. Once inside the cell, vacuolar zinc transporters Cot1, Zrt3, and Zrc1 regulate the transport of zinc into or out of the vacuole. The heteromeric complex formed by Msc2 and Zrg17 facilitates the transport of cytoplasmic zinc to the endoplasmic reticulum in cases of excess zinc. Excessive zinc can lead to the generation of reactive oxygen species (ROS), inducing oxidative stress and activating scavenging genes. Autophagy is proposed to play a significant role in maintaining cellular zinc homeostasis by transporting excess zinc to the vacuole.[51]

In the context of cobalt toxicity, the absence of Yap1 results in increased sensitivity to cobalt. Yap1 regulates the expression of genes involved in iron homeostasis, including those related to plasma membrane iron uptake (FIT2, FIT3, and FET4), mitochondrial iron import (MRS4), and iron-sulfur protein synthesis (ISU2). Microarray analyses show Yap1 as a negative regulator of FET4 expression (Fig. 9). Interestingly, cobalt levels decrease in the yap1 mutant strain relative to the wild type, and the double mutant yap1fet4 does not exhibit increased resistance to cobalt compared to the single mutant yap1. Approximately 46% of cellular cobalt enters the cell via Pho84, suggesting that Yap1 partially controls cobalt and phosphorus uptake through the regulation of PHO84.[52]

8. Cell modelling

In the pcYeast (proteome constraint yeast) model, the emphasis is placed on key protein compartments crucial for central metabolism. These include the plasma membrane, cytosol, mitochondrion, and mitochondrial membrane. Notably, certain cell compartments like the nucleus or endoplasmic reticulum are not explicitly defined in the model yet, though they do occupy space within the cytosol. This selective focus on specific compartments streamlines the computational complexity while still capturing essential aspects of cellular function, particularly in the context of central metabolism.

Biochemical reactions in the whole cell model, their interdependence and constraints (Fig. 10) Metabolic reactions v_i are proportional to enzyme concentrations e_i that are synthesised at rate $v_{syn,i}$ by the ribosomes R. Individual protein can be degraded with rate $v_{deg.i} = k_{deg}.e_i$ or diluted by growth rate $v_{dil.i} = \mu.e_i$. Compartment-specific constraints are indicated in the light-blue boxes. **B.** Optimisation problem with the key constraints, including (1) steady-state mass balances; (2) production of biomass components such as DNA, lipids, cell wall and polysaccharides. Proteins are excluded as their synthesis rates are optimisation variables (3) enzyme capacity constraints that couple metabolic flux to catalytic rate $k_{cat,i}$ and the enzyme level, whose value at steady state is determined by its synthesis rate. Note we use equalities and hence enzymes work at their maximal rate and minimal required protein levels are computed; (4) ribosome capacity that defines an upper bound for protein synthesis rate; (5) compartment-specific proteome

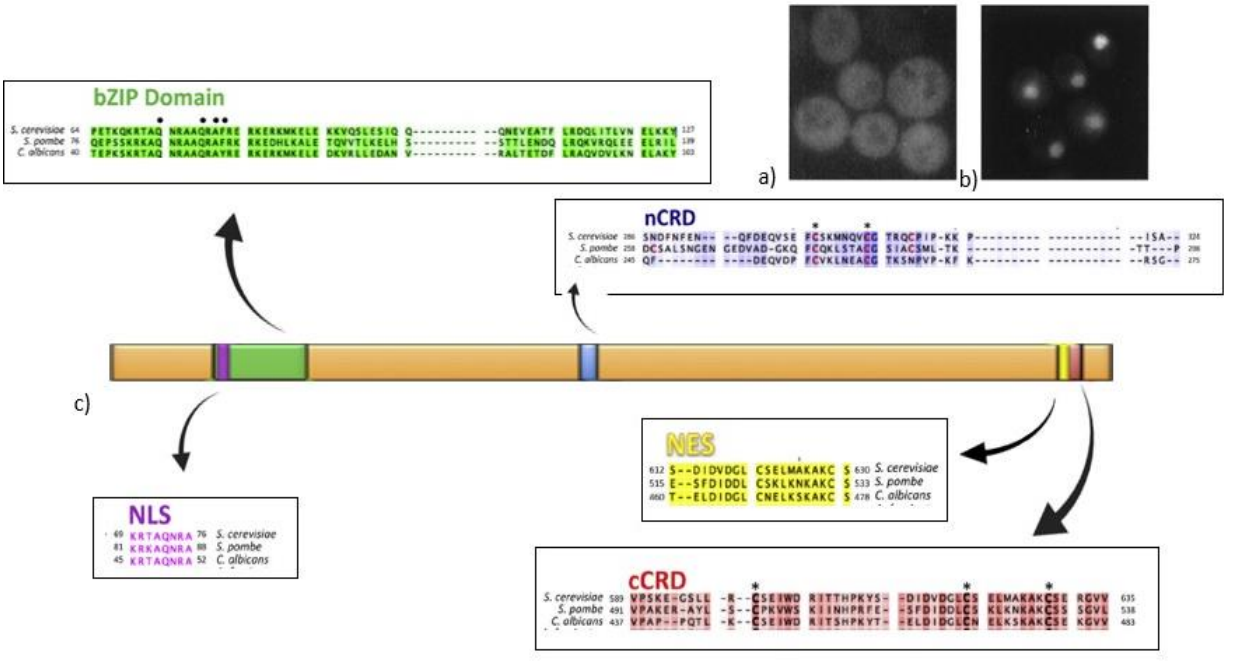

Fig. 9 YAP1 mediates redox regulation.

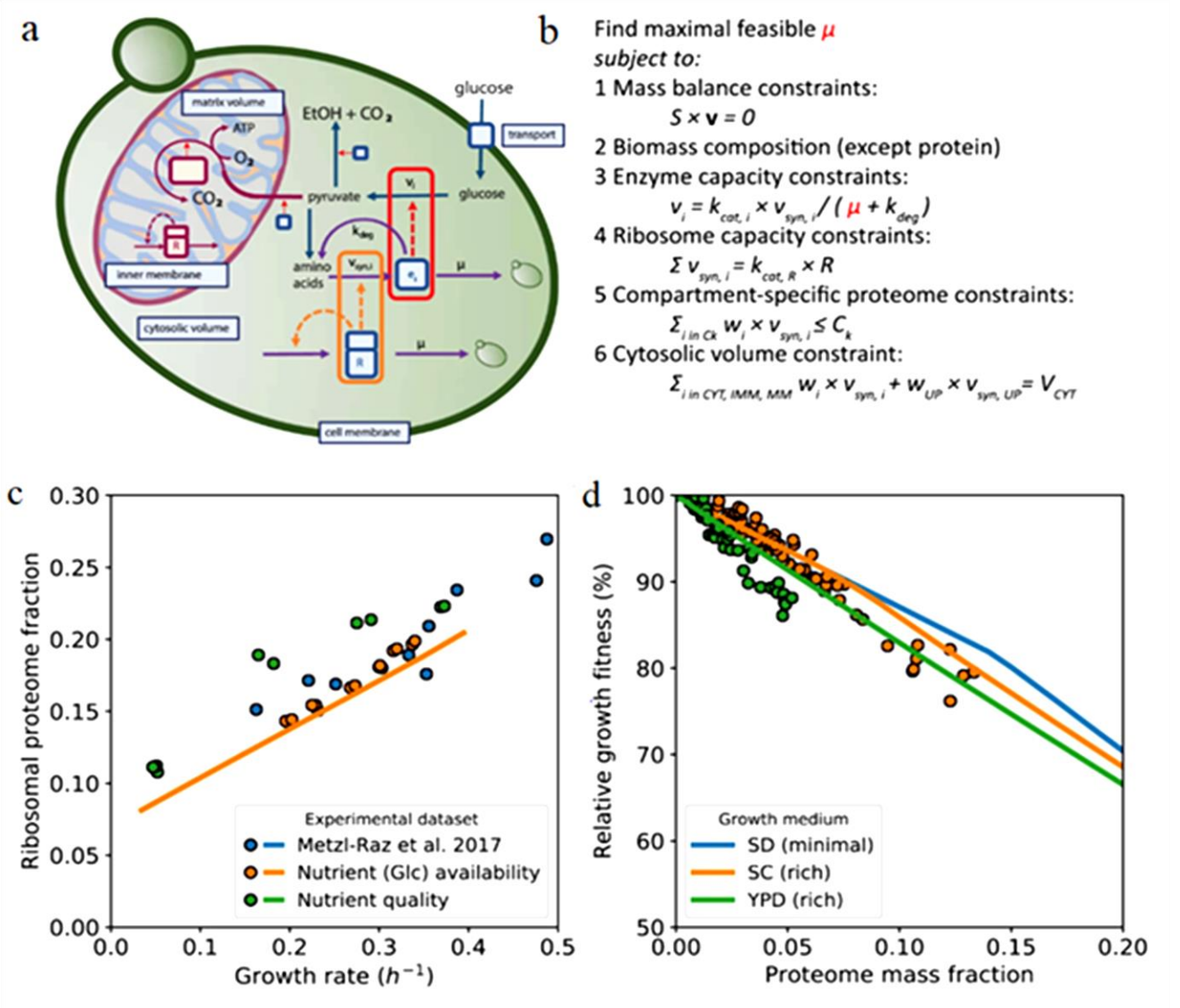

Fig. 10 pcYeast model formulation and calibration of protein synthesis parameters. Reproduced with the permission from [53], Copyright 2022 The Author(s)

constraints that define the maximal concentration of proteins that can be contained in that compartment, with the specific volume or area of protein i; (6) a cytosolic protein density constraint that has the same function as that of proteome constraints, but whose equality forces the cell to fill up any vacant proteome space with unspecified protein UP. **C** Growth rate was varied through sugar type (trehalose, galactose, maltose, glucose) or glucose concentration, and ribosomal protein fraction was determined by proteomics.[53] The translation rate was calibrated on the literature data. **D** Impact of mCherry protein overexpression on growth rate. Symbols show experimental data, solid lines show model predictions based on glucose minimal (SD) medium or rich SC/YPD media. Model predictions were obtained by varying the proteome mass fraction, occupied by mCherry, and determining the maximal predicted growth rate at each value of the mass fraction. The relative growth fitness represents the ratio between the growth rate at certain mCherry expression level vs. the unperturbed state (no mCherry expression). Source data for panels **c** and **d** are provided as a Source Data file.

Metabolic enzymes are categorized into specific compartments such as cytosol, plasma membrane, mitochondrial matrix, or inner-mitochondrial membrane. The transport of mitochondrial proteins necessitates additional protein transport complexes. The comprehensive model encompasses 1523 proteins, with life cycles described by 16,304 reactions, covering processes like translation initiation, elongation, and termination (Table 1). The model accounts for factors like ribosomal assembly, protein-specific folding facilitated by chaperones, and degradation reactions. Additionally, it considers the energetic costs associated with

translation initiation based on 5'UTR length.

Table 1. Whole-cell modelling in yeast predicts compartment-specific proteome constraints that drive metabolic strategies. Reproduced with the permission from [53]. Copyright 2022, The Author(s).

Process/compartment	No of reactions	No of proteins
Total	24422	1520
Metabolic network	5774	913
from Yeast7.6	5738	909
manually added metabolic reactions	36	4
Cytoplasm	2349	778
Plasma membrane	529	114
Mitochondria	1089	272
Endomembrane system	2127	133
Metabolic complex formation, disassembly, dilution	2787	-
tRNA turnover and modification	2194	56
Protein synthesis and turnover	13312	403
Cytoplasmic translation	1512	138
Mitochondrial translation	8	89
Protein folding	1515	31
Protein degradation	1607	42
Protein misfolding and refolding	6061	73
Protein transport	1324	30
Protein dilution by growth	1285	-
Formation of macromolecular complexes	355	196

Environmental perturbations in a bioprocess directly affect the intracellular environment of a yeast cell. The biosensors present enable real-time monitoring of changes in intracellular parameters, such as glycolytic flux (with GlyRNA), oxidative stress (with OxPro), ribosome production (with RibPro), intracellular pH (with sfpHluorin), and ATP concentration (with QUEEN-2m). This was studied using fluorescent proteins (Fig. 11).[54]

RNA, being a foundational molecular entity, not only functions as a template for protein synthesis but also assumes a pivotal regulatory role in gene expression and carries out diverse functions contingent upon its type (Fig. 12). The intricacies of RNA processing are intricately governed, necessitating the involvement of a myriad of regulatory proteins, notably RNA binding proteins (RBPs).[55] Within this intricate framework, errors may manifest, potentially giving rise to human diseases, with a particular emphasis on genetic disorders (Table 2). It is approximated that deficiencies within the regulatory genomic regions or the noncoding segments of genes contribute to as much as 50% of instances of genetic diseases.

Yeast stands as a frequently utilized and easily maintainable bioassay species, presenting a valuable model that circumvents ethical concerns. Despite its simplicity, yeast exhibits sensitivity to a broad spectrum of metabolic and membrane-modulating agents, making it a versatile and ethical choice for experimental investigations.[56]

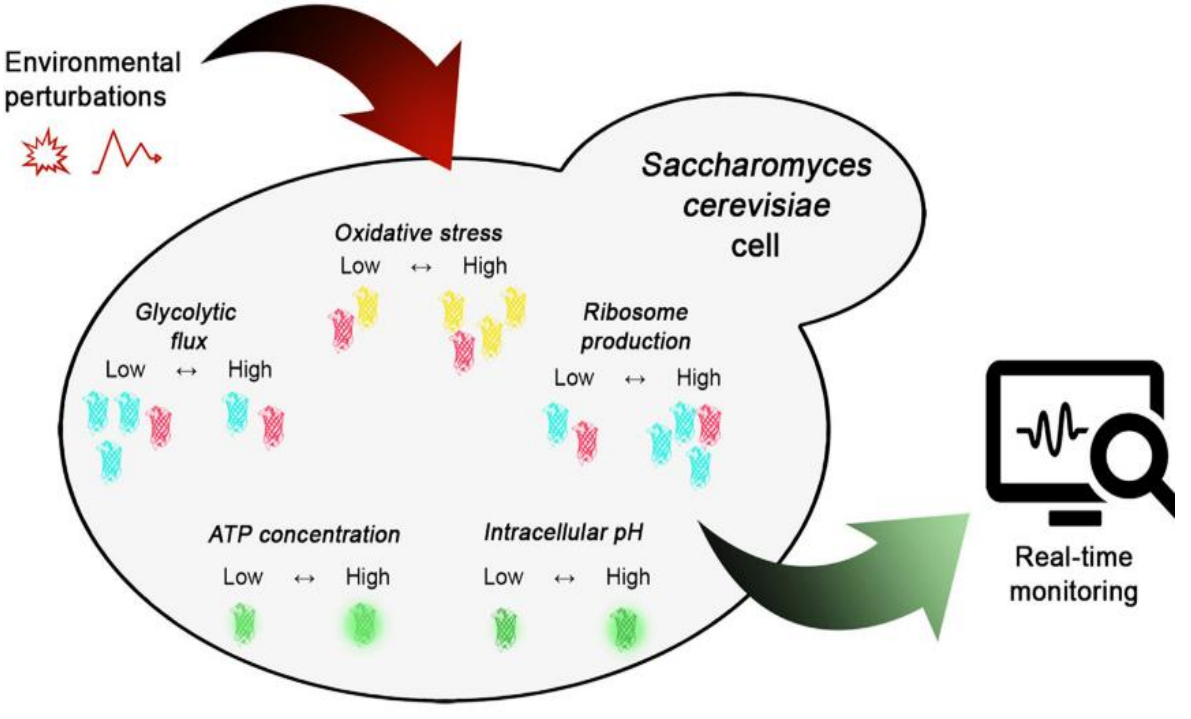

Fig. 11 Environmental perturbations in a bioprocess and real time monitoring.

Table 2. RNA-mediated diseases and their study in yeast model. Reproduced with the permission from [55], 2021 Wiley Periodicals LLC.

Human disease	Human protein	Yeast model
Amyotrophic lateral sclerosis	TDP-43	Exogenous overexpression of human wild type and mutant TDP-43. Genetic screens Oxidative stress/Heat shock induced aggregation Stress granule dynamics TDP-43 phosphorylation
	FUS	Exogenous expression of human wild type and mutant FUS bPY-type nuclear localization signal Genetic screens FUS phosphorylation Liquid-liquid phase separation
	Matrin-3	Exogenous expression of human Matrin-3
Huntington's disease	Huntingtin	Exogenous expression of mutant human Huntingtin containing poly-glutamine expansion repeats of different lengths Genetic screens
Parkinson's disease	α-Synuclein	Exogenous expression of human α-Synuclein RBP-yTRAP (yeast Transcriptional Reporting of Aggregating Proteins) Genetic screens
	LARK 2	Overexpression of human LRRK2 enzymatic core leading to mitochondrial dysfunction Oxidative Stress (both protective and deleterious depending on mutation)
	DJ-1	Yeast ortholog Hsp31
Alzheimer's disease	Tau	Exogenous expression of human Tau
	Amyloid Beta	Exogenous expression of human Amyloid Beta
	Ubiquitin	Exogenous expression of human ubiquitin mutant
Aging	LSM4	Yeast ortholog Lsm4 Cloning using yeast DNA library RNA oxidation
	S6K	Yeast ortholog Sch9
	DIS3L2	Yeast ortholog Ssd1 Polysome profiling *SSD1* overexpression/deletion
	Sirtuins	Yeast orthologs Sir2 & Sir4 rDNA silencing in the nucleolus
	BLM/WRN	Yeast ortholog Sgs1 *SGS1* deletion Transcriptional silencing of *HMLα*.
Uveal melanoma	EIFIAX	Yeast ortholog Tif11
Breast cancer	BRCA1	Exogenous expression of human BRCA1
Medulloblastoma	DDX3X	Yeast ortholog Ded1 Medulloblastoma associated mutants of *DED1*
COVID-19	SARS-CoV-2	Viral genome reconstruction
	Proteome	Vaccine manufacturing High-throughput screening

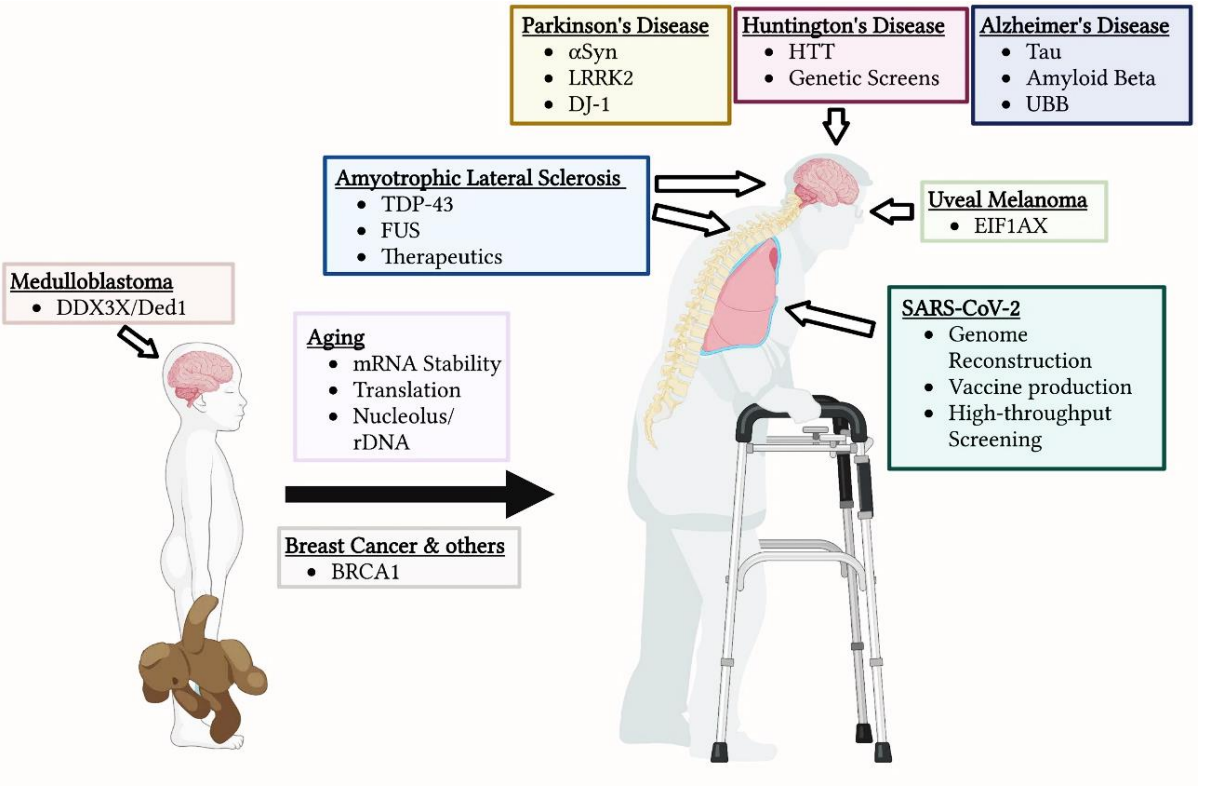

Fig. 12 The budding yeast model system serves as a robust approach to understand the complexities of human diseases mediated by RNA . Reproduced with the permission form [55], Copyright Authors.

9. Limitations of yeast as a model

A crucial observation in this context is that the subset of housekeeping genes specific to humans not only exhibits associations with fewer functional terms but also demonstrates a lesser degree of significance in these associations. Two contributing factors account for this effect. Firstly, it is acknowledged that some genes identified as human-specific may result from methodological artifacts. For instance, the belief propagation (BP) method, while requiring sequence similarity for gene alignment, deems it necessary but not sufficient, potentially leading to the artificial classification of human genes lacking sequence similarity with yeast genes. Secondly, and more significantly, a majority of functional annotations for human genes are initially ascribed in other species, notably yeast, and subsequently transferred across ortholog groups. In our framework, human-specific genes are defined as a subset of housekeeping genes lacking orthology with yeast. Consequently, it is anticipated that these genes traverse the obscured dimensions within the functional space of human genes, representing regions that are under-annotated.[57,58]

10. Conclusions

The review highlights the increasing importance of yeast-based functional genomic and proteomic technologies, elevating its role as a model organism in the realm of drug discovery. In vitro systems offer distinct advantages in exploring the molecular, cellular, and physiological mechanisms associated with chemically induced toxicity. This is particularly relevant when examining the toxicity of known target organs and species, enabling the investigation of specific questions about toxic effects that might be challenging to address in vivo. Yeast cells serve as a suitable model for investigating toxicity due to their convenient genetic manipulability, short life cycle, and ready access to genomic sequences, making them relatively easy to culture. The factors influencing sensitivity to toxic materials encompass cellular thiols, such as glutathione, phytochelatins, labile sulphide, and metallothioneins. Genes also contribute to the transfer, partition, or sequestration of metallic or other toxic substances. These features, along with associated pathological conditions, have been explored and validated in the genomic sequence of Saccharomyces cerevisiae. Moreover, the molecular mechanisms regulating the toxic potential of metals or other harmful substances can be effectively investigated using this model.

Conflict of Interest

There is no conflict of interest.

Supporting Information

Not applicable.

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