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REVIEW

From Model Organism to Pharmaceutical Powerhouse: Innovative Applications of Yeast in Modern Drug Research

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ABSTRACT: Yeast-based models have become a powerful platform in pharmaceutical research, offering significant potential for producing complex drugs, vaccines, and therapeutic agents. While many current drugs were discovered before fully understanding their molecular mechanisms, yeast systems now provide valuable insights for drug discovery and personalized medicine. Recent advancements in genetic engineering, metabolic engineering, and synthetic biology have improved the efficiency and scalability of yeast-based production systems, enabling more sustainable and cost-effective manufacturing processes. This paper reviews the latest developments in yeast-based technologies, focusing on their use as model organisms to study disease mechanisms, identify drug targets, and develop novel therapies. We highlight key platforms such as the yeast two-hybrid system, surface display technologies, and optimized expression systems. Additionally, we explore the future integration of yeast engineering with artificial intelligence (AI), machine learning (ML), and advanced genome editing technologies like CRISPR/Cas9, which are expected to accelerate drug discovery and enable personalized therapies. Furthermore, yeast-based systems are increasingly employed in large-scale drug production, vaccine development, and therapeutic protein expression, offering promising applications in clinical and industrial settings. This paper discusses the practical implications of these systems and their potential to revolutionize drug development, paving the way for safer, more effective therapies.

KEYWORDS: Yeast; model organism; pharmaceutical research; drug discovery; synthetic biology; metabolic engineering; vaccine development; therapeutic proteins; machine learning; biopharmaceuticals

1 Introduction

Yeasts are eukaryotic, single-celled microorganisms classified as members of the fungus kingdom. The first yeast originated hundreds of millions of years ago, and over 1500 species have been identified to date, with new species continuously being discovered through ongoing taxonomic research and molecular techniques [1,2]. Yeasts are estimated to constitute about 1% of all described fungal species. As a eukaryotic model organism, yeasts were the first eukaryotic organism to have its genome sequenced, ushering in a new era for humankind's in-depth understanding of the functions of eukaryotic cells and the molecular mechanisms of their life activities [3,4].

Compared to mammalian cells, yeast (e.g., *Saccharomyces cerevisiae*, *Hansenula polymorpha*, *Yarrowia lipolytica*, *Pichia pastoris*) offers several unique advantages in biotechnology. *Saccharomyces cerevisiae*

(baker's yeast), a genetic model organism model organism in genetics, is widely used due to its well-characterized genetic background, ease of culture, and comprehensive gene manipulation tools. Its ability to undergo both asexual (budding) and sexual reproduction makes it particularly versatile for genetic studies. It has been used extensively in genetic research, protein production, and fermentation processes and is a cornerstone in industrial biotechnology [5,6]. *Hansenula polymorpha*, a methylotrophic yeast, is especially valued for its ability to utilize methanol as a sole carbon source, making it ideal for the high-level expression of recombinant proteins. It is commonly used to produce biopharmaceuticals and enzymes [7]. *Yarrowia lipolytica*, an oleaginous yeast, is renowned for its ability to accumulate lipids, making it useful in biofuel production and the synthesis of fatty acids. It is also employed in the production of organic acids and industrial enzymes [8]. *Pichia pastoris*, another methylotrophic yeast, has gained widespread use in biotechnology due to its strong, tightly regulated alcohol oxidase (AOX1) promoter, which facilitates the high-level expression of recombinant proteins. It is especially useful for producing proteins with complex post-translational modifications, such as glycosylation, and is commonly used in both research and industrial applications [9].

These yeasts offer the advantage of being genetically tractable and capable of efficient heterologous gene expression. Their protein glycosylation pathways are highly conserved with those in mammalian cells, making them suitable for producing human therapeutic proteins with the desired post-translational modifications [10,11]. Additionally, the yeast system benefits from mature tools and extensive databases. E.g., the *S. cerevisiae* knockout collection, maintained by the *Saccharomyces* Genome Database (SGD) (https://www.yeastgenome.org/, accessed on 12 December 2024), provides a comprehensive resource for genome-wide knockout studies and protein interaction analyses [12]. This collection contains strains where each gene in the *S. cerevisiae* genome has been systematically deleted, enabling large-scale functional genomics studies (Table 1) [13].

Further, *S. cerevisiae* and other yeast species, such as *Pichia pastoris*, *Yarrowia lipolytica*, and *Hansenula polymorpha*, have fully sequenced genomes, offering an invaluable resource for exploring gene regulatory regions and identifying potential targets for genetic manipulation [14]. The *S. cerevisiae* genome was the first eukaryotic genome to be sequenced, and subsequent efforts have led to the creation of various databases, including the SGD and the Yeast Gene Order Browser, which catalog gene annotations, regulatory regions, and gene interactions [15,16]. These resources enable researchers to conduct in-depth analyses of yeast genetics, regulatory networks, and metabolic pathways, facilitating advancements in synthetic biology and metabolic engineering [16].

However, when using yeast in new pharmaceutical research, there are also some drawbacks: firstly, there is limited complexity: yeast cells have lower complexity than human cells, which may restrict the study and understanding of specific drug effects [14,17]. Secondly, there is a lack of tissue structure: yeast, a single-celled organism, lacks cellular tissue structure compared to complex multicellular tissues, which may affect the testing and assessment of certain drugs' effects [18]. Thirdly, there are limited metabolic pathways: yeast cells have metabolic pathways that differ from human cells, meaning they may not fully mimic conditions within the human body when assessing drug metabolism and drug interactions, as presented in Table 1 [5].

Table 1: Advantages and disadvantages of using yeast in new pharmaceutical research

Aspect	Yeast cells	Mammalian cells
Advantages		
Genetic manipulation [19]	Easy and cost-effective genetic manipulation	More complex and expensive genetic manipulation
Growth rate [20]	Fast growth and high cell density	Slower growth and lower cell density
Culture conditions [21]	Simple and inexpensive cultural conditions	Complex and costly cultural conditions
Model organism [22]	Well-established model organism with extensive tools	Closer to human physiology
Metabolic engineering [23]	Easier metabolic pathway engineering	More challenging metabolic engineering
High-throughput screening [24]	Suitable for high-throughput screening	Limited scalability for high-throughput applications
Post-translational modifications [25]	Lower-cost post-translational modifications	More accurate human-like post-translational modifications
Cost [21]	Lower production and maintenance costs	Higher production and maintenance costs
Disadvantages		
Post-translational modifications [25]	Limited in human-like post-translational modifications	Capable of complex human-like modifications
Protein folding [26]	May not properly fold all human proteins	Proper folding of human proteins
Cellular environment [21]	Different from the human cellular environment	Similar to the human cellular environment
Disease modeling [23]	Less suitable for modeling human diseases	Better suited for modeling human diseases
Drug metabolism [23]	Different drug metabolism pathways	Human-like drug metabolism pathways
Immune system studies [27]	Cannot be used for immune system studies	Suitable for immune system studies

Nevertheless, advances in yeast technology have paved the way for a variety of new genome-wide screening approaches for new drug discovery. In the past, studies using yeasts enabled breakthroughs in understanding basic cellular and molecular processes [22,28]. In recent years, yeasts are experiencing a 'rebirth' in fundamental and applied pharmaceutical research [16]. Various experimental strategies employing yeast aim to elucidate disease-related molecular events and uncover novel drugs [17,18]. This paper summarizes the impact of yeast as an experimental tool for new pharmaceutical research and evaluates biomedical research utilizing the yeast two-hybrid system. The recently applied and promising approach of yeast surface display (YSD) technology in new drug discovery is also discussed.

2 Yeast Genome Analysis and New Targets for Pharmaceutical Research

In 1996, Goffeau et al. [29] summarized the genome of baker's yeast *Saccharomyces cerevisiae* laboratory strain S288c, consisting of 12 million base pairs and over 6000 genes. This marked significant progress in understanding eukaryotic organisms and established yeast as a model organism with a well-defined genetic background in biomedical research. The availability of more than 6000 mutant strains of *Saccharomyces cerevisiae*, created through various mutation methods, has greatly facilitated research across different fields.

Through the analysis of open reading frames (ORFs) in the *Saccharomyces cerevisiae* genome, researchers later found that the functions of 60% of the ORFs were unknown [30]. When these ORFs were compared with the functional genes of humans and other mammals (e.g., rats, mice, cows, and sheep) reported in GenBank, it was discovered that 31% of them had high homology with mammalian functional genes. Notable examples include the yeast genes *RAS1* and *RAS2* (which are homologous to mammalian RAS genes), *MSH2* (yeast homolog: *MSH2*, mammalian homolog: *MSH2*), *MLH1* (yeast homolog: *MLH1*, mammalian homolog: *MLH1*), *IRA2* (yeast homolog: *IRA2*, mammalian homolog: *IRAK2*), *SGS1* (yeast homolog: *SGS1*, mammalian homolog: *WRN*), and *TEL1* (yeast homolog: *TEL1*, mammalian homolog: *ATM*) [31,32]. This indicates a significant level of similarity between these yeast ORFs and their mammalian counterparts. The homology percentage was calculated at the DNA level and is estimated to be between 60% and 85%, depending on the gene. By constructing deletion mutants of these homologous genes in yeast, it becomes possible to screen compounds for activity, leveraging the conserved functions between yeast and mammalian systems [33,34]. Lum et al. [35] established a yeast mutant library containing 3503 heterozygous allelic deletions. They used the principle that different mutants have varying sensitivities to drugs, thus affecting their growth, to analyze 78 antimicrobial and antiviral drugs, including 5-fluorocytosine. They screened some of these drugs for their targets. They found that the enzyme lanosterol synthase in the sterol biosynthesis pathway was the target of anti-cardiovascular drugs. At the same time, Ribosomal ribonucleic acid (rRNA) processing was identified as a potential target of the cell growth inhibitor 5-fluorouracil [35–37]. Establishing a method for screening drug targets using heterozygous alleles in yeast has provided new insights for drug research and accelerated the study of drug activity, side effects, and chemical toxicity. Besides, the study of yeast genes such as *GAL4*, *LEU*, *TRP*, *HIS*, *ADE*, and *CDC25* has provided additional screening markers for establishing a yeast two-hybrid system, yeast surface display system, and yeast expression system [35,38].

Additionally, recent advances in genome editing technologies, such as CRISPR-Cas9, could facilitate the use of yeast as a model organism for advancing drug discovery. CRISPR-Cas9 requires whole genome sequences to design and guide RNA (gRNAs) in targeting relevant genes. This system is very effective in *Saccharomyces cerevisiae* and other yeasts, such as *Pichia pastoris*, *Yarrowia lipolytica*, and *Hansenula polymorpha*, primarily due to their efficient homology-directed DNA repair mechanisms. Interestingly, other unconventional yeasts have also reported high success rates, suggesting that many genomes can be modified using the CRISPR-Cas9 system (Fig. 1) [39,40]. For example, in the heat-tolerant methylotrophic yeast *Ogataea polymorpha*, CRISPR-Cas9-assisted multiplexed genome editing successfully introduced all the genes required for resveratrol biosynthesis, as well as genes for the biosynthesis of human serum albumin and cadaverine [39]. These new biotechnological applications provide a promising avenue for developing subsequent drugs [41].

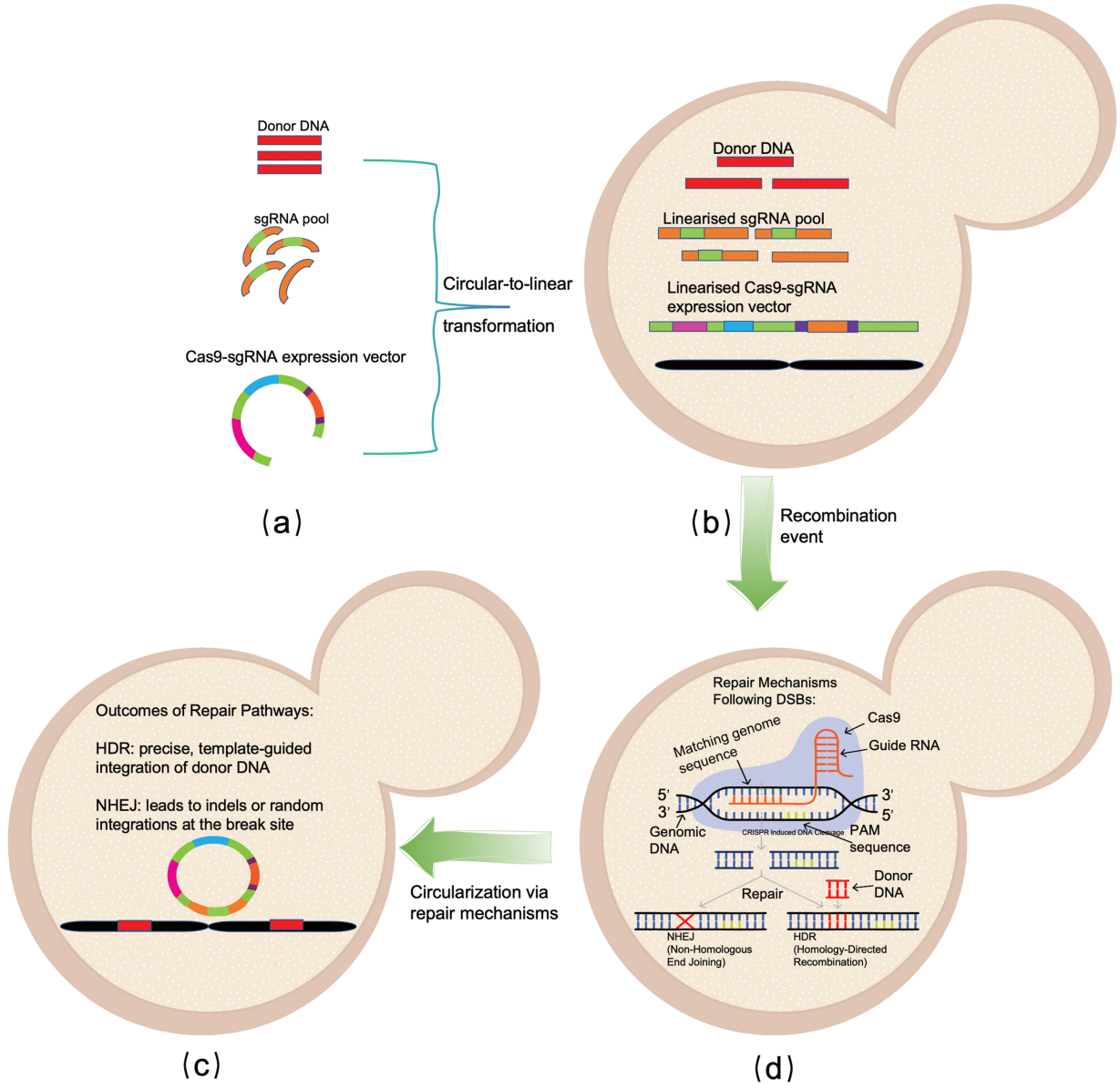

Figure 1: Schematic Overview of Cas9-Mediated DNA Editing and Repair Pathways in Yeast. a. Input: the initial inputs include donor DNA templates for homology-directed repair (HDR), a pool of single guide Ribonucleic acid (sgRNAs) targeting specific loci, and a Cas9-sgRNA expression vector. These constructs are typically circular before the transformation process. b. Transformation: circular DNA constructs are linearized and introduced into yeast cells through transformation. Linearized donor DNA, sgRNA pools, and Cas9-sgRNA expression vectors are prepared for subsequent recombination events. c. DSB Induction and Repair: the Cas9-sgRNA complex induces double-strand breaks (DSBs) at target genomic sites. Cellular repair mechanisms are activated with two main pathways: (1) Homology-Directed Repair (HDR), which uses donor DNA as a template for precise integration, and (2) Non-Homologous End Joining (NHEJ), which is error-prone and may result in insertions or deletions. d. Repair Outcomes: depend on the pathway utilized. HDR facilitates precise integration of donor DNA, while NHEJ leads to random or imprecise modifications. The circularization of DNA may occur as part of the repair mechanisms, ensuring vector stability

3 Yeast Two-Hybrid (Y2H) System in Biomedical Research

Brückner et al. [42] established a yeast two-hybrid system (Y2H) to detect protein-protein interactions by using the Gal4 transcriptional activator of the yeast *Saccharomyces cerevisiae*. The Gal4 protein activates the transcription of a gene involved in galactose utilization, forming the basis of selection [43]. In a Y2H system, the premise is that the activation of the downstream reporter gene(s) occurs through the binding of a transcription factor to an upstream activating sequence (UAS) [44]. The transcription factor is split into two fragments: the DNA-binding domain (BD) and the activating domain (AD). The BD binds to the UAS, while the AD activates transcription [45]. A known functional protein is typically used as the 'Bait,' and proteins that may interact with the 'Bait' are used as the 'Prey.' The interaction between the 'Bait' and the 'Prey' is verified by the expression and detection of reporter genes [46].

To date, yeast two-hybrid technology has been expanded to study a broader range of intermolecular interactions [47]. Variants include the reverse two-hybrid system for studying interactions between proteins and small-molecule inhibitors, the single-hybrid system for studying interactions between DNA and proteins, the three-hybrid system for studying interactions between RNA and proteins, and another three-hybrid system for studying interactions between small molecules and proteins in a ligand-dependent manner [48]. The differences among these systems lie in selecting reporter genes and the 'Prey.' Additionally, they differ in selecting the reporter gene and the 'Bait' or 'Prey' expression vector. Yeast two-hybrid systems have been widely used in biomedical research [49]. Their widespread application is expected due to their relative affordability, lack of need for specialized large equipment, and feasibility in any molecular biology laboratory with reasonable throughput (Fig. 2) [50].

One of the primary applications of the yeast two-hybrid (Y2H) system in drug discovery is the identification of protein-protein interactions that are critical for disease progression [51]. By mapping these interactions, researchers can pinpoint potential drug targets. The yeast two-hybrid system continues to be a cornerstone in the field of drug discovery. Its ability to uncover protein-protein interactions and facilitate the screening of potential inhibitors makes it an essential tool for identifying and validating new drug targets [52]. As technology advances, its role in drug discovery is likely to become even more prominent, contributing to the development of more effective and targeted therapies [53]. In the following section, we will discuss the latest advancements and progress in yeast two-hybrid systems within biomedical research.

3.1 Study of Protein Interactions

Protein-protein interactions (PPIs) occur throughout a cell and are essential for understanding cellular functions. Studying these interactions in model organisms enhances our understanding of biological processes, aids in deciphering disease mechanisms, and helps in identifying potential drug targets and screening new therapeutics [54]. By constructing DB-Bait and AD-Prey yeast expression vectors, interactions between proteins can be detected using reporter genes. In this context, we will refer to the protein used in the DB vector as 'Bait' and the protein used in the AD vector as 'Prey' in the subsequent sections. Additionally, indirect interactions between different proteins can be investigated through host endogenous protein-mediated methods [55]. For example, early studies demonstrated that the HIV-encoded Rev protein interacts with the yeast nucleoprotein *Rip1* [56]. Further research using the yeast two-hybrid system revealed that this interaction is mediated by Crm1p in yeast [54].

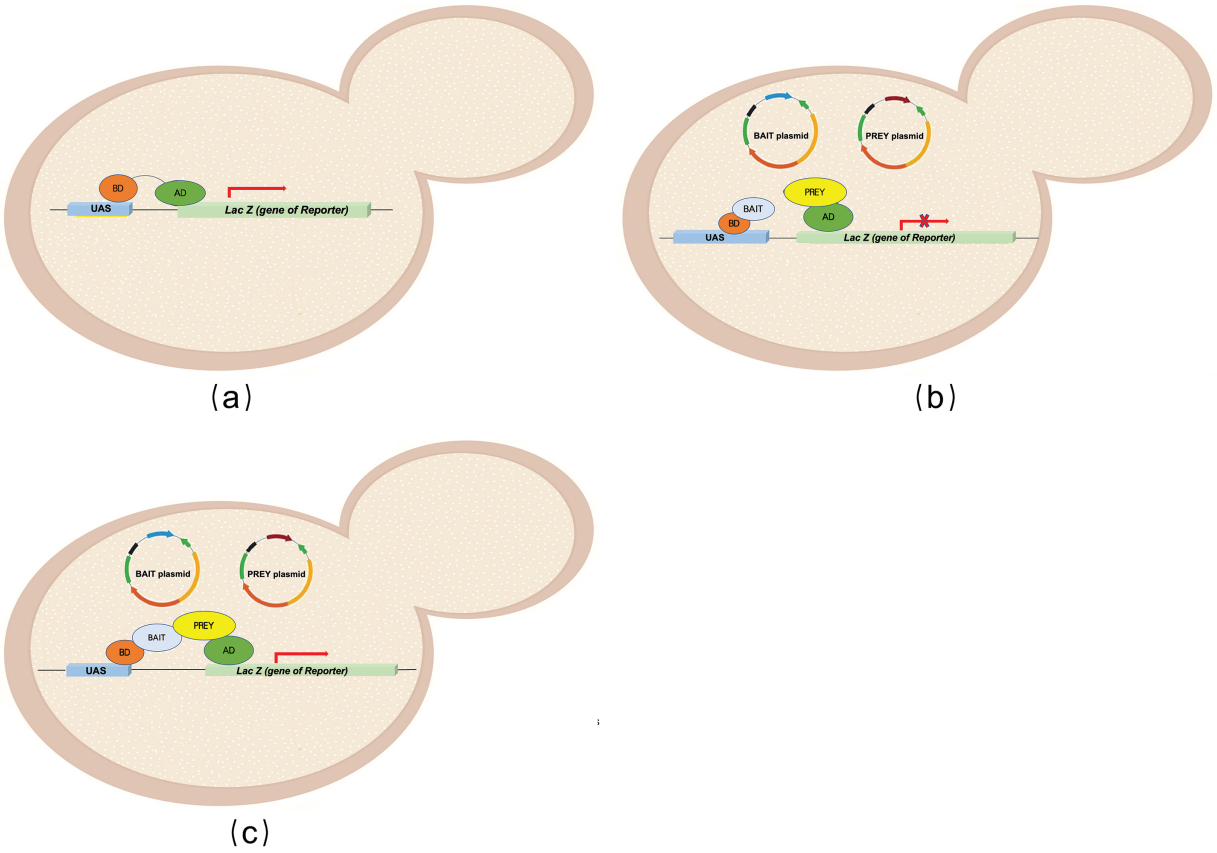

Figure 2: Overview of yeast two-hybrid system (Y2H), a genetic technique that detects protein-protein interactions: in a Y2H system, the expression of a reporter gene requires activation through the binding of a transcription factor to an upstream activating sequence (UAS). The UAS consists of two independent domains: the DNA-binding domain (BD) and the activation domain (AD). The BD binds to the UAS, while the AD activates transcription (a). The protein fused to the BD is called the Bait, and the one fused to the AD is referred to as the Prey. Without Bait-Prey interaction, the AD domain cannot initiate gene expression (b). However, when the Bait and Prey interact, the BD binds to the DNA, localizing the AD upstream of the reporter gene, resulting in the expression of the reporter gene (c)

3.2 Discovery of New Proteins and New Functions of Proteins

Using a protein with a known function as 'Bait', new proteins can be discovered from cDNA libraries as 'Prey', and new functions of known proteins can also be identified. For example, Fañanás-Pueyo et al. [57] transformed all predicted open reading frames (ORFs) in yeast into MATa (mating type a) and MATα (mating type alpha) strains as 'Bait' or 'Prey' by ligating them with DB or AD, respectively. In *S.cerevisiae*, MATa and MATα represent the two mating types, where MATa is the a-type and MATα is the alpha-type, each having distinct roles in the mating process. These strains are used to facilitate the identification of protein interactions in the yeast two-hybrid system. They then transformed these constructs into MATa and MATα yeasts to form 'Bait pools' and 'Prey pools'. By selecting 96 positive clones from each pool and systematically comparing the results, they identified 183 pairs of interacting proteins, more than half of which were previously unreported [58]. These new protein interactions may be related to different transport modes of substances in yeast [59]. Besides, other targets for screening aptamers using yeast two-hybrid systems include the transcription factor signal transducer and activator of transcription 3 (Stat3) and the tyrosine kinase avian erythroblastic leukemia viral oncogene homolog 2 (ErbB2). Constitutive activation of

Stat3 has been observed in various tumors, while ErbB2 is overexpressed in numerous cancers, including breast, ovarian, bladder, and lung cancers [60]. Therefore, investigating the oncogenic functions of these two proteins through a yeast two-hybrid system may open new therapeutic avenues for cancer research [61].

3.3 Antigen-Antibody Interaction Studies

Existing techniques for detecting antigen-antibody interactions are based on *in vitro* immunoreactivity. However, the interactions of drugs, especially peptides and proteins, with normal components in the body are highly complex and challenging to determine using conventional immunological studies [62]. Therefore, evaluating drug immunotoxicity and/or immunogenicity during safety assessments is difficult with standard methods. Mehta et al. [63] linked various tumor suppressor p53 and T antigen mutants (produced via PCR) to DB or AD to form fusion proteins, using LacZ as the reporter gene to establish a new reliable detection method. They detected interactions between 34 types of mutant p53 and the T antigen, and these mutant p53 proteins were also found in tumor patients. The gradual improvement of this method is expected to bring significant advancements to the preclinical safety evaluation of drugs.

Zhang et al. [62] developed an antigen-antibody co-display (AACD) system for detecting interactions between G-protein-coupled receptors (GPCRs) and single-chain variable fragments (scFvs) using a split-ubiquitin-based yeast two-hybrid (YTH) system. This system was engineered by fusing a transmembrane peptide to anchor scFv antibodies to cell membranes, allowing co-display of the GPCRs on cell membranes. They further optimized the topology and key elements of the scFv fusion proteins, creating an AACD system that can rapidly determine the association between GPCRs and their candidate antibodies. This innovation shortens the research cycle for off-target detection and epitope recognition [64].

3.4 Screening for New Drugs

The transfer process of pathogenic bacteria or viruses after invasion is related to specific proteins on their surfaces. Some proteins on the surface of host cells may become the 'targets' for these pathogens. Compounds that bind to these specific proteins on bacteria or viruses could potentially prevent them from invading normal tissues or cells, achieving therapeutic and preventive purposes [65]. Using various screening methods to identify compounds that bind to specific proteins on the surface of these pathogens makes it possible to prevent their invasion of normal tissues or cells. This targeted binding can serve both therapeutic and preventive functions [66].

A gene fragment encoding a specific protein from a pathogen can be used as 'Bait' by linking it to a DNA-binding domain (DB), while a cDNA library or random sequences serve as 'Prey' by attaching to an activation domain (AD). These constructs are transformed into yeast, enabling the identification of 'Prey' proteins or peptides that bind to the bait. This approach was first demonstrated by Zhu et al. [67] using a yeast two-hybrid system to screen for hormone-receptor interactions, identifying a proinsulin mutant with high binding affinity to the Insulin-like Growth Factor 1 (IGF-1) receptor, a potential candidate for drug screening in diabetes treatment. Similarly, researchers have also optimized a high-throughput assay for HBx-DDB1 interactions in *S. cerevisiae*, suggesting its potential for discovering therapeutic agents for chronic hepatitis B [68].

4 Yeast Surface Display System and New Drug Screening

Yeast surface display (YSD) is a "whole-cell" platform used for the heterologous expression of proteins immobilized on the yeast's cell surface. From 10,000 to 1,000,000 copies of the fusion protein can be expressed on the surface of each yeast cell [69]. Substances that interact with these gene products can be identified by displaying target gene products with unknown functions or products related to specific pathological

processes on the yeast cell wall [70]. This approach helps to study the functions of unknown genes and can lead to the discovery of new drug targets or the screening of new lead compounds for disease treatment. YSD is a promising technology that is not yet optimized for biotechnological applications [71].

Yeast surface display technology is based on the study by Teparić et al. [72] on a glycoprotein in the yeast cell wall. They showed that a- and α-agglutinin are two types of mannose proteins in the yeast cell wall. The a-agglutinin consists of two parts: Aga1 and Aga2. Aga1 is covalently linked to β-glucan in the inner layer of the yeast cell wall, while Aga2 binds to Aga1 through two disulfide bonds and is displayed on the cell surface. The most commonly used yeast surface display system originally developed by Wang, a single-chain variable fragment (scFv) was genetically engineered to be fused to the C-terminus of Aga2, with a GAL4 promoter inserted upstream of Aga2 [73]. Under galactose induction, the scFv was expressed and displayed on the surface of the yeast. This setup allowed the corresponding ligands to be easily detected from a library of random mutants using flow cytometry (Fig. 3) [73]. Orr et al. [74] further developed this method by ligating the Vbeta8 domain of a soluble T-cell receptor and an antibody mutant targeting the c-myc epitope to the C-terminus of scFv, displaying it on the surface of yeast cells. They then used flow cytometry with a fluorescent marker to detect scFv mutants with higher affinity for the T-cell receptor [74]. These studies demonstrated that this method can be used to analyze antigenic epitopes, particularly for binding sites of eukaryotic secretory or cell surface proteins.

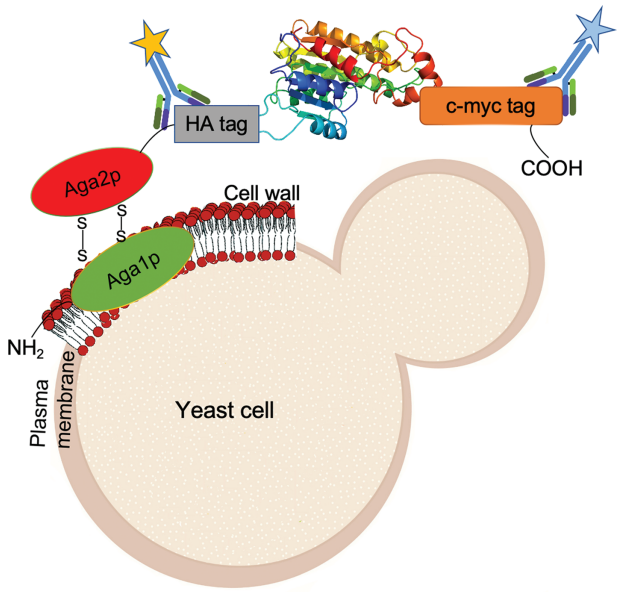

Figure 3: Overview of the Yeast Surface Display (YSD) system, a biotechnological technique used for the cell surface expression of heterologous proteins, which are fused to the C-terminus of the mating agglutinin protein Aga2. YSD construct includes two epitope tags: a hemagglutinin (HA) tag between Aga2p as well as a c-myc tag. Aga1p is anchored to the inner layer of the yeast cell wall, while Aga2p binds to Aga1p through two disulfide bonds. Induction of protein expression results in surface display of the fusion protein through disulfide bond formation of Aga2p to Aga1p

Other applications for YSD include library screening, whole-proteome studies, bioremediation, vaccine and antibiotic development, biosensor production, ethanol production, biocatalysis, and new drug discovery [75]. In a recent study, Wang et al. [73] developed a new method for high-throughput rapid extracellular antigen profiling using yeast surface display technology to discover autoantibodies against medically relevant autoimmune targets. By displaying 2688 barcoded human extracellular proteins on the yeast surface

and comparing them to patient serum immunoglobulins, they identified relevant antigens from sorted yeast using next-generation sequencing. This approach led to the identification of several autoantibodies present in autoimmune polyglandular syndrome type 1 and systemic lupus erythematosus conditions in autoimmune patients [76,77]. In addition, Lopez-Morales et al. [78] used YSD to study the evolution of the SARS-CoV-2 receptor-binding domain (RBD) and the propensity of mutations to evade immunological recognition. By displaying a library of receptor-binding domains (RBDs) on the surface of yeast cells, researchers can analyze the antibody-binding properties of various viral variants through deep mutation scanning and next-generation sequencing. In the case of SARS-CoV-2, the RBD is part of the spike protein, which binds to the angiotensin-converting enzyme 2 (ACE2) receptor on human cells. By mapping mutations in the RBD, researchers have identified several mutations that enhance ACE2 binding, providing insights into the potential for viral immune escape. This approach has been particularly valuable for studying SARS-CoV-2 variants. By optimizing the YSD workflow, researchers have mapped binding affinities between the SARS-CoV-2 RBD and different classes of antibodies, offering predictive insights into how mutations may impact antibody recognition and suggesting potential future variants of concern. The study of viral mutations and their capacity to evade immune recognition is critical, and yeast models offer a robust platform for investigating these mutations at a high throughput. Additionally, the ability to screen for novel antibodies or vaccine candidates in yeast systems may help mitigate the risks associated with emerging viral variants.

Interestingly, yeast two-hybrid and Yeast surface display techniques focus on protein-protein interactions (including ligand-receptor interactions), and the combination of these two techniques will facilitate the development of vaccines as well as new pharmaceutical research [27]. E.g., to obtain a soluble antibody with high stability and correct folding after intracellular expression, Tristán-Manzano et al. [27] attached the scFv fragment of the antibody to the activation domain (AD) of the transcription factor VP16 and the antigen to the DNA-binding domain (DB) of LexA. They established a yeast surface display method to demonstrate the interaction between the antigen and the antibody using *His3* and *LacZ* as reporter genes. When an antigen-antibody interaction occurs, the combination of AD and DB initiates the expression of the reporter genes, which can be detected using a nutrient-deficient medium or the blue-white spotting method. This method allows for the screening of antigen-specific antibody fragments from the scFv mutant display library, providing a valuable platform for vaccine development.

5 Optimizing the Yeast Expression System for New Drug Development

The use of genetic engineering to address the issue of 'drug sourcing' is a focal point and a hot topic in the field of biomedicine. Various peptides or proteins have been successfully expressed in prokaryotic cells, eukaryotic cells, animal mammary glands, plants, and other systems [79]. Yeast, as a model organism with extensive genetic background research, has emerged as a prominent system for exogenous gene expression. Its rapid growth, ease of genetic manipulation, and ability to complete post-translational processing of eukaryotic proteins or peptides make yeast a major platform for exogenous gene expression [80]. Brooks et al. [81] were the first to express an exogenous gene in *Saccharomyces cerevisiae*. They ligated the *Liver-expressed antimicrobial peptide D* (*LeIF-D*) gene upstream of the ethanol dehydrogenase I gene, resulting in the expression of biologically active LeIF-D in yeast at a rate of 1×10^6 molecules/cell. *Saccharomyces cerevisiae* has been utilized for expressing heterologous proteins for a significant period, owing to its extensive history in the fermentation industry. The completion of the yeast's full sequence analysis further solidified its position as a popular expression system. In a study by Bhattacharya et al. [82], a *S.cerevisiae* mutant deficient in four enzymes related to ergosterol metabolism was genetically engineered. This mutant was then modified to incorporate an enzyme derived from *Hypericum perforatum* (St. John's Wort), a plant known for its use in hydrocortisone synthesis, enabling the yeast to produce hydrocortisone. When cultured in a simple carbon

medium, the mutant exhibited high expression levels. Subsequently, the cultured mutant produced a steroid hormone suitable for treating patients with arthritis and adrenocortical insufficiency. This innovative work holds promise for producing small molecule compounds through biotransformation technology.

However, researchers have identified some limitations in using *Saccharomyces cerevisiae* as a model organism for expressing exogenous proteins: the exogenous gene vector commonly used in the transgenic system of *Saccharomyces cerevisiae* is a 2 μm add-on plasmid. This vector is genetically unstable and prone to loss during cell proliferation, leading to instability in engineered strains and ultimately affecting the production of recombinant proteins [83]. Additionally, *Saccharomyces cerevisiae* contains an α1-3 glycosidic bond at the end of its core polysaccharide. The presence of this glycosidic bond increases the antigenicity of recombinant proteins, posing a significant challenge in developing these proteins or peptides into drugs [84].

Recently, the yeast *Pichia pastoris* has become more widely used in the production of exogenous proteins. This yeast features a robust promoter, the alcohol oxidase 1 (AOX1) promoter, which is activated in the absence of a repressive carbon source such as glucose and utilizes methanol as the sole carbon source. This promoter ensures strict regulation and high efficiency in the expression of exogenous genes [85]. Additionally, the expression system often employs integration plasmids as vectors for exogenous genes, allowing the transfected genes to integrate into the yeast genome and replicate synchronously with genomic DNA. This integration reduces the likelihood of gene loss, resulting in stable engineered strains [86].

Moreover, since this expression system commonly utilizes integration plasmids as exogenous gene vectors, the transferred genes can integrate into the yeast genome and replicate in parallel with genomic DNA, reducing the likelihood of loss and resulting in relatively stable engineered strains capable of achieving high expression levels [87]. For example, the novel vector pIB4α developed by Gurkan et al. [88] enables the expression of C6.5, a functional single-chain antibody fragment targeting the surface glycoprotein HER2 (human epidermal growth factor receptor 2) found on ovarian and breast cancer cells, at up to 70 mg/L. This level of expression holds promise for commercial production applications.

Castro et al. [89] observed that although the N-terminus of human interferon β1 secreted and expressed in *Pichia pastoris* was identical to that of the natural product, the secreted protein was partially N-glycosylated, potentially affecting its biological activity. To address this issue, Hamilton et al. [90] developed a strategy to delete endogenous yeast genes associated with the glycosylation pathway. They then introduced five enzymes or proteins, including mannosidases (I and II), N-acetylglucosaminyltransferases (I and II), and UDP-N-acetylglucosaminyltransferase, into the yeast genome. This led to the formation of a human-like glycosylation pathway, specifically the N-acetylglucosamine (GlcNAc)2-mannose (Man)3-N-acetylglucosamine (GlcNAc)2 (GlcNAc2Man3GlcNAc2) pathway, within the yeast cells. This modification facilitated the expression of human N-glycosylated glycoproteins, improving the quality of the expressed proteins. This establishment of the expression system serves as a foundation for the large-scale production of human glycoproteins for medicinal purposes and offers a valuable tool for studying the conformational relationships of glycoproteins.

Furthermore, since the plasmid vector with a yeast gene AOX promoter was utilized in the expression system of *Pichia pastoris*, requiring methanol for inducing the expression of exogenous genes, it posed challenges for industrial production due to methanol's flammability and volatility [91]. To address this issue, Arjmand (2024) [92] proposed replacing the AOX promoter with the Formaldehyde Dehydrogenase 1 (FLD1) promoter. This promoter can induce the expression of exogenous genes when methanolamine is the sole nitrogen source. Additionally, the FLD1 promoter offers advantages such as high tunability, making it a promising alternative for industrial applications.

In addition to protein and peptide expression and production, transgenic yeast holds potential for various applications such as vaccine development, drug screening model construction, and pathogenesis studies. For instance, pathogenic bacteria and fungi frequently form biofilms upon invading host tissues, which provide protection and render drug treatments ineffective [93]. Biofilm formation is a well-documented mechanism in pathogens like *Pseudomonas aeruginosa* and *Candida albicans*, contributing to their resistance to both the host immune response and antimicrobial therapies. Further research into biofilm formation mechanisms could aid in developing new antimicrobial drugs [94]. Bouyx et al. [95] investigated biofilm formation and genesis using *Saccharomyces cerevisiae* as a model and found that yeast adhesion is related to its surface glycoprotein. Interestingly, when knocking out this glycoprotein-encoding gene (*FLO11*) along with its regulatory gene (*FLO8*), the adhesion between mutant yeast and other yeasts was eliminated. Since homologues of the *FLO11* gene also exist in some pathogenic fungi like *Candida albicans*, *Saccharomyces cerevisiae* could serve as a model for studying pathogenic bacteria invasion and pathogenesis, as well as for the rapid screening of antimicrobial drugs [96].

6 Conclusion Remarks

The rapid advancements in engineering yeast for pharmaceutical research have revolutionized the field of drug discovery and development. The comprehensive analysis of the yeast genome has provided invaluable insights into new targets for pharmaceutical research, leveraging the remarkable genetic similarities between yeast and higher eukaryotes [97]. The identification and functional analysis of yeast genes homologous to human disease genes have opened new avenues for understanding disease mechanisms and identifying potential drug targets [98].

The Y2H system has emerged as a powerful tool for studying protein-protein interactions, offering a high-throughput method to map complex interaction networks. This system has significantly contributed to identifying drug targets, understanding viral pathogenesis, and exploring the molecular underpinnings of various diseases, including neurodegenerative disorders and cancer [99]. The integration of Y2H with next-generation sequencing and CRISPR-Cas9 technology further enhances its potential, providing a more detailed and dynamic view of protein interactions and genetic interactions [70].

The YSD system has become an essential platform for new drug screening and development. By displaying peptides and proteins on the yeast cell surface, this system allows for the rapid screening of large libraries for binding affinity and specificity, facilitating the identification of therapeutic candidates. The YSD system's ability to mimic mammalian post-translational modifications and present complex antigens has made it an invaluable tool in vaccine development and antibody engineering [100].

Optimizing the yeast expression system has been crucial for producing high yields of recombinant proteins, essential for developing new drugs and therapeutic agents. Advances in metabolic engineering, synthetic biology, and genome editing have led to more efficient and scalable production processes [23]. The flexibility and robustness of yeast as a production platform make it ideal for manufacturing a wide range of pharmaceuticals, from simple peptides to complex biologics [20].

In conclusion, engineering yeast has become a cornerstone of modern pharmaceutical research, offering versatile and powerful platforms for drug discovery, screening, and production. The continued development and refinement of yeast-based technologies promise to enhance the efficiency and sustainability of pharmaceutical manufacturing processes, ultimately leading to safer and more effective therapies for treating human diseases. As research progresses, the integration of these yeast-based systems with emerging technologies will likely yield even more significant breakthroughs, paving the way for innovative therapeutic solutions and transforming the landscape of modern medicine.

7 Future Perspectives

The future of engineering yeast for pharmaceutical research is poised to bring transformative changes to drug discovery and development. As we continue to harness the power of yeast, several promising avenues and emerging technologies are expected to shape the next generation of pharmaceutical research.

7.1 Integration with Artificial Intelligence and Machine Learning

The integration of Artificial Intelligence (AI) and Machine Learning (ML) with yeast-based systems is rapidly transforming the landscape of pharmaceutical research. By harnessing the power of AI and ML, researchers can analyze vast and complex datasets generated from yeast genome studies, protein-protein interaction networks, and high-throughput screening assays [101]. These technologies enable the identification of novel drug targets, prediction of therapeutic efficacy, and optimization of experimental workflows, ultimately accelerating the drug discovery process.

AI and ML algorithms are particularly adept at uncovering hidden patterns and correlations in large-scale biological data. For example, ML models can analyze genetic sequences and protein interactions to predict the functional outcomes of specific genetic modifications in yeast [102]. This ability to predict the effects of genetic alterations in yeast models enables a more targeted approach to drug development and pathway engineering. Furthermore, AI-driven approaches are being used to optimize yeast strains for the efficient production of therapeutic proteins, improving yields and reducing costs associated with biopharmaceutical manufacturing [103].

One of the key areas where AI and ML are making an impact is in the design of synthetic biology circuits within yeast. These circuits often involve complex gene interactions, and predicting their behavior is a challenging task. AI and ML models, particularly deep learning techniques, can simulate these gene regulatory networks, providing insights into how synthetic constructs might behave *in vivo*. This allows for the design of more robust and predictable yeast-based biosystems for drug production [102].

In high-throughput drug screening, AI-powered tools can enhance the analysis of large datasets by identifying potential drug candidates with higher accuracy and efficiency. These tools can analyze patterns in compound-library screening results, identify promising drug-like molecules, and predict their interactions with yeast-expressed target proteins [101]. Additionally, AI algorithms can assist in the identification of off-target effects, toxicity risks, and the potential for drug resistance-critical factors in the drug development process.

The integration of AI and ML also enables adaptive learning in yeast engineering processes. As data from experimental trials are collected, ML models can continuously learn from these experiments, improving their predictive capabilities over time. This iterative approach can lead to more refined yeast strains and optimized fermentation processes, enhancing the scalability and productivity of therapeutic molecule production [103].

Moreover, the combination of AI with next-generation sequencing and genome editing technologies such as CRISPR-Cas9 has the potential to revolutionize the precision of yeast-based genetic engineering. AI tools can predict the most effective CRISPR guide RNAs, thereby reducing off-target effects and improving the efficiency of gene editing. This synergy between AI and advanced genome-editing techniques is likely to play a crucial role in the development of next-generation yeast platforms for pharmaceutical applications [104].

In summary, AI and ML have the potential to significantly enhance the capabilities of yeast-based systems in drug discovery and production. Their ability to analyze complex biological data, predict genetic outcomes, and optimize experimental designs will not only streamline the drug development process but also enable more efficient and sustainable manufacturing of therapeutics. As these technologies continue

to evolve, they will play an increasingly vital role in shaping the future of pharmaceutical research and biotechnology.

7.2 Advanced Genome Editing Techniques

The ongoing advancements in genome editing technologies, particularly CRISPR-Cas systems, will further enhance the precision and efficiency of genetic modifications in yeast. These tools will enable more complex and targeted manipulation of yeast genomes, facilitating the study of intricate gene functions and interactions [39]. Additionally, the development of novel CRISPR variants and delivery methods will expand the applicability of genome editing in diverse yeast strains [87].

7.3 Synthetic Biology and Metabolic Engineering

The field of synthetic biology will continue to play a crucial role in engineering yeast for pharmaceutical applications. The design and construction of synthetic gene circuits and metabolic pathways will enable the production of novel and complex biomolecules. Innovations in metabolic engineering will optimize yeast metabolism for higher yields and improved scalability of biopharmaceuticals [105]. The development of orthogonal systems and synthetic promoters will provide greater control over gene expression and metabolic flux.

7.4 Expanding the Yeast Toolkit

The exploration and utilization of non-conventional yeast species will diversify the toolkit available for pharmaceutical research. Yeast species such as *Pichia pastoris*, *Yarrowia lipolytic*a, and *Kluyveromyces lactis* offer unique metabolic capabilities that make them particularly valuable for various biotechnological applications [106]. *Pichia pastoris* is known for its high capacity for methanol utilization, making it a robust expression system for recombinant protein production, particularly for complex proteins requiring post-translational modifications such as glycosylation. *Yarrowia lipolytica*, an oleaginous yeast, excels in lipid metabolism, allowing it to efficiently produce lipids and biofuels, as well as valuable pharmaceuticals like biopolymers and enzymes [107]. Additionally, *Kluyveromyces lactis* is a strong candidate for dairy-related applications, capable of efficiently fermenting lactose and producing recombinant proteins with high yield [108]. By leveraging these species' specific metabolic advantages, researchers can develop tailored solutions for a wide range of pharmaceutical applications, including protein production, vaccine development, and bioremediation [109].

In conclusion, the potential of engineering yeast for pharmaceutical research remains vast and promising. As technological advancements continue to evolve, yeast-based systems are expected to become more refined, offering enhanced capabilities for the production of safer, more effective, and scalable therapeutic agents. By leveraging these innovations, researchers can accelerate the development of novel pharmaceutical treatments, optimizing drug discovery processes and improving the overall efficiency of therapeutic development [110]. These advancements hold the key to overcoming current challenges and driving progress in modern medicine, ultimately facilitating the creation of more targeted and personalized therapies [111].

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