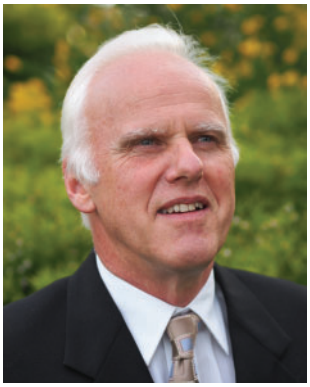
Yeast as a model organism for the pharmaceutical and nutraceutical industries



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Considerable knowledge about how we function has come through the use of the unicellular microbe yeast. Yeasts are eukaryotes like us and the similarity between us and yeasts is readily visible at the molecular level. This places yeast as an important tool for industries involved in health research, including pharmaceutical and nutraceutical discovery.

A number of advances to understand the biology of human life have been recognised by the awarding of Nobel Prizes and the studies have involved yeast[1]. Examples include:

- the discovery of key regulators of the cell cycle to Lee Hartwell, Tim Hunt and Paul Nurse (2001);
- studies of the molecular basis of eukaryotic transcription to Roger Kornberg (2006);
- discovery of the enzyme telomerase and how chromosomes are protected by telomeres to Elizabeth Blackburn, Carol Greider and Jack Szostak (2009);
- discovery of machinery regulating vesicle traffic to James Rothman, Randy Schekman and Thomas Südhof (2013); and
- studies on autophagy to Yoshinori Ohsumi (2016).

These discoveries are important for humans since we can extrapolate from yeast studies, where we have knowledge from mutants and genetic engineering, to make statements like 'in this particular aspect this is how we function'. So human proteins can be identified by the similarity to a yeast protein whose function is characterised and in many cases there can be complementation with a human protein in a yeast deletion mutant.

There is higher complexity in humans but it is often due to our multicellular nature. More cell types mean proteins may have multiple isoforms for specialisation and novel functions. Conversely, there is a well-recognised genome duplication of *Saccharomyces cerevisiae* that adds complexity to this model yeast. However, our knowledge of that duplication and the capability to remove sequences at will means that this model eukaryote can be engineered to produce the human proteins in the context that we want to study, enabling us to test ideas and develop new therapies. For example, this engineering might include the functional replacement of the duplicated *RAS1/2*, *HMG1/2*, or *TOR1/2* genes, with the equivalent human genes for cancer, cholesterol pathway, or protein turnover studies in *S. cerevisiae*.

Another great example of yeast utility for human protein studies are yeast two hybrid analyses[2] that enable studies of human protein-protein interactions as well as interactions between viral proteins and our proteins. Yeast with such interactions can be screened for compounds that disrupt the interactions in the reverse two hybrid screen[3]. Compared to *in vitro* approaches (e.g. surface plasmon resonance), a big advantage of such screening is that interactions are genuine, no protein isolation is required, and bioavailability is demonstrated.

Yeast to study chronic diseases

Chronic diseases, including cancer, heart disease, fibromyalgia, Parkinson's disease and Alzheimer's disease (AD), are of interest to us because they are common and their incidence increases with age, important factors that drive the search for cures. There are huge economic benefits that can result from advances in treatment of such chronic diseases. These diseases are a huge burden to our society, but to industry they represent valuable opportunities for revenue, since people may require treatments for the remainder of their life.

As a model organism, yeast can provide information in many ways. A few selected examples are listed in Table 1 and some examples are described in a little more detail below. In addition, several recent reviews also discuss the use of yeast as model[17–19].

Yeast can contribute to cancer research providing information on cell growth and cell death as well as the mechanisms of resistance to cancer drugs such as methotrexate where yeast can become resistant through over-production of dihydrofolate reductase[11]. But apart from being a model organism yeast has already provided significant advances in cancer prevention: yeast produces viral subunit vaccines that protect against hepatitis B and human papillomavirus [see the article by Penumarthi and Smooker in this issue] that cause cancers. In the future, yeast may produce cancer antigens that may trigger protective responses and they may also produce therapeutic antibodies that target cancer antigens.

For prevention of cardiovascular disease by the cholesterol-lowering statins, we have learnt from yeast that statins target the same pathway in yeast and markedly affect mitochondrial function[17].

Table 1. Some examples of human diseases where yeast (mostly the budding yeast, *Saccharomyces cerevisiae*) can serve as a model.

Disease	Yeast model and behavior	Some further reading
Cardiovascular disease	Statins target HMG CoA reductase which is also present in yeast. Side-effects on mitochondrial function can be observed.	4
Alzheimer's disease	Autophagy of Aβ can be studied: compounds promoting Aβ removal can be screened.	5–7
Parkinson's disease	alpha synuclein is toxic.	8
Prion disease	Yeast have prions (affecting just yeast) that can be 'cured'.	9
Cancer	Cell cycle can be studied in *Schizosaccharomyes pombe*, a yeast that divides by fission. Resistance to cancer drugs like methotrexate can be studied in yeast.	10,11
Malaria	*Plasmodium* drug targets and resistance (e.g. DHPS and DHFR) can be modelled in yeast.	12
HIV	HIV-1 Vpr causes a growth arrest in yeast and humans: inhibitors of that effect can be screened in yeast.	13
Biogenic amines	Dopamine and phenylethylamine toxicity in yeast can be overcome with antioxidants.	14,15
Aging	Yeast exhibit aging symptoms including telomere shortening, mtDNA deletions and reduced autophagy.	16

Figure 1. Materials for screening a library of ~4500 viable homozygous gene deletion strains (from the EUROSCARF diploid collection) for sensitivity to the antimalarial, artesunate.

This can explain muscle fatigue and why most athletes discontinue their prescribed statins.

In the case of AD, the effects of the AD-associated amyloid beta (Aβ) protein can be studied in genetically engineered yeast that produce Aβ. Compounds that increase the clearance of such a deleterious protein in yeast may be useful to test in humans[5–7].

While compound screening is often performed in large-scale high throughput *in vitro* assays the pre-screening of compounds in yeast (Figure 1) has distinct advantages. One learns very quickly about cytotoxicity and bioavailability of compounds, but the assay needs to ask the right questions. Assays are guided by our understanding of the molecular processes of a disease. For example, high levels of the toxic Aβ peptide are associated with AD. Therefore, removing Aβ or overcoming its toxic effects in yeast forms the basis of most assays. Immunotherapies have been very successful with many cancers and inflammatory diseases, but for AD they are not the best option for a disease where prevention is the main therapeutic option. Another, less expensive and more practical way for reducing toxicity, is to find low MW compounds to promote Aβ clearance. The screening of such compounds in yeast can be aided if Aβ is fused with a green fluorescent protein (GFP) so that clearance can be monitored *in vivo*. Thus high throughput cell-based assays can be developed[7].

Age-related diseases and nutraceutical testing

Aging is responsible for many chronic diseases: as we age we see increases in a spectrum of diseases. It is impossible to stop aging but if we could slow it we could reduce the burden of many diseases. The cause of aging is debatable but it is clear that old cells exhibit reduced lifespan, accumulation of aberrant proteins, reduced telomeres and mitochondrial dysfunction. The budding yeast *Saccharomyces cerevisiae* also exhibits these behaviours and can be used as a model to look for compounds that restore health to old cells.

Studies with the yeast model for AD provide insights into the use of yeast as model for aging. While all cells in the yeast population are producing the Aβ-GFP fusion protein constitutively, the young yeast degrade it[7]. Thus, Aβ-GFP is only observed in older cells that have two or more buds (equivalent to grandparents and great grandparents). Compounds that can aid the removal of the Aβ-GFP from the older cells may be actually anti-aging compounds, and promote clearance of other deleterious compounds.

While effects on mitochondrial function in humans are deleterious to growth, yeast with no mitochondrial function and even no mitochondrial DNA can grow on sugars. This marks yeast as being an excellent organism to distinguish agents that affect mitochondria. In addition, yeast is ideal for observations on mtDNA loss and mitochondrial genotoxicity.

Telomere shortening can also be studied in yeast.

The power of numbers in microbes can provide answers to the problems

Another aspect of microbes is that they can usually develop mechanisms to deal with the stresses they are challenged with. So when the population is challenged with an antibiotic or a toxic protein, some cells in the very large population might acquire a mutation that causes resistance to the stress. Identification of such mutations can teach us how the disease functions, how the yeast deals with it, and how we might therefore approach the human disease problem. For examples, the malaria parasite *Plasmodium falciparum* can become resistant to antifolates through mutations in the genes encoding enzymes involved in folate synthesis and utilisation and cancer can be targeted with antifolates but cancer cells can become resistant through amplification of the enzyme involved in folate utilisation, or through mutations that cause increased drug efflux[20]. These phenomena can be replicated and modelled in yeast.

Conclusions

Similarities in yeast and human cells mean that yeast can be surrogates to model disease processes and treatments. Yeast offer many opportunities for physiological, genetic and molecular manipulation, so in many instances they offer mimicry of processes where we are seeking pharmaceutical or nutraceutical interventions.

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Biograpy

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