Biodiscovery of chemo preventatives of Alzheimer's disease using yeast



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Yeasts have been used as models for just about everything, from basic studies in molecular biology and genetics to cancer and studies of drug resistance. Initially Alzheimer's disease (AD) was seen as an extracellular disease, so it was hard to see how yeast might make a contribution to AD research. However, there is now recognition that the main player, Aβ, has intracellular and extracellular effects. Furthermore, it is now clear that yeast studies are relevant to AD.

AD is the most common form of dementia and is usually age-related. In addition to the burden it places on those directly affected, there is a huge economic burden. Amyloid Aβ, a peptide of 42 amino acids, is generally regarded as the major player in AD and there are considerable efforts to inhibit Aβ oligomerisation and Aβ toxicity, both of which are associated with AD. Most efforts to study these effects and to search for inhibitors of these effects utilise transgenic mice that overexpress Alzheimer's Precursor Protein (APP), from which Aβ is derived. Current models use multiple mutations in APP that result in accumulation of Aβ in a matter of months, leading to deposition of Aβ plaques in the brain, and relevant behavioural traits such as the ability to learn mazes. Such mice are used for the assessment of chemo preventatives that inhibit Aβ deposition and learning disorders.

However, Aβ formation can be assessed in yeast in much less complicated ways, including assays that determine unprocessed APP levels (without Aβ) and Aβ (without APP). Assessments of Aβ formation in a yeast model (1) help the understanding of molecular events, and (2) enable screening for chemo preventatives that alleviate adverse events in the molecular processes.

in vivo Aβ oligomerisation

Several yeast systems have been used to assess the oligomerisation of Aβ. The first study used a two-hybrid system in yeast to study the interaction of Aβ monomers by linking Aβ42 to LexA DNA binding domain (bait) and also B42 transactivation domain (prey) [1]. Others used Aβ/Sup35p fusions [2,3]. Sup35p is a well characterised yeast translational termination factor, which can be efficiently used as a reporter system for studying protein interactions. For our studies we used Aβ peptide C- or N-terminally fused to green fluorescent protein (GFP). Cells expressing GFP/Aβ fusions also showed lower growth yield and an increased heat shock response, indicative of its toxic nature in yeast cells [4]. Each of these yeast systems are useful tools in the study of Aβ oligomerisation, for screening compounds affecting the aggregation process and also to decipher the molecular pathways triggered by Aβ-aggregation.

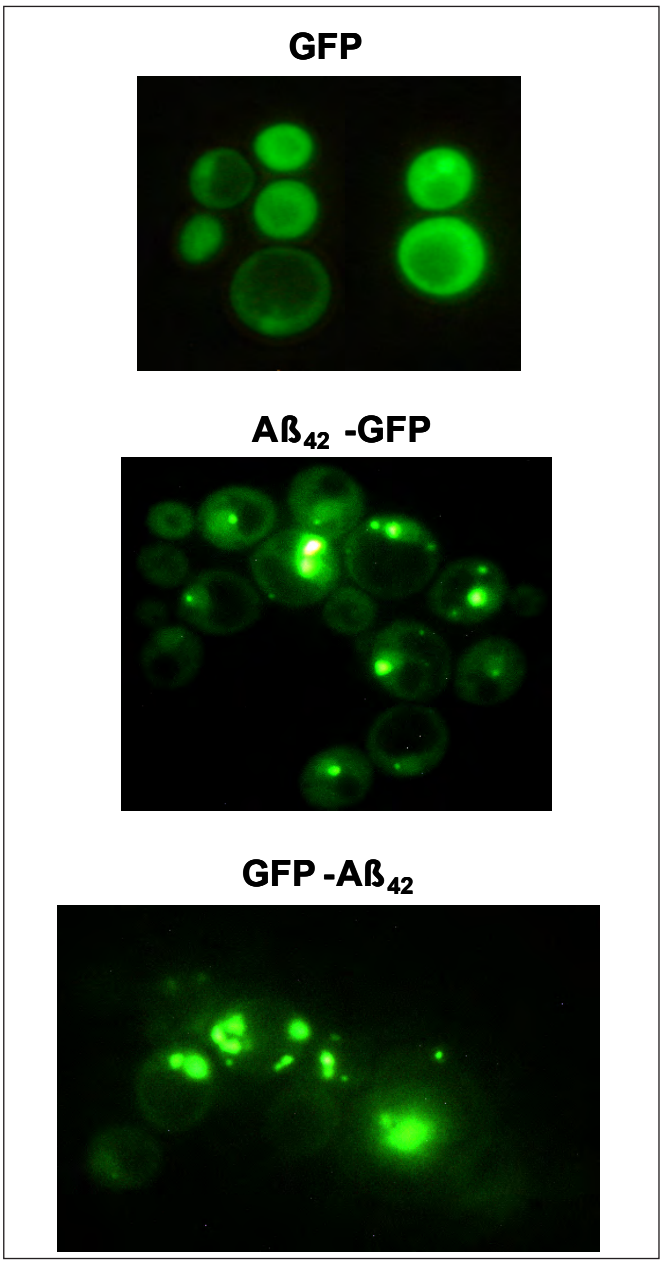
Yeast cells that produce Aβ–GFP or GFP-Aβ are quite unlike those that produce unfused GFP. In the latter, essentially all cells exhibit uniform cytoplasmic green fluorescence. In contrast, only a minor population of transformants producing Aβ-GFP or GFP-Aβ exhibit green fluorescence, and in these cases it is confined to punctate patches in larger, generally older cells (Figure 1). In terms of assay development, the addition of some agents (chemical or food fractions) to the yeast cultures induces an increased proportion of cells to fluoresce. One reason for increased fluorescence is the inhibition of oligomerisation of Aβ. Compounds that have this effect are presumed to have crossed the cell membrane reaching the cytoplasm to have their effect on newly synthesised Aβ-GFP or GFP-Aβ. Such compounds have potential in the prevention of AD.

In addition to finding numerous novel compounds in CSIRO's chemical and food libraries that cause increased fluorescence, two known compounds associated with the chemo prevention of AD have also been examined. The first of these, folate, is linked with chemoprevention of AD [5] and was shown to increase fluorescence of Aβ-GFP or GFP-Aβ in yeast [6]. Yeast normally synthesises abundant levels of folate, so this experiment required the construction of yeast mutants deficient in folate synthesis. In these mutants Aβ42-GFP aggregation could be decreased upon treatment with folinic acid, a synthetic form of folate [6]. The underlying molecular mechanisms for the protective effects of folate remain unknown; however, it is likely that homocysteine toxicity, which is linked to folate metabolism, is involved.

The second potential AD chemopreventative is a group of cholesterol-lowering drugs known as statins, which have been shown to modulate APP/cholesterol metabolism, Aβ accumulation/clearance, and cognition in mammalian AD

models [7,8]. Simvastatin appears to be the most effective member of the group, giving rise to an AD incidence that is just 30% of untreated population [9]. Therefore, statins have been suggested as a potential AD chemo preventative [10,11]. Preliminary data from yeast assays also shows simvastatin increases relative green fluorescence in GFP/Aβ expressing cells, indicative of Aβ disaggregation. It is inferred from experimental evidence that simvastatin has the same target in yeast and man [12,13]; however, the underlying mechanism of simvastatin's action needs to be investigated. While reduced prenylation of β-secretase, and consequent reduced processing of APP and reduced production of Aβ, has been considered to be the reason for simvastatin's effects, yeast studies, where APP processing is not a factor, suggests an alternative mechanism. We suggest that cholesterol lowering should be examined as well as the inhibition of mitochondrial respiratory function [12], which would lead to fewer reactive oxygen species (ROS). ROS is strongly implicated in AD, and the ability of yeast to survive without mitochondrial respiratory function offers a convenient approach to unravel the contribution of mitochondria in AD.

Figure 1. ***In vivo*** **Aβ oligomerisation in yeast.** Fluorescent microscopy of exponentially growing yeast cells expressing GFP, Aβ-GFP and GFP-Aβ. Diffuse cytosolic fluorescence is observed with GFP; however, punctate patches are observed with GFP/Aβ fusions.



Extracellular Aβ toxicity

Increased levels of Aβ42 protein and deposition in the brain have been shown to correlate with the acute cognitive decline observed in AD [14,15]. Studies have attributed Aβ42 aggregation and toxicity as one of the primary causes of AD-caused dementia [16]. However, the exact isoform and mechanism of Aβ42-caused neuronal dysfunction is still unknown. Chemically-synthesised Aβ42 peptide has been extensively used for studying its pathological role in AD. Identifying a particular Aβ42 toxic species has been a major problem in mammalian cell culture studies as they require media and serum to maintain viability. Recently, a new method to study Aβ42 cellular toxicity in water, achieved through the use of yeast, was developed [17]. In this method Aβ42 was stably maintained in either oligomeric or fibrillar form, thereby enabling a study of the specific effects of different isoforms of Aβ42. Using a survival plating cell viability assay, oligomeric Aβ42 was found to have a concentration-dependent toxicity and significantly more toxicity than fibrils. This observation is consistent with Aβ42 studies in mammalian cell lines, suggesting soluble oligomeric Aβ42 as the main determinant of neuronal dysfunction and cognitive decline in AD.

Micromolar concentrations of oligomeric Aβ42 cause significant toxicity in neuronal cell culture assays. Similar Aβ42 concentrations cause significant cell death in the yeast assay [17]. Notably, some of the novel compounds that increase fluorescence (modulation of Aβ aggregation) in the yeast Aβ oligomerisation assay also appear to rescue yeast in the Aβ toxicity assay. The identification of leads from the above assays is a considerable advance over existing assays that involve highly artificial *in vitro* tests that do not provide information about bioavailability, and tests in animal models that are often related to suppression of Aβ-induced toxicity or cognitive tests.

Conclusion

Yeast provides a number of unique opportunities to study AD:

- APP effects and Aβ effects can be examined separately.
- Cellular environments can be easily and comprehensively manipulated.
- Gene expression can be closely monitored.
- Genomic backgrounds can be manipulated.
- Mutant selection systems can be arranged.

Therefore, yeast studies are proving very useful for the study of AD with great potential in the development of AD chemo preventatives [11]. Currently extracellular and intracellular molecular pathways associated with AD are both capable of being addressed in yeast.

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Biographies

Ian Macreadie has worked with yeast genetics and molecular biology for 33 years, at Monash University, University of Texas Health Science Centre at Dallas, and at CSIRO. He is currently the Chief Scientific Officer of Sienna Cancer Diagnostics and Editor of Microbiology Australia.

Professor Ralph Martins career in Alzheimer's disease over 24 years has resulted in over 200 publications. He established the McCusker Alzheimer's Research Foundation and the Centre of Excellence for Alzheimer's Disease Research and Care. In 2004, he was appointed inaugural Chair in Ageing and Alzheimer's at Edith Cowan University. He is a senior editor for the *Journal of Alzheimer's Disease* and editorial board member for *CNS and Neurological Disease.* Martins is a board member of three research foundations and Alzhyme, a biotech company developing anti-amyloid drugs, which has taken a lead compound from concept to successful animal trials in four years. He is the WA Australian of the Year for 2010.

Prashant Bharadwaj is a doctoral student at Centre of Excellence for Alzheimer's Disease Research, Edith Cowan University, WA affiliated to CSIRO Molecular and Health Technologies, VIC. His research is focussed into developing yeast as a model for studying beta amyloid (Aβ) peptide.

Generating interspecific wine yeast hybrids for funky wines

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When we think of *Saccharomyces cerevisiae*, fermentation immediately comes to mind, but this is not the only trait that makes this yeast the organism of choice for bread, beer and wine production. The winemaking industry, for example, requires robust strains, capable of converting sugar to ethanol in challenging conditions; high osmotic stress and low pH in the initial grape must, followed by high ethanol concentration at the later stages of fermentation. Winemakers also look for ways of using fermentation to introduce aroma and flavour diversity to their wines as a means of improving style and for product differentiation. Choice of wine yeast from the plethora of strains available to winemakers is one way of achieving this, particularly with the new breed of interspecific hybrid yeast strains currently being generated.

Economies of scale, the risk of indigenous microorganisms of the grape must spoiling a wine and the desire to produce consistency in wines between vintages have led to the modern winemaking practice of using an inoculum of a single robust yeast strain, typically of *S. cerevisiae,* for each fermentation. While wines made using this practice produce consistently sound fermentations, there are indications that the contributions of many different microorganisms in spontaneous fermentations deliver greater diversity of flavour profiles and better palate structure to wine. Many winemakers would like to produce more complex wines, but don't want to risk their fermentations to an unknown yeast population that might cause spoilage.

One strategy used to generate robust wine yeast strains that produce a wider range of cellular metabolites, and hence a more complex wine profile, has been to cross-existing, commercial *S. cerevisiae* strains with other, non-*cerevisiae,* members of the *Saccharomyces* genus. *Saccharomyces* species are diverse, but they share a common mating system and, therefore, some species can mate with each other, and this is a natural, non-GM (non-genetically modified), process. In fact, a small number of available commercial lager, wine and cider yeast strains, originally isolated from natural environments, have been identified as interspecific hybrids between members of the *Saccharomyces* senu stricto complex, a group of closely related species within the *Saccharomyces* genus [1-5].