Saccharomyces cerevisiae as a conduit to providing insights into cellular responses using hypothesis-generating technology

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In the post-genomic era, hypothesis-generating methods of data collection have facilitated rapid advances in many areas of research. Using *Saccharomyces cerevisiae* in conjunction with these methods we have been able to gain insight into the 'bigger picture' of our particular research fields. This has provided the means of generating more 'informed' hypotheses that have subsequently been used in traditional hypothesis-driven research. As a result, important issues have been elucidated in the areas of flavour profile management in industrial fermentations, identification of cellular pathways involved in the biological activity of anticancer drugs and gaining a holistic view of the diverse molecular systems in which cells combat the negative effect of reactive oxygen species.

S. cerevisiae (yeast), was the first eukaryotic model organism to have its genome sequenced [1]. The development of analytical techniques to probe and quantify the expression levels of a large number of individual genes has facilitated a major step forward in the ability to monitor expression of the complete yeast genome [2]. The ease with which yeast can be genetically engineered has also resulted in it being the only organism in which a genome-wide mutant library of all non-essential genes has been constructed [3]. The sheer volume of data that can be generated using these methods, coined 'functional genomics', has not just given insights into the broader picture of scientific endeavours but has enabled the identification of important molecular markers [4] and also facilitated gene function discovery [5].

The complexity and variability of the raw materials used in industrial fermentations make it difficult to identify and understand the main processes important in the completion of a stable and consistent end product. The dynamic nature of this process limits the usefulness of classical research methods and results in the acceptance of 'scientific dogmas' that eventually are found to be 'old wives tales'. This is not the scientific basis that a researcher wants to be using to formulate hypotheses.

In view of producing more informed hypotheses, research collaborations between the University of Western Sydney, The University of New South Wales (UNSW) and Carlton & United Beverages have used changes in yeast gene expression to provide insights into the processes important for successful beer fermentations. As a result, the first genome-wide expression analysis carried out on yeast in an industrial fermentation process was attempted [6]. Three clusters of genes (ergosterol biosynthesis, redox homeostasis and metabolism) were identified as being highly induced in the first hour of fermentation compared to the twenty-third hour (Figure 1 [A, B, C]). The thioredoxin and glutathione systems that protect yeast cells from reactive oxygen species [7] were also induced during this period (Figure 1 [B]). This resulted in the discovery that ergosterol has an important role in protection against oxidative stress damage to cells [8]. This research has led to an awareness of the importance of careful

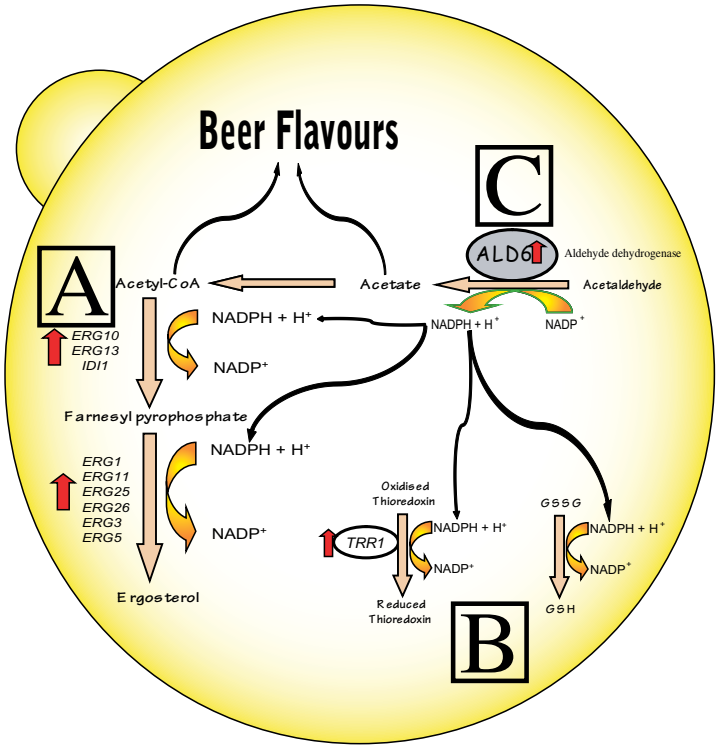

Figure 1: Characterisation of the metabolic pathways active in the first hour of a beer fermentation compared to the twenty-third. Red arrows indicate genes that were induced during the initial stages of the fermentation. (A) Ergosterol biosynthesis pathway. (B) Thioredoxin and glutathione systems important for redox homeostasis. (C) Conversion of acetaldehyde to acetate by Ald6p.

management of fermentation aeration regimens so as to provide enough oxygen for ergosterol biosynthesis but not a level that aggravates the effects of reactive oxygen species causing oxidative stress.

The most highly induced gene during the initial period of fermentation was *ALD6* (Figure 1 [C]), which codes for the cytosolic aldehyde dehydrogenase protein [9]. The Ald6p converts acetaldehyde to acetate and produces the reducing agent, NADPH, that is evidently needed for the ergosterol biosynthesis and the reduction of oxidised anti-oxidants, which were highly active in the initial stages of the fermentation (Figure 1 [B]). Ald6p also provides the acetyl-CoA that is not only needed for ergosterol biosynthesis but is an important precursor for the production of ethyl acetates and other volatiles important for the flavour in beer. Initial results measuring the flavour profile of ald6 mutants have shown that this protein is very important in flavour production and implicate the importance of ergosterol and redox management of beer fermentations on the flavour profile of the finished product.

Genome-wide surveys of changes in transcripts have provided insight into regulatory responses to free radicals generating compounds. However, the relevance of these genes to survival, repair of damage or cellular recovery has not yet been revealed. To complement our interests in protection against reactive oxygen species the complete set of viable deletion strains in *S. cerevisiae* was screened to identify cell functions involved in resistance to oxidative stress. Approximately 10% of the mutant library (456) was sensitive to at least one of five different types of oxidant, many of which were not previously known to have a role in resistance to reactive oxygen species. The degree of specificity of cellular responses to different oxidants was surprising with only two mutants sensitive to all oxidants examined and only twelve sensitive to at least four. This demonstrated the different spectra of deletants that were sensitive to different oxidants and highlighted that no single oxidant is representative of a general oxidative stress [10].

The genome-wide set of *S. cerevisiae* deletion strains has also been shown to be useful in elucidating the cellular mechanisms involved in the selectivity of the angiogenesis inhibitor arsenical 4-(N-(S-glutathionylacetyl)amino) phenylarsenoxide (GSAO) prior to new gene expression [11]. A recent UWS study explored the mechanisms responsible for the highly toxic nature of a novel anticancer drug also using the genome-wide set of *S. cerevisiae* deletion strains as a model system. Surprisingly initial results have indicated that although it is platinum-based like cisplatin, its mechanism of toxicity is completely different to this widely used anticancer drug, making it a promising candidate as a possible treatment for tumours that have acquired resistance against cisplatin.

Despite the belief of some factions that hypothesis-driven methods are the only significant means of scientific advance, the combination of data- and hypothesis-driven research (a topic discussed in more detail by Kell and Oliver [12]) has given us a way forward in enhancing scientific discovery in our fields of study.

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