Functional genomics in yeast

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Completion of the *Saccharomyces cerevisiae* genome sequencing project in 1996 led to an incredible explosion of research on basic cellular processes and has provided the opportunity to determine how genes and their products are regulated and function on a genome-wide scale. The technologies that were developed from this provided an incredible array of tools to study cellular processes in great detail and were a paradigm for developments from subsequent sequencing projects.

On completion of the *S. cerevisiae* sequencing project it was estimated that there was understanding of the role of only about 50% of the genes identified, and for many genes this knowledge was only partial. The completed sequence provided the catalyst for development of a wide range of technologies that allow analysis of how the entire complement of genes in the genome participates in particular processes. Over the intervening decade the proportion of genes for which there is some understanding of function has risen considerably with a light-hearted estimate of the completion of the annotation process by 01 April 2007 in time for the 23rd International Conference on Yeast Genetics and Molecular Biology (Melbourne, 01 – 06 July 2007).

This understanding is now leading to interesting developments in the systems biology approach to modelling how an organism functions, as exemplified by the analysis of how yeast cells respond to osmotic stress [1]. These technologies have also been valuable in studying cellular functions in eukaryotic organisms other than *S. cerevisiae*, including humans. About 60% of yeast genes have a human homologue, and in many cases the human gene can function in yeast. Moreover, nearly 400 proteins in *S. cerevisiae* are orthologues of proteins associated with human diseases [2]. Therefore, *S. cerevisiae* can provide a platform to study molecular processes and structure-function relationships, and to analyse the toxicity and effectiveness of drugs in a more rapid and convenient way than in the particular organism under study.

The technologies developed include microarray for gene expression studies, with extension to ChIP-chip analysis for each transcription factor to determine where it binds in the entire yeast genome [3]. By labelling every gene individually with *GFP* the location of each gene product in the cell can be determined and a database is available on the internet. Several protein-protein interaction screens have provided valuable databases that inform the fundamental subassemblies that make up the cellular machinery [4,5,6]. However, this article discusses those approaches that have led to the generation of mutations affecting every gene in the yeast genome since these have been very fruitful, not only in studying yeast genes, but also in studying structure-function relationships for other, higher eukaryotes.

Genome-wide mutation analysis

The use of mutants as a specific way to study gene function has been very widely used for a long time. With the availability of the genomic sequence came the opportunity to generate mutants on a genome-wide scale to try and find a phenotype for the mutations that would provide clues to the function of unknown genes and to identify the role of all genes in specific cellular processes.

With *S. cerevisiae* there were several such ambitious programs. An initial program (Eurofan), inspired by the sequencing partners in Europe, set out to delete 1000 genes (of the ~6000 identified) in a cooperative way. This was superseded by a joint US/European program that led to the generation of mutants in every non-essential yeast gene, and conditional mutants in essential genes. This set of yeast mutants has every mutant specifically and individually 'tagged' with unique 20 bp 'barcodes' at the start and end of the kanamycin resistance cassette used to generate the deletion. These mutants have been used individually to identify phenotypes. For example, in our laboratory, initial screening of the genome-wide deletion collection on five different oxidants identified about 456 mutations affecting cellular responses to reactive oxygen species, and increased the number of known mutants by more than ten-fold [7]. Many of the unknown mutants identified were as sensitive as other well-characterised mutants obtained by random mutagenesis and tedious screening procedures that were obviously far from saturating.

Since the tags (molecular bar codes) in the deletion strains can be identified by hybridisation of DNA from the population to

custom microarrays, these strains can also be used to determine the contribution that a gene makes to the 'fitness' of the organism. By growing a mixed population of all mutant strains under a particular condition (e.g. in the presence of a toxic metabolite) one can detect strains that have been lost by the absence of the bar code hybridisation signal. With the large number of screens that have been done of how cells respond to various antibiotics and toxic compounds, it is possible in some cases to identify the target of an unknown compound by comparison to the spectrum of responses to known drugs [8]. Each mutation is available in haploid *a* and alpha strains, in the heterozygous diploid and in the homozygous diploid state. Heterozygous diploids have been exploited in haploinsufficiency studies to identify gene functions and these are reported to give a clearer understanding of gene involvement in metabolic processes, since they identify genes that need to be fully expressed for a function to occur normally [9].

At about the same time, the laboratories of Mike Snyder and Shirleen Roeder set out to generate transposon insertion mutations in every yeast gene. By clever design of the transposable cassette (Figure 1) the mutants can be used not only to determine the phenotype of the insertion mutant, but also the regulation of the gene due to the presence in the transposon of a *lacZ* reporter gene lacking an ATG start codon. The location of the gene product in the cell could be determined by using 3X haemagglutinin epitope tags fused to the yeast gene as part of the transposon. An interesting refinement was to engineer *loxP* sites, arranged so that in any mutant expression of *cre* recombinase would remove all but 39 bp of the transposon up to the haemagglutinin tags. This could generate 'hypomorphic' alleles (i.e. with reduced or conditional activity) instead of null alleles providing further tools for analysis of gene function. Basically this procedure provided three complementary hits to functional analysis in the one system: analysis of the loss of function of the gene; analysis of its expression; and identification of the location of the gene product [10].

Within five years of them becoming available, over 100 experimental conditions were analysed and more than 5000 novel phenotypic traits were assigned to yeast genes using these various mutant collections [11], and these numbers have increased substantially since then. This has led to the identification of numerous previously unknown components of well-known

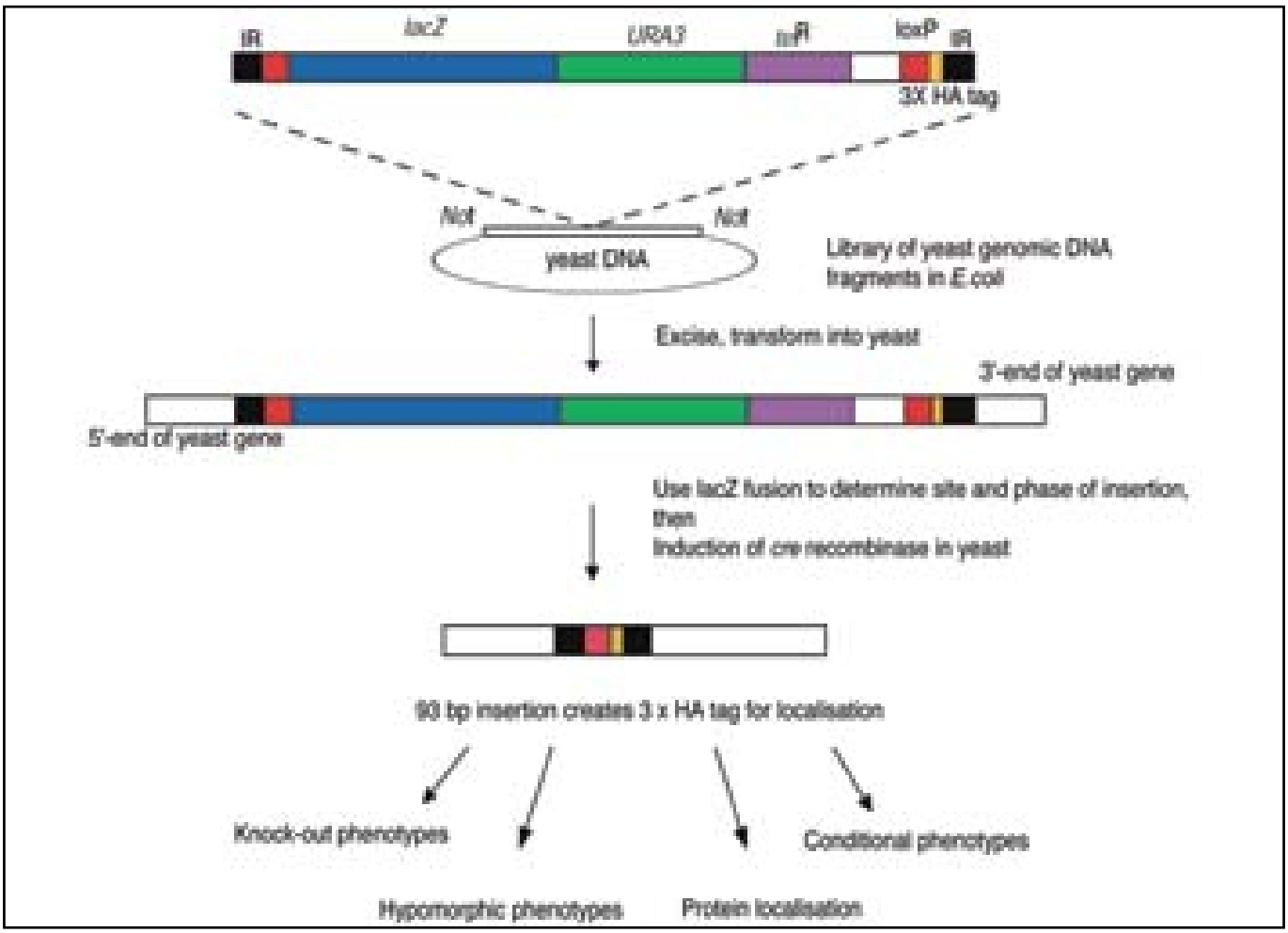


Figure 1.

pathways and processes, such as cell division, protein trafficking, meiosis and spore formation and germination.

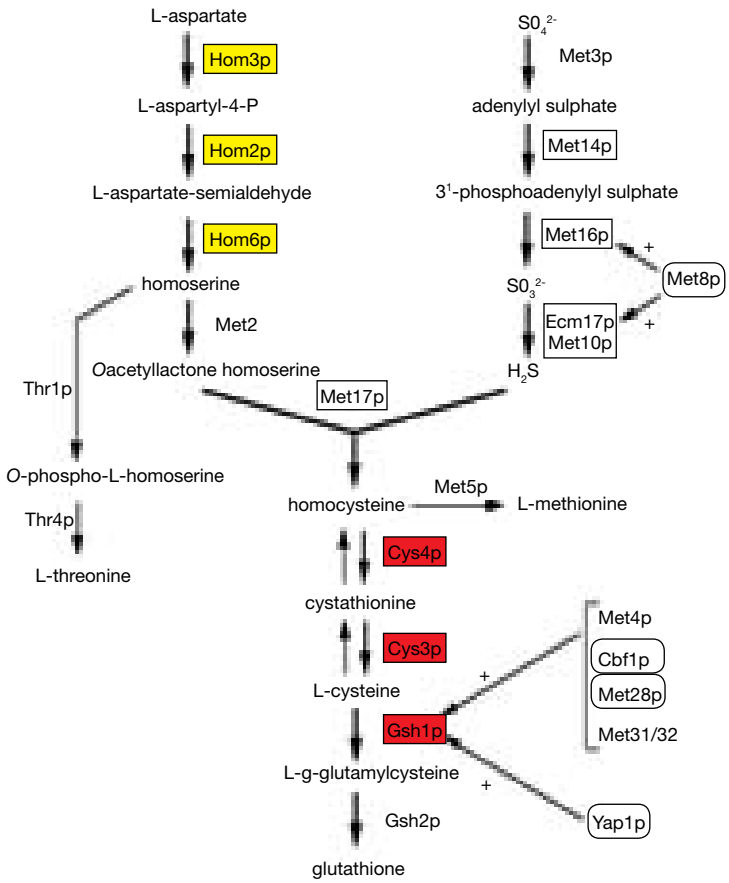
The deletion mutant collection has also been used to analyse functional aspects of genes from other organisms. A clear example is the study by Lindquist's group of the effects of over-expressing the human alpha-synuclein gene that is implicated in several neurodegenerative disorders, such as Parkinson's disease, in every individual member of the mutant set [12]. When expressed in yeast, alpha-synuclein selectively associated with the plasma membrane, before forming cytoplasmic inclusions. This system provided an opportunity to dissect the molecular pathways underlying normal alpha-synuclein biology and the pathogenic consequences of its misfolding. A similar systematic genome-wide study has been done to identify host functions affecting replication of the positive-strand Brome mosaic virus [13].

In collaboration with Phil Hogg's group in the Centre for Vascular Biology we screened the anticancer compound GSAO (glutathionarsenoxide, which functions to inhibit angiogenesis) against the deletion set. This identified every mutant in the glutathione biosynthesis pathway as among the most sensitive, and the most sensitive mutant was in the *YCF1* gene encoding the transporter that detoxifies glutathione-xenobiotic conjugates by transporting them from the cytoplasm to the vacuole (Figure 2). *YCF1* is a homologue of the human MRP multidrug resistance transporter family, and subsequent work in higher eukaryotic cells confirmed that specific members of this family were the basis for the selectivity of the GSAO against endothelial cells [14]. This highlights the very effective use of the yeast mutant collection to rapidly provide clues about what is happening in higher eukaryotes.

Synthetic lethality screening

Synthetic lethality is the genetic interaction of two non-lethal mutations that lead to inviability in combination. The related process, synthetic fitness, describes the interaction of two non-lethal mutations that lead to a non-lethal growth defect in combination. Although synthetic lethality and fitness have been used commonly for many years, first in *Drosophila* [15] and later extensively in yeast [16], the technique really achieved its potential for the elucidation of pathway topology with the work of Tong *et al* [17,18] using the genome-wide knockout collection.

In an ordered approach, Tong crossed the viable haploid strains of the knockout collection with a strain carrying a 'query' mutation. The resultant diploids where sporulated and the



Deleted Gene	IC50, mM	Function of gene product
YCF1	0.027	Metal resistance, similarity to MDR proteins
CYS4	0.032	First enzyme in cysteine biosynthesis
CYS3	0.430	Catalyses cysteine for cystathionine
PEP3	0.045	Protein trafficking through vacuolar membrane
SOD1	0.049	Cu/Zn superoxide dismutase
GSH1	0.054	First step of γ-glutamyl cycle of glutathione biosynthesis
YDL041w	0.062	Unknown function
MET8	0.070	Expression of PAPS and sulfite reductases
MET14	0.073	Sulfate assimilation and methionine biosynthesis
ECM17	0.080	Sulfite reductase β subunit
MET16	0.080	Sulfate assimilation and methionine metabolism
MET10	0.083	Conversion of sulfite into sulfate
KCS1	0.096	Vacuole biogenesis and environmental stress response
MET28	0.100	Transcription factor involved in sulfur metabolism
PHO85	0.108	Phosphate and glycogen metabolism and cell cycle control
PRM7	0.110	Unknown function
MET22	0.120	Salt tolerance, methionine synthesis and sulfate assimillation
YDJ1	0.125	Heat shock protein involved in mitochondrial protein import
CCR4	0.130	Carbon catabolite repression, oxidative stress
BRE1	0.135	Ubiquination of histone H2B
YDL023c	0.135	Unknown function
RHR2	0.140	Glycerol phosphatase
RSM23	0.150	Mitochondrial ribosome small subunit component
HOM3	0.180	First step in common methionine and threonine biosynthesis
VID28	0.188	Vacuolar import and degradation
HOM2	0.190	Methionine and threonine biosynthesis
MET17	0.200	Required for sulfur amino acid synthesis

Figure 2.

*MAT***a** haploids that carried both the original library mutation and the query mutation were isolated. Genes that conferred synthetic lethality resulted in loss of the strain from the plate. Ooi simplified the technique by integratively transforming pools of deletion strains, growing the cells *en masse,* and isolating the total genomic DNA to screen the pool using microarrays that can identify the barcodes that are built into the knockout collection [19]. The technique was termed SLAM for 'synthetic lethality analysis by microarray'. Recently described is an improvement on this technique, termed dSLAM for 'heterozygote-based diploid synthetic lethality analysis by microarray', in which the knockout construct is integrated into a mixed population of heterozygous diploid knockout strains, which are then sporulated and the progeny selected for both the single and double knockouts for use in microarray analysis [20]. Use of the more genetically stable diploids and the single mutant controls in the analysis results in an increase in the reliability of the data. This technique can also be adapted to examine the less common phenomenon of synthetic haploinsufficiency, further adding to our understanding of pathway dynamics.

In a much more adventurous approach one group has developed the concept of the synthetic genetic array analysis in which every viable deletion mutant is crossed with every other, leading to approximately 11 million double knockout combinations [21].

Whilst synthetically lethal point mutations may be the result of either loss of redundant pathways or the cumulative effect of two debilitating mutations in the same pathway, synthetic lethality analysis of knockouts unambiguously pinpoints parallel pathways. Although *Saccharomyces* duplicated its genome after the divergence of *Saccharomyces* from *Kluyveromyces* approximately 10^8 years ago leading to approximately 400 duplicated gene pairs [22], results indicated that the majority of genes conferring synthetic lethality (and therefore having redundant function) examined so far, arise from non-homologous genes rather than paralogs [23] indicating that parallel pathways are often nonparalogous, possibly to increase their robustness.

Concluding comment

The genome-wide analysis of single gene knockouts and the use of synthetic lethality screens can be seen as orthogonal dimensions to the data that can be obtained from genome-wide expression analysis (DNA microarray) and protein-protein interactions (PPI). While the latter are useful and powerful techniques, in many ways the mutational systems are far more powerful because they can discern the function of genes that are not regulated at the level of transcription (or only weakly so) or that require significant physical interactions. In contrast to the physical interaction map of the proteome provided by PPI techniques, synthetic lethality analysis generates a genetic interaction map that can discern gene products in parallel pathways that are not physically coupled or even in the same subcellular compartment. Despite these comments, it has been clear from our experience and that of other groups studying cellular responses to the environment at the genomic techniques of microarray, that gene over-expression and mutant analysis are very complementary. Each technique identifies a very different subset of genes, which provides further clues to function. For example, in the response to oxidants, deletion screening identifies functions that are required to maintain resistance when an oxidant is applied, whereas microarray analysis identifies those genes that are induced as a result of oxidant damage and these are very different sets of genes [7].

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