From Petri Plates to Petri Nets, a revolution in yeast biology

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In the beginning was... the genome.

The 30th of January, 1991 found me outside Temple Underground Station on the Thames Embankment in London. John Sgouros of the Martinsried Institute for Protein Sciences, who had flown over from Munich that morning, emerged from the station carrying seven A0 sheets in a roll. They were copies of Figure 1 for the paper 'The complete DNA sequence of yeast chromosome II', for which we were determined to get a 1991 submission date. We walked around the corner to the Nature offices in Little Essex Street and handed in these giant Figures together with envelopes containing the manuscript and the other Figures, which I had brought down from Manchester. Nature then lost the copies of Figure 1 but, by 27th March 1992, the paper was accepted and appeared in the journal on the 7th of May 1992 (Oliver et al. 1992).

This paper reported the first complete DNA sequence of a chromosome from any organism and, with it, everything changed—certainly for me, for yeast genetics, but also for the wider biomedical research community's view of the value of genome sequencing. The complete sequence of this chromosome taught us some important lessons in two major areas.

First, in the area of eukaryotic chromosome organisation and evolution. We found that the relationship between the genetic distance between loci, as measured in terms of recombination frequency, and that of physical distance, as measured by the DNA sequence, was far from linear. In fact, the ratio of the genetic distance (in cM) to the physical distance (in kb) showed a > 40-fold range for different intervals along the chromosome, being smallest close to the centromere and greatest half-way down each chromosome arm (Figure 3 in Oliver et al. 1992, 1993). It also indicated the role of retrotransposons in the generation of redundancy in the yeast genome by duplicating portions of the chromosome (Wickstead et al. 1994) and increasing the copy number of tRNA genes (Eigel and Feldmann 1982, Genbauffe et al. 1984).

Second, the complete sequence of this chromosome taught us that we knew much less about the genetics of Saccharomyces cerevisiae than we thought. The yeast genetics research community, at this time, was justly proud of the genome map (Mortimer et al. 1989) that had been constructed by classical genetic techniques supplemented by recombinant DNA analyses. In fact, there was even talk that for some functions, e.g. DNA repair, the map was saturated. However, inspection of the chromosome III sequence showed that there were 182 potential protein-encoding genes (open reading frames, ORFs, > 100 amino acids in length) of which only 37 were known from classical studies (Oliver et al. 1992). There were 35 laboratories involved in the sequencing of this first chromosome, and the Yeast Genome Consortium, ably led by André Goffeau, went on in this 'cottage industry' approach to sequence the other 15 chromosomes until the first complete genome sequence of a eukaryotic organism was published in 1996 (Goffeau et al. 1996), just a year after that of the first complete bacterial genome sequence (Fleischmann et al. 1995).

There were individual papers reporting the sequence of the other 15 chromosomes (Dujon et al. 1994, Feldmann et al. 1994, Johnston et al. 1994, Bussey et al. 1995, Murakami et al. 1995, Galibert et al. 1996, Bowman et al. 1997, Churcher et al. 1997, Dietrich et al. 1997, Jacq et al. 1997, Philippsen et al. 1997, Tettelin et al. 1997), many of these were published, together with a bioinformatics overview (Mewes et al. 1997), in a special issue of Nature entitled The Yeast Genome Directory (for which the European Commission paid handsomely) that did not appear until the following year. The regular issue of Nature that appeared at the same time carried a ‘News & Views’ piece on the yeast genome from Craig Venter and his colleagues (Clayton et al. 1997) that, in the short run, got more attention than any of those in the Directory. I think that it is difficult for today’s researchers, used to the facility of next-generation sequencing and automated methods of genome assembly, to appreciate just how much work was put in by the different yeast labs involved in the project. When the project began, the sequencing was done by hand (only 7% of the chromosome III sequence was obtained using automated methods (Oliver et al. 1992) and sequence assembly was largely a hand-crafted exercise. Although all yeast researchers, and very many others, make direct or indirect use of the yeast genome sequence every day, it has received the ultimate accolade of “citation eclipse”, being rarely referred to in their papers.

Functional genomics

The complete yeast genome sequence revealed 6200 putative genes encoding 5885 proteins, 275 tRNAs, and 40 small nuclear (sn) RNAs (Goffeau et al. 1996). This may be compared to the 769 loci on the S. cerevisiae genetic map at the outset of the sequencing...
project (Mortimer et al. 1989). Bioinformatic analyses performed at the time of completion of the genome sequence (Mewes et al. 1997) revealed that only ca. 40% of the genes revealed by the sequence could be attributed a function, either from direct experimental evidence, or their products’ membership of well-defined protein families, or their significant amino-acid sequence homology to proteins of known function.

Thus, 7–8 years of genome sequencing had not only revealed eight times as many genes than 40 years of classical analyses, but a large proportion of these putative genes appeared to be completely unknown to biological science.

Even with just the sequence of chromosome III in hand (some 30 years ago), it was immediately obvious that the normal course of genetics would need to be turned on its head. Instead of isolating mutants with defective or altered phenotypes and using classical genetic analyses to define functional genes and map their relative locations on chromosomes, now genes would be both identified and mapped by genome sequencing, and it would be necessary to work in the opposite direction to discover their functions (Oliver et al. 1993, Oliver 1996a, 1997). It was also evident that this new approach to genetic analysis would require techniques that were every bit as comprehensive as that of genome sequencing itself. By 2000, it was possible to lay out the basic levels of ‘omic analysis of genome, transcriptome, proteome, and metabolome (Oliver 2000).

The magnitude and complexity of the task of elucidating the functions of all the novel genes revealed by the complete yeast genome sequence suggested that such an enterprise was beyond the skills and resources of any single laboratory or institution and that we should build on the network approach to large-scale projects that had been the vision of André Goffeau. Accordingly, a new European Consortium, EUROFAN (European Functional Analysis Network; https://cordis.europa.eu/project/id/BIO4950080), comprising some 145 laboratories from 14 countries, was established at the beginning of 1996 with the aim of determining the role of 1000 S. cerevisiae genes of unknown function that had been identified from the genome sequence (Oliver 1996b). The basic strategy was to generate a library of single-gene deletants with which to seek for null phenotypes. Following the generation of the mutants, in which all laboratories participated initially, EUROFAN did phenotypic analyses that were organised in a hierarchical manner that was designed to approach the biological function of each gene with ever-increasing specificity as it moved down the pathway of analysis.

The deletion mutants were generated by PCR-mediated gene replacement using the kanMX cassette constructed by Achim Wach and Peter Philippsen (Wach et al. 1994). This cassette is designed such that it has no sequence homology anywhere in the yeast genome. This means that it can be targeted to a specific chromosomal location by PCR-generated extension sequences that are homologous to sequences flanking the ORF, i.e. to be deleted. Cells with this cassette successfully integrated could be selected using the drug geneticin (G418), resistance to which was conferred by the kan<sup>6</sup> gene carried by the cassette. This cassette, itself, conferred no measurable phenotype on the recipient strain other than drug resistance (Baganz et al. 1997). The phenotypic analyses undertaken were both qualitative and quantitative in nature. While competition experiments were identified as being particularly useful for quantitative analyses, the initial methods of discriminating between mutant strains in mixed cultures were rather cumbersome (Baganz et al. 1998). This problem was quickly resolved by Ron Davis’s innovation of incorporating gene-specific molecular bar-codes into the PCR primers used to amplify the deletion cassettes (Shoemaker et al. 1996). This meant that each deletant was uniquely identified by a pair of bar-codes and the advantages of this were so obvious that a new international project was formed between labs in the US, Canada, and EUROFAN that resulted in the generation of a complete library of strains containing bar-coded deletions for all protein-encoding genes predicted from the genome sequence (Giaever et al. 2002).

At the outset of these phenotypic analyses, the most obvious phenotype was cell death, and ca. 19% of yeast’s protein-encoding genes were found to be essential, i.e. haploid segregants lacking the gene fail to form a colony on a YEPD agar plate. However, it should be recalled that essentiality, like any other phenotype, is context dependent. The ORF YDL120 provides a good example of the way this hierarchical analysis of gene function worked, and a rare case where a given gene was taken all the way through the hierarchy of tests and the detailed biochemical lesion resulting from the deletion was not only identified, but also related to a human disease. Initial tests on a ydl120A mutant showed that it failed to grow on glycerol, and so it was passed to the Mitochondrial Node for more detailed analysis. There, Françoise Foury and her colleagues were able to locate the fault to mitochondrial iron transport. Moreover, the amino-acid sequence of this yeast ORF’s protein product showed similarity to that of the human protein frataxin, which is the product of the human gene determining the neurodegenerative disease, Friedreich’s ataxia. The yeast lesion could be complemented by the expression of a cDNA copy of the human coding sequence, confirming functional homology and elucidating, for the first time, the biochemical basis of the human disease (Foury and Cazzalini 1997, Rotig et al. 1997).

Quantitative phenotypic analyses exploited the unique identifiers that the molecular bar-codes provided in order to carry out competition experiments between the complete library of deletion mutants, usually as heterozygous (hemizygous) diploids so that all protein-encoding genes should be interrogated. Studies were carried out both in batch (Deutschbauer et al. 2005) and continuous (Delneri et al. 2008) culture. The latter (Pir et al. 2012) had the advantage of allowing competitions to be carried out under different nutrient limitations or at different growth rates (using chemostat culture) or in nutrient-unconstrained conditions (using turbidostat culture). Genes whose hemizygous mutants showed a significant change in their growth rate, compared to the wild type, were termed high flux control (HFC) genes. These HFC genes may be divided into two classes: a haploinsufficient (HI) set, where the hemizygous mutants grow slower than the wild type, and a haploproficient (HP) set, in which the hemizygotes grow faster than the wild type. The HI set is enriched for genes involved in the processes of gene expression, while the HP set is enriched for genes concerned with the cell cycle and genome integrity. Since haploproficiency was observed in turbidostat culture, this means that diploid cells lacking one copy of an HP gene grow at a rate in excess of the maximum specific growth rate previously observed in wild-type cells (Pir et al. 2012). This implies that the control of growth rate in yeast represents a trade-off between the selective advantages of rapid growth and the need to maintain the integrity of the genome.

For a subset of HP genes, heterozygous deletion was found to be sufficient to cause aberrant cell cycling and altered rates of apoptosis, phenotypes associated with cancer in mammalian cells (de Clare and Oliver 2013). Most of these yeast genes are the orthologs of mammalian cancer genes, and hence, our studies suggest that gene copy number variation (CNV) may lead to tumorigenesis in human cells. Using this yeast gene set as a model, it was shown that the response to a range of anticancer drugs is strongly de-
dependent on gene dosage, such that low or intermediate concentrations of the drugs can actually increase yeast’s growth rate. These data suggest that the identification of CNVs in tumor cells may assist both the selection of anticancer drugs and the dosages at which they should be administered. This is yet another example of how the systematic analysis of gene function in yeast (or functional genomics; Hieter and Boguski 1997) can shed light on human diseases.

For all its successes, EUROFAN’s hierarchical approach to the elucidation of gene function did not decipher the role of as many of yeast’s novel genes as had been hoped. This may be attributed to two main problems and yet more genome sequencing is providing at least a partial answer to them both. The first problem is our lack of knowledge of yeast ecology. This limits our ability to devise simple phenotypic tests at the lower end of the hierarchy of functional analysis. The second is the remarkable amount of redundancy in such a small eukaryotic genome as that of S. cerevisiae. Differences, between members of a gene family, in the regulation of gene expression or the cellular localisation of protein products can mean that this redundancy is more apparent than real (Delneri et al. 1999). However, a major source of redundancy in the S. cerevisiae genome is the whole-genome duplication (WGD) that the ancestor of this and related species underwent during their evolution. This major evolutionary event was first inferred by Ken Wolfe and Dennis Shields (Wolfe and Shields 1997) by analyzing the S. cerevisiae genome sequence (Goffeau et al. 1996) and was subsequently confirmed by sequencing the genomes of two members of the Saccharomycetaceae that diverged from the Saccharomyces lineage prior to the WGD—Kluyveromyces waltii (Kellis et al. 2004) and Ashbya gossypii (Dietrich et al. 2004).

Since the discovery of the WGD, a huge amount of effort has gone into the isolation of strains of S. cerevisiae and its close relatives from the wild and the sequencing of their genomes. These efforts again involved a collaborative approach and the formation of research consortia such as Généolevures, led by Jean-Luc Souciet and Bernard Dujon (Sherman et al. 2004, Souciet et al. 2009), and the 1,000 Genomes project led by Gianni Liti and Joseph Schacherer (Peter et al. 2018). These studies have not only thrown light on the evolutionary history of the Saccharomycetaceae, including the domestication of S. cerevisiae, but the provenance of the wild isolates has also contributed to our understanding of yeast ecology. Despite all these efforts, both in the laboratory and the field, there remain some 18% of protein-encoding genes of S. cerevisiae that are still of unknown function (Wood et al. 2019). Such ‘orphan’ genes are often dismissed as being specialised genes that are unique to a particular species; in fact, more than one-third of them are conserved across the tree of evolution from yeasts to humans (Wood et al. 2019). Thus, the functional genomics agenda remains unfinished.

**Systems biology**

The endpoint of the functional genomics agenda should be a complete list of the working parts of the yeast cell, such as you might find in the back of a workshop manual of a complex machine (Oliver 2002). However, this parts inventory is not sufficient to allow the servicing of the machine or to permit us to engineer it to fulfil new purposes. What is required is to know how those working parts interact to make a functional machine, and how those interactions are controlled, just as, for instance, we would need the wiring diagram of a radio in order to understand the interactions of its parts and how its performance is controlled (Lazebnik 2002). There were valiant attempts to take a systems approach to biology in the 1960s (see Waddington 1968). While it was recognised that this would involve the cooperation of experimental biologists and mathematical modellers, there were simply insufficient experimental data to permit the construction of robust and predictive models. In the event, the modellers went ahead without the essential data and, consequently, mathematical modelling got a bad name—at least among molecular geneticists.

One of the early steps required in a systems approach to yeast biology was to integrate the different levels of ‘omic analysis (genomic, transcriptomic, proteomic, and metabolomic) by carrying out what have come to be known as ‘multi-omic’ experiments (Castrillo et al. 2007). Since that time there have been enormous advances in the technology of ‘omic analyses, with the transition from microarrays to RNAseq for transcriptomic experiments (Waern et al. 2011, Hesketh 2019) and the extensive array of mass spectrometry techniques employed for both proteomics (Rees and Lilley 2011, Nightingale et al. 2019) and metabolomics (Winder and Dunn 2011, Chaleckis et al. 2019). For metabolomics, this has meant that the promise of being able to reveal gene functions by comparing the metabolic profiles of single-gene mutants (Raamsdonk et al. 2001) has at last been realised (Müller et al. 2016)—although it did require the conversion of the entire yeast deletion collection to prototrophy in order to achieve it (Müller et al. 2012).

For all the advances in these analytical technologies, it was two approaches that exploited the ‘awesome’ power of yeast genetics that made major contributions to the early development of yeast systems biology. The first of these was the yeast two-hybrid system developed by Stan Fields (Fields and Song 1989), which allowed the high-throughput analysis of protein–protein interactions. This could be applied, not only to yeast proteins (Uetz et al. 2000, Ito et al. 2001), but also to proteins of other species, e.g. humans (Rual et al. 2005), expressed in yeast. Whilst the system has its limitations, for instance it measures interactions within the yeast nucleus, a number of variations to circumvent them have been devised. For all that, Y2H analyses have provided a hugely valuable dataset when combined with studies that used other techniques (von Mering et al. 2002), including biochemical methods exploiting mass spectrometry (Gavin et al. 2002), and rigorous statistical analyses (Yu et al. 2008). The second approach has been arguably of even greater value since it identifies functional interactions. This is the high-throughput detection of gene-gene (epistatic) interactions by using the synthetic genetic array (SGA) methodology developed by Charlie Boone, Brenda Andrews, and colleagues in Toronto (Tong et al. 2001, 2004, Costanzo et al. 2010). At first, the synthetic phenotypes were monitored as a growth/no growth qualitative output, but subsequently colony growth rate was measured (Baryshnikova et al. 2010) and even cell morphology (Ohya et al. 2015, Mattiazzii Usaj et al. 2020). Similarly, the methods could, at first, only be applied to measuring interactions between pairs of nonessential genes but it has now been extended to include the essential genes through the use of titratable promoters (Mnaimneh et al. 2004) and temperature-sensitive alleles (Li et al. 2011). These methods of matching both physical and functional interactions have proved enormously valuable to system modellers, not only to constrain their models, but also to test their predictions against empirical datasets (see below).

As with Functional Genomics, the advent of Systems Biology saw the establishment of networks of European yeast researchers to pursue major programmes of work—the Yeast Systems Biology Network (YSBN, led by Jens Nielsen; https://cordis.europa.eu/project/id/18942) and UNICELLSYS (led by Stefan Hohmann; https://cordis.europa.eu/project/id/201142). YSBN had a coordi-
nating role in systems biology research using yeast as a model organism and aimed to employ both mathematical analyses and computational tools to integrate experimental data; it set out to develop common resources and standards. UNICELLSYS had the more focussed aim of achieving a quantitative understanding of how cell growth and proliferation are controlled and coordinated by both extracellular and internal signals. These two networks operated in a very synergistic manner.

Stefan Hohmann's organisation of UNICELLSYS showed considerable vision at a time when not everyone was convinced of the need for Systems Biology, or even that it represented ‘respectable’ science. Moreover, working together with Hiroaki Kitano, Stefan played a major role in coordinating and promoting Systems Biology on a global, scale. The work in UNICELLSYS involved some advances in proteomic technology to improve quantitation (Selesekh et al. 2015) and, also, a shift in emphasis from protein synthesis to post-translational modifications (Kapuy et al. 2009, Amoutzias et al. 2012) and protein–protein interactions (Nandy et al. 2010). Work on the responses to external stimuli concentrated on the response to osmotic stress, with experimentalists joining with modellers to elucidate the circuitry involved (Nadal-Ribelles et al. 2012, Tiger et al. 2012, Talemri et al. 2016). Work on the control of cell proliferation in response to internal signals concentrated on the cell cycle and had two strands—in one, the experimentalists worked in close collaboration with the modellers with some individuals becoming adept in both approaches (Barberis et al. 2011), while the other comprised a purely theoretical approach (He et al. 2011).

YSBN had the aim of establishing some common standards between research groups, not only in experimental methods (a problem already encountered in EUROFAN, Brown et al. 2001), but also in how both models and data were represented and stored. The standardisation of experimental methods (Canelas et al. 2010) was a massive undertaking that involved a comparison of the behaviour of two yeasts strains—CEN.PK113-7D, a lab strain that was widely used by yeast physiologists and that had already been extensively characterised by an earlier multi-lab experiment (van Dijken et al. 2000), and YSBN2, a new reference strain constructed in the s288c background (Winston et al. 1995) but with the auxotrophic markers replaced by wild-type copies of the genes. Batch and continuous fermentations were carried out in Jack Pronk's laboratory in Delft and samples distributed to the participating laboratories. These undertook analyses of the transcriptome (by four different methods in four laboratories), enzyme activities (three different protocols in two labs), and the metabolome (three different technologies in seven labs). This produced an extensive reference dataset that, regrettably has been underused (Canelas et al. has < 400 citations at the time of writing), perhaps because the transcriptome analyses were not carried out using RNAseq technology (Waern et al. 2011).

A major contribution of YSBN was to establish a consensus stoichiometric model of the yeast metabolic network (Herrgård et al. 2008). A genome-scale model of the yeast metabolic network had already been constructed by Jens Nielsen, Bernard Palsson, and their colleagues (Förster et al. 2003) building on Palsson's E. coli model (Edwards and Palsson 2000). However, the consensus model (which was constructed at a YSBN ‘jamboree’ in Manchester) benefitted from the input of a wide range of experts, standardised and unambiguous representations of chemical structures, its adherence to the Systems Biology Mark-up Language (SBML, Hucka et al. 2003), and its availability on a publicly accessible database (now at https://sysbiochalmers.github.io/yeast-GEM/).

This model (Yeast1) was rapidly updated even during the course of YSBN (Nookaew et al. 2008, Dobson et al. 2010, Heavner et al. 2012), not least because it did not permit the performance of simulations and Flux Balance Analysis (FBA). The current version is Yeast8 (available at the website above) and the evolution of genome-scale models of the S. cerevisiae metabolic network is the subject of an excellent recent review (Yu et al. 2022).

Other systems of modelling yeast metabolism have been developed. Reiser et al. (2001) constructed a logical model of the yeast metabolic network. This is a very simple model: it has no dynamics and it does not even contain the stoichiometry of the reactions. However, each reaction is linked to a gene in the complete S. cerevisiae genome sequence (Goffeau et al. 1996) and this revealed a number of ‘orphan’ reactions, ones for which there was good evidence that they must occur in yeast, but for which no gene encoding the enzyme catalysing the reaction had yet been identified. Adam, a ‘Robot Scientist’, was then provided with this model and with access to the public sequence databases. This permitted Adam to use artificial intelligence to design, and robotics to execute, experiments that identified the genes associated with some of these orphan reactions—the first example of a machine discovering new science (King et al. 2009).

The most important developments of the genome-scale stoichiometric model of yeast metabolism have involved the exploitation of transcriptomic (Lee et al. 2012) or proteomic (Sánchez et al. 2017, Lu et al. 2019) data to further constrain the model and increase the accuracy of its predictions. Future developments are likely to involve the use of machine-learning techniques to reduce the uncertainties in enzyme kinetic constants, such as $k_{cat}$ or $k_{m}$ values (Kroll et al. 2021, Li et al. 2021). Uncertainties can be accommodated by other modelling systems such as Flexible Nets (a development from Petri Nets, hence the title of this piece), which can also deal with regulation of both reactions and the industrial processes which rely on them (Júlvez et al. 2018). In the other direction, Szappanos et al. (2011) used machine learning techniques to reconcile contradictions between the predictions of the yeast genome-scale metabolic model and the experimental genetic interaction data of Costanzo et al. (2010). In this way, they were able to correct errors in the model, demonstrating that there was only one route to NAD biosynthesis in yeast, and not two as there are in E.coli (Edwards and Palsson 2000). We can expect to see an increased use of machine learning and other forms of artificial intelligence in systems biology modelling. However, it is important that we ensure that our models have explanatory power as well as predictive accuracy.

**Final thoughts**

Much of the work described in this article is the result of open-ended research programmes that aimed to produce useful data. In other words, they were hypothesis generating, rather than hypothesis testing. Science has always needed both these kinds of research (Kell and Oliver 2004), and data-generating research should not be dismissed as mere ‘stamp-collecting’. Indeed, early attempts to develop a systems approach to biology failed due to a lack of quantitative and comprehensive data (see above). Today, however, such data are in plentiful supply and there is much enthusiasm for investigations based on ‘Big Data’ (Pal et al. 2020). It is important to remember that the results of such analyses can be skewed by incomplete or biased data sets (for a topical example, see Bradley et al. (2021)). Moreover, despite the so-called ‘data avalanche’, there are data that are critical to the generation of accurate predictions by systems biology models that are either incomplete or unavailable. For example, our knowledge of the biochemical composition of yeast biomass under different physiolog-
ical conditions is in a parlous state (Dikicioglu et al. 2015). Another crucial data set, the kinetic parameters of enzymes, will either be hard won through exhaustive (and exhausting) experimental work (Smallbone et al. 2013) or require the use of advanced deep-learning methods to leverage the scattered and heterogeneous experimental data available (Kroll et al. 2021, Li et al. 2021). Other essential resources that must be generated and maintained if the potential of Big Data analyses in systems biology is to be realised are properly curated databases—dumps of electronic lab notebooks are not useful. Such databases require not only the skills and commitment of professional curators, but also the direct involvement of experimentalists in the curation process (Oliver et al. 2016). A major problem is that the generation of these missing data and the maintenance of high-quality databases are often considered mundane activities that tend to be under-resourced by funding bodies. However, given the resources, the combination of the intrinsic advantages of yeasts as model organisms, and the demonstrated ability of the international community of yeast researchers to work together for the common good, will ensure that yeasts will remain in the vanguard of the revolution in biology and build upon the achievements of pioneers like Stefan Hohmann. Stefan was not only open to new technologies and new concepts in his science, but he was also open to collaboration and had a talent for organisation and coordination; we are all in his debt.

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**References**

Amoutzias GD, He Y, Lilley KS et al. Evaluation and properties of the budding yeast phosphoproteome. Mol Cell Proteomics 2012;11 M111.009555.

Baganz F, Hayes A, Farquhar R et al. Quantitative analysis of yeast gene function using competition experiments in continuous culture. Yeast 1998, 14 1417–27.

Baganz F, Hayes A, Marren D et al. Suitability of replacement markers for functional analysis studies in Saccharomyces cerevisiae. Yeast 1997;13 1563–73.

Barberis M, Beck C, Arnaudoussvai A et al. A low number of SIC1 mRNA molecules ensures a low noise level in cell cycle progression of budding yeast. Mol Biosyst 2011;7 2804–12.

Baryshnikova A, Costanzo M, Kim Y et al. Quantitative analysis of fitness and genetic interactions in yeast on a genome scale. Nat Methods 2010;7 1017–24.

Bowman S, Churcher C, Badcock D et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome XII. Nature 1997;387 90–3.

Bradley VC, Kuriwaki S, Isakov M et al. Underrepresented big surveys significantly overestimated US vaccine uptake. Nature 2021;600 695–700.

Brown APJ, Planta RP, Restuhabi F et al. Transcript analysis of 1003 novel yeast genes using high-throughput northern hybridisations. EMBO J 2001;20 3177–86.

Bussey H, Kackab DB, Zhong WW et al. The nucleotide sequence of chromosome I from Saccharomyces cerevisiae. Proc Natl Acad Sci USA 1995;92 3809–13.

Bussey H, Storms RK, Ahmed A et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome XVI and its evolutionary implications. Nature 1997;387 103–5.

Canelas AB, Harrison N, Fazio A et al. Experimental systems biology: lessons from an integrated, multi-laboratory study in yeast. Nat Commun 2010;1 145.

Castrillo JI, Zeef LA, Hoyle DC et al. Growth control of the eukaryote cell: a systems biology study in yeast. J Biol 2007 6: 4.

Chaleckis R, Ohashi K, Meister I et al. Metabolomic analysis of yeast and human cells: latest advances and challenges. Methods Mol Biol 2019;2049 233–46.

Churcher C, Bowman S, Badcock K et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome IX. Nature 1997;387 84–7.

Clayton RA, White O, Ketchum KA et al. The first genome from the third domain of life. Nature 1997;387 459–62.

Costanzo M, Baryshnikova A, Bellay J et al. The genetic landscape of a cell. Science 2010;327 425–31.

de Claire M, Oliver SG. Copy-number variation of cancer-gene orthologs is sufficient to induce cancer-like symptoms in Saccharomyces cerevisiae. BMC Biol 2013;11 24.

Delneri D, Gardner DCJ, Oliver SG. Analysis of the seven-member AAD gene set demonstrates that genetic redundancy in yeast may be more apparent than real. Genetics 1999;153 1591–600.

Delneri D, Doyle DC, Gkargkas K et al. Identification and characterisation of high flux control (HFC) genes of Saccharomyces cerevisiae through competition analyses in continuous cultures. Nat Genet 2008;40 113–7.

Deutschbauer AM, Daniel F, Jaramillo DF et al. Mechanisms of haploinsufficiency revealed by genome-wide profiling in yeast. Genetics 2005;169 1915–25.

Dietrich FS, Mulligan J, Hennessey K et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome IV. Nature 1997;387 78–81.

Dietrich FS, Voegeli S, Brachat S et al. The Ashbya gossypii genome as a tool for mapping the ancient Saccharomyces cerevisiae genome. Science 2004;304 304–7.

Dikicioglu D, Kirdar B, Oliver SG. Biomass composition: the “elephant in the room” of metabolic modelling. Metabolomics 2015;11 1690–701.

Dobson PD, Smallbone K, Jameson D et al. Further developments towards a genome-scale metabolic model of yeast. BMC Syst Biol 2010;4 145.

Dujon B, Albermann K, Aldea M et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome XV and its evolutionary implications. Nature 1997;387 96–102.

Dujon B, Alexandraki D, André B et al. Complete DNA sequence of yeast chromosome XI. Nature 1994;369 371–8.

Edwards JS, Palsson BØ. The Escherichia coli MG1655 in silico metabolic genotype: its definition, characteristics, and capabilities. Proc Natl Acad Sci USA 2000;97 5528–33.

Eigel A, Feldmann H. Ty and 4 elements occur next to several tRNA genes in yeast. EMBO J 1982;1 1245–50.

Feldmann H, Aigle M, Aljinovic et al. Complete DNA sequence of yeast chromosome II. EMBO J 1994;13 5795–809.

Fields S, Song OK. A novel genetic system to detect protein–protein interactions. Nature 1989;340 245–6.

Fleischmann R, Adams M, White O et al. Whole-genome random sequencing and assembly of Haemophilus influenzae Rd. Science 1995;269 496–512.
Förster J, Famili I, Fu P et al. Genome-scale reconstruction of the Saccharomyces cerevisiae metabolic network. Genome Res 2003;13:244–53.

Foury F, Cazzalini O. Deletion of the yeast homologue of the human gene associated with Friedreich’s ataxia elicits iron accumulation in mitochondria. FEBS Lett 1997;411:373–7.

Galibert F, Alexandraki D, Baur A et al. Complete nucleotide sequence of Saccharomyces cerevisiae chromosome X. EMBO J 1996;15:2031–49.

Gavin AC, Bösche M, Krause R et al. Functional organization of the yeast proteome by systematic analysis of protein complexes. Nature 2002;415:341–7.

Genbauffe FS, Chisholm GE, Cooper TG. Tau, sigma and delta. A family of repeated DNA sequences in yeast. J Biol Chem 1984;259:518–25.

Giaever G, Chu AM, Ni L et al. Functional profiling of the Saccharomyces cerevisiae genome. Nature 2002;418:387–91.

Golfeau A, Barrell BG, Bussey H et al. Life with 6000 genes. Science 1996;274:546–67.

He E, Kapuy O, Oliveira RA et al. System-level feedbacks make the anaphase switch irreversible. Proc Natl Acad Sci 2011;108:10016–21.

Heavner BD, Smallbone K, Barker B et al. Yeast 5 – an expanded reconstruction of the Saccharomyces cerevisiae metabolic network. BMC Syst Biol 2012;6:55.

Herrgård MJ, Swainston N, Dobson P et al. A consensus yeast metabolic network reconstruction obtained from a community approach to systems biology. Nat Biotechnol 2008;26:1155–60.

Hesketh A. RNA sequencing best practices: experimental protocols and data analysis. Methods Mol Biol 2019;2049:113–30.

Hieter P, Boguski M. Functional genomics: it’s all how you read it. Nature 1997;387:601–2.

Hucka M, Finney A, Sauro HM et al. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. Bioinformatics 2003;19:524–31.

Ito T, Chiba T, Ozawa R et al. A comprehensive two-hybrid analysis to explore the yeast protein interactome. Proc Natl Acad Sci 2001;98:4569–74.

Jacq C, Alt-Mörbe J, André B et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome IV. Nature 1997;387:75–7.

Johnston M, Andrews S, Brinkman R et al. Complete nucleotide sequence of Saccharomyces cerevisiae chromosome VIII. Science 1994;265:2077–82.

Johnston M, Hillier L, Riles L et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome XII. Nature 1997;387:84–7.

Jülvez J, Dikicicoglu D, Oliver SG. Handling variability and incompleteness of biological data by flexible nets: a case study for Wilson disease. Npj Sys Biol Appl 2018;4:7.

Kapuy O, Barik D, Domingo Sananes MR et al. Bistability by multiple phosphorylation of regulatory proteins. Prog Biophys Mol Biol 2009;100:47–56.

Kell DB, Oliver SG. Where is the evidence, now what is the hypothesis? The complementary roles of inductive and hypothesis-driven science in the post-genomic era. Bioessays 2004;26:99–105.

Kellis M, Birren BW, Lander ES. Proof and evolutionary analysis of ancient genome duplication in the yeast Saccharomyces cerevisiae. Nature 2004;428:617–24.

King RD, Rowland J, Oliver SG et al. The automation of science. Science 2009;324:85–9.

Kroll A, Engvigst MKM, Heckmann D et al. Deep learning allows genome-scale prediction of Michaelis constants from structural features. PLos Biol 2021;19:e3001402.

Lazebnik Y. Can a biologist fix a radio? – Or, what I learned while studying apoptosis, Cancer Cell 2002;2:179–82.

Lee D, Smallbone K, Dunn W et al. Improving metabolic flux predictions using absolute gene expression data. BMC Syst Biol 2012;6:73.

Li F, Yuan L, Lu H et al. Deep learning based kcat prediction enables improved enzyme constrained model reconstruction. bioRxiv 2021. DOI: 10.1101/2021.08.06.455417.

Li Z, Vizeacoumar FJ, Bahr S et al. Systematic exploration of essential yeast gene function with temperature-sensitive mutants. Nat Biotechnol 2011;29:361–7.

Lu H, Li F, Sánchez B et al. A consensus S. cerevisiae metabolic model Yeast8 and its ecosystem for comprehensively probing cellular metabolism. Nat Commun 2019;10:1–13.

Mattaizzi Usaj M, Sahin N, Friesen H et al. Systematic genetics and single-cell imaging reveal widespread morphological pleiotropy and cell-to-cell variability. Molec Sys Biol 2020;16:e9243.

Mewes HW, Albermann K, Bahr M et al. Overview of the yeast genome. Nature 1997;387:7–65.

Mnaimneh S, Davierwala AP, Haynes J et al. Exploration of essential gene functions via titratable promoter alleles. Cell 2004;118:31–44.

Mortimer RK, Schild D, Contopoulou CR et al. Genetic map of Saccharomyces cerevisiae, edition 10. Yeast 1989;5:321–404.

Mu Ileder M, Calvani E, Alam MT et al. Functional metabolomics describes the yeast biosynthetic regulome. Cell 2016;167:553–65.

Müller E, Capuano F, Pir P et al. A prototrophic deletion mutant collection for yeast metabolomics and systems biology. Nat Biotechnol 2012;30:1176–8.

Murakami Y, Naitou M, Hagiwara H et al. Analysis of the sequence of chromosome VI from Nat Genet 1995;10:261–8.

Nadal-Ribelles M, Conde N, Flores O et al. Hog1 bypasses stress-mediated down-regulation of transcription by RNA polymerase II redistribution and chromatin remodeling. Genome Biol 2012;13:R106.

Nandy SK, Jouhten P, Nielsen J. Reconstruction of the yeast protein-protein interaction network involved in nutrient sensing and global metabolic regulation. BMC Syst Biol 2010;4:68.

Nightingale DJH, Oliver SG, Lilley KS. Mapping the Saccharomyces cerevisiae spatial proteome with high resolution using hyperLOPIT. Methods Mol Biol 2019;2049:165–90.

Nookaw I, Jewett MC, Meechai A et al. The genome-scale metabolic model iN800 of Saccharomyces cerevisiae and its validation: a scaffold to query lipid metabolism. BMC Syst Biol 2008;2:1–15.

Ohtsu N. Evolution Through Gene Duplication. London: Allen & Unwin, 1970.

Ohya Y, Kimori Y, Okada H et al. Single-cell phenomics in budding yeast. Mol Biol Cell 2015;26:3920–392.

Oliver S. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.

Oliver SG. ‘To-day, we have naming of parts…’. Nat Genet 1992;1:38–46.
Oliver SG. From DNA sequence to biological function. Nature 1996a;379:597–600.

Pal S, Mondal S, Dasc G et al. Big data in biology: the hope and present-day challenges in it. Gene Reports 2020;21:100869.

Peter J, De Chiara M, Friedrich A et al. Genome evolution across 1,011 Saccharomyces cerevisiae isolates. Nature 2018;556:339–44.

Philippson P, Kleine K, Pöhlmann R et al. The nucleotide sequence of Saccharomyces cerevisiae chromosome XIV and its evolutionary implications. Nature 1997;387:93–8.

Pir P, Gutteridge A, Wu J et al. The genetic control of growth rate: a systems biology study in yeast. BMC Syst Biol 2012;6:4.

Raamsdonk LM, Teusink B, Broadhurst D et al. A functional genomics strategy that uses metabolome data to reveal the phenotype of silent mutations. Nat Biotechnol 2001;19:45–50.

Rees J, Lilley K. Enabling technologies for yeast proteome analysis. Meth Molec Biol 2011;759:149–78.

Reiser PGK, King RD, Kell DB et al. Developing a logical model for yeast metabolism. Electron Trans Artif Intell 2001;5:223–44.

Rotig A, deLonlay P, Chretien D et al. Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich’s ataxia. Nat Genet 1997;17:215–7.

Rual JF, Venkatesan K, Hao T. Towards a proteome-scale map of the human protein–protein interaction network. Nature 2005;437:1173–8.

Sánchez BJ, Zhang C, Nilsson A et al. Improving the phenotype predictions of a yeast genome-scale metabolic model by incorporating enzymatic constraints. Mol Syst Biol 2017;13:935.

Selevsek N, Chang C-Y, Giot L et al. Reproducible and consistent quantification of the Saccharomyces cerevisiae proteome by SWATH-mass spectrometry. Mol Cell Proteomics 2015;14:739–49.

Sherman D, Durrens P, Beyre E et al. Génolevures: comparative genomics and molecular evolution of hemiascomycetous yeasts. Nucleic Acids Res 2004;32:D315–318.

Shoemaker DD, Lashkari DA, Morris D et al. Quantitative phenotypic analysis of yeast deletion mutants using a highly parallel molecular bar-coding strategy. Nat Genet 1996;14:450–6.

Smallbone K, Messiha HL, Carroll KM et al. A model of yeast glycolysis based on a consistent kinetic characterization of all its enzymes. FEBS Lett 2013;587:2832–41.

Souciet JL, Dujon B, Gaillardin C et al. Comparative genomics of prototrophic Saccharomyces cerevisiae. Genome Res 2009;19:1696–709.

Szappanos B, Kovács K, Szamecz B et al. An integrated approach to elucidate the organization principles of genetic interaction networks in yeast metabolism. Nat Genet 2011;43:656–62.

Talemi S, Tiger C-F, Andersson M et al. Systems level analysis of the yeast osmo-stat. Sci Rep 2016;6:30950.