THE ART AND DESIGN OF GENETIC SCREENS: YEAST

Susan L. Forsburg

Understanding the biology of complex systems is facilitated by comparing them with simpler organisms. Budding and fission yeasts provide ideal model systems for eukaryotic cell biology. Although they differ from one another in terms of a range of features, these yeasts share powerful genetic and genomic tools. Classical yeast genetics remains an essential element in discovering and characterizing the genes that make up a eukaryotic cell.

ASCOMYCETE
Free-living fungus that reproduces sexually through the formation of spores packaged in a sac called an ascus. Some taxonomists include non-sexually reproducing fungi with DNA sequences, which indicates a close degree of relatedness.

COMPLEMENTATION TEST
Determines whether two recessive mutations are in the same functional unit or gene. Two recessive mutant strains, *a1* and *a2*, crossed together complement each other if the resulting diploid has a wild-type phenotype; as each provides the function missing in the other, they are assumed to affect independent genes. If, instead, the diploid has the mutant phenotype, then *a1* and *a2* do not complement and are assumed to affect the same gene.

Molecular and Cell Biology Laboratory, The Salk Institute, 10010 N. Torrey Pines Road, La Jolla, California 92037, USA. email: forsburg@salk.edu

Yeast geneticists half-mockingly talk about the cult of APYG: the 'awesome power of yeast genetics'. But mocking aside, these simple, single-celled fungi have proven themselves to be the workhorses of cell biology because of the ease of their genetic manipulation. The budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* are quite different in their biology (FIG. 1), but they share a similar tool set that makes the process of gene discovery, and subsequent characterization of gene function, remarkably easy.

Historically, *S. cerevisiae* has been the more popular experimental system. The first eukaryote to be transformed by plasmids, it was also the first eukaryote for which precise gene knockouts were constructed, and the first to have its genome sequenced[1–3]. The cell biological issues that have been explored in this ASCOMYCETE range from signal transduction to cell-cycle control, chromosome structure to secretion. The identified genes have been used as probes to uncover further pathways and to identify metazoan homologues. Despite the completion of its genome sequence several years ago[1], the roles of many of its 6,000+ genes remain unclear. The process of mutant analysis and discovery of gene function continues with the added tools of genomics[4,5].

By contrast, the experimental history of *S. pombe* involves a smaller, but growing, community. Its genome sequence is essentially complete but shares no conserved synteny (gene order) with the budding yeast in its 4,900+ genes[6]. Although yeast phylogeny is still unclear, *S. pombe* is thus quite distinct from *S. cerevisiae* and filamentous fungi[7–9]. The fission yeast has a symmetrical pattern of cell division and has been particularly popular for studies of cell growth and division, and chromosome dynamics. Other cell biological questions have been addressed more recently, inspired by the power of a comparative approach between these two superficially similar organisms. Each of them offers unique insights as a model organism for elucidating the biology of more complex cell types[10].

Both yeasts are adaptable to several forms of genetic analysis that allow the identification of new genes, or the functional analysis of previously identified genes[11,12]. Because both can grow and divide as haploids, recessive mutations are easily recovered. However, a diploid sexual cycle exists for both, allowing facile genetic analysis, including tests of COMPLEMENTATION, RECOMBINATION and EPISTASIS. The identification of replication origin sequences allows plasmids to be

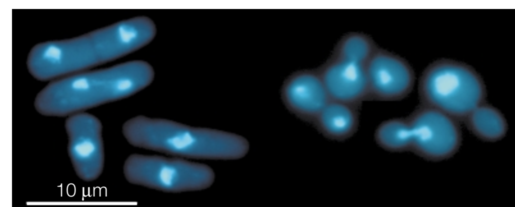

Figure 1 | **Morphology of the two yeasts.** The fission yeast *Schizosaccharomyces pombe* and the budding yeast *Saccharomyces cerevisiae* are free-living haploid cells that are easily grown in the laboratory. Left, fission yeast; right, budding yeast; visualized with the DNA stain DAPI (4′,6-diamidino-2-phenylindole) to highlight the nucleus.

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Table 1 | **Nomenclature in the two yeast species**

	S. cerevisiae	*S. pombe*
Wild-type gene	*YFG1*	*yfg1*+
Deletion (null) mutant	*Δyfg1 yfg1Δ*	*Δyfg1 yfg1Δ*
Recessive mutant	*yfg1-1*	*yfg1-1*
Dominant mutant	*YFG1-2*	*yfg1-2*
Protein	Yfg1 YFG1p	Yfg1 yfg1p

Yfg typically means 'your favourite gene'. The 'p' designation for proteins (for example, Yfg1p) is occasionally used. *S. cerevisiae*, *Saccharomyces cerevisiae* or budding yeast; *S. pombe*, *Schizosaccharomyces pombe* or fission yeast.

RECOMBINATION
Any process in a diploid or partially diploid cell that generates new gene or chromosomal combinations not found in that cell or in its progenitors. At meiosis, recombination (or crossing over) is the process of reciprocal exchange between homologous chromosomal segments that generates a haploid product genotypically distinct from the two haploid genotypes of the original meiotic diploid.

EPISTASIS
The phenotype caused by a mutation in one gene is masked by a mutation in another gene. Epistatic analysis requires that two mutants have distinguishable phenotypes. It can be used to determine the order of gene function by testing whether the phenotype of the double mutant *ab* is similar to that of mutant *a*, or mutant *b*.

HETEROLOGOUS RECOMBINATION
Recombination between DNA molecules with significantly different sequences, for example when a transgenic construct integrates randomly in the genome.

maintained as free episomes, and these are easily introduced into the cell by transformation. In addition, both species have high rates of homologous recombination, allowing precise manipulation of the genome for the construction of gene disruptions and allele-specific replacements. Their nomenclature is distinct (TABLE 1), but they benefit from similar genetic and molecular tools (see examples in TABLE 2), which provide the basic requirements for the genetic screens described below.

With the genome sequences of both species now completed, the challenge is to identify the functions associated with their genes. The classical genetic analysis described here complements the newest genomic approaches[13]: genetics moves from a function, defined by mutation, to identify the gene responsible, whereas genomics moves from the catalogue of genes to identify their function. The true power of genetics is its predictive value; genetic interactions predict physical interactions, and these can be tested using standard molecular and biochemical techniques. Genetics is, therefore, one of a trio of methods, the others being molecular biology/biochemistry and cell biology, which are required to understand the function of individual genes *in vivo*. In this review, I discuss principles and examples of genetic screening methods in both yeasts that facilitate functional gene discovery, and that continue to provide crucial insights into the mechanisms that make up a single cell.

Table 2 | **Corresponding tools in the two yeast species**

	S. cerevisiae	*S. pombe*
Regulated promoter	*GAL* (galactose regulated)	*nmt* (thiamine regulated)
Plasmid replication origins	*ARS1* or 2μ	*ars1*
Auxotrophic markers		
Uracil, orotidine 5′-phosphate decarboxylase Select against with 5-FOA	*URA3*	*ura4*+
Leucine, β-isopropylmalate dehydrogenase	*LEU2*	*leu1*+
Adenine, phosphoribosyl-aminoimidazole carboxylase Accumulates red colour	*ADE2*	*ade6*+

2μ (2 micron), an endogenous plasmid DNA molecule found in some yeast cells, with a circumference of 2μ; 5-FOA, 5′-fluoro-orotic acid. *S. cerevisiae*, *Saccharomyces cerevisiae* or budding yeast; *S. pombe*, *Schizosaccharomyces pombe* or fission yeast.

Classical genetics and mutant isolation

The essence of any genetic screen or selection is to identify a process of interest, to predict the likely phenotype of a mutant unable to carry out that process, and to devise a method of identifying mutants with that phenotype. The precise methods used vary according to the needs of the investigator and the limitations associated with each approach, but the broad goals remain the same across different screens.

A particular advantage of the yeasts as genetic models is their haploid life cycle. This facilitates the recovery of recessive mutations; these are usually loss-of-function alleles and are particularly useful in determining the normal function of the gene. Null mutations that result in a complete loss of gene function can be generated in several ways. Specific genes can be disrupted using a construct that contains a nutritional or drug marker and homologous sequences. If no gene-specific sequences are used, the construct can integrate randomly throughout the genome. For example, fission yeast has recently been subject to random insertional mutagenesis[14,15] — a technique that is assisted by its relative promiscuity in HETEROLOGOUS RECOMBINATION[16]. The advantage is the ease of identifying the gene of interest, which is tagged by the inserted cassette, and the knowledge that the gene function is abolished. The disadvantage is that essential genes cannot be examined, because the mutated cells will be inviable; so, the approach is useful for any non-essential cell activity (for example, meiosis) or redundant gene, or for gene activity that is sensitive to dosage.

However, even lethal mutations can be maintained if the wild-type copy is also present and this is accomplished using diploid heterozygotes. A recent development in the budding yeast community is the use of a pool of strains in which all the open reading frames have already been knocked out (see link to the *Saccharomyces* genome deletion project) or fused to a transposon (see link to TRIPLES — a database of transposon-insertion phenotypes, localization and expression in *Saccharomyces*). These collections therefore contain mutations in essential as well as non-essential genes. But there is an additional twist — not all viable heterozygotes in this collection are normal. Several such heterozygous diploids have abnormal chromosome content, presumably as a result of internal selection[17]. This is presumably because budding yeast often has several versions of the same gene[18], reflecting ancient genome duplication events; loss of one gene might impose a growth selection that leads to increased copy number of the chromosome that contains another version of the same gene, because it confers increased growth rate. (This leads to the important caveat that any time an investigator grows a yeast strain in the laboratory, the cells are under some form of selection!) Nevertheless, these heterozygous strains can be turned to an advantage and the pool of diploid disruptions can be used for novel screens. For example, the reduced dosage associated with a heterozygous null can generate useful phenotypes owing to HAPLOINSUFFICIENCY, and this has been successfully used in screens for drug sensitivity[19].

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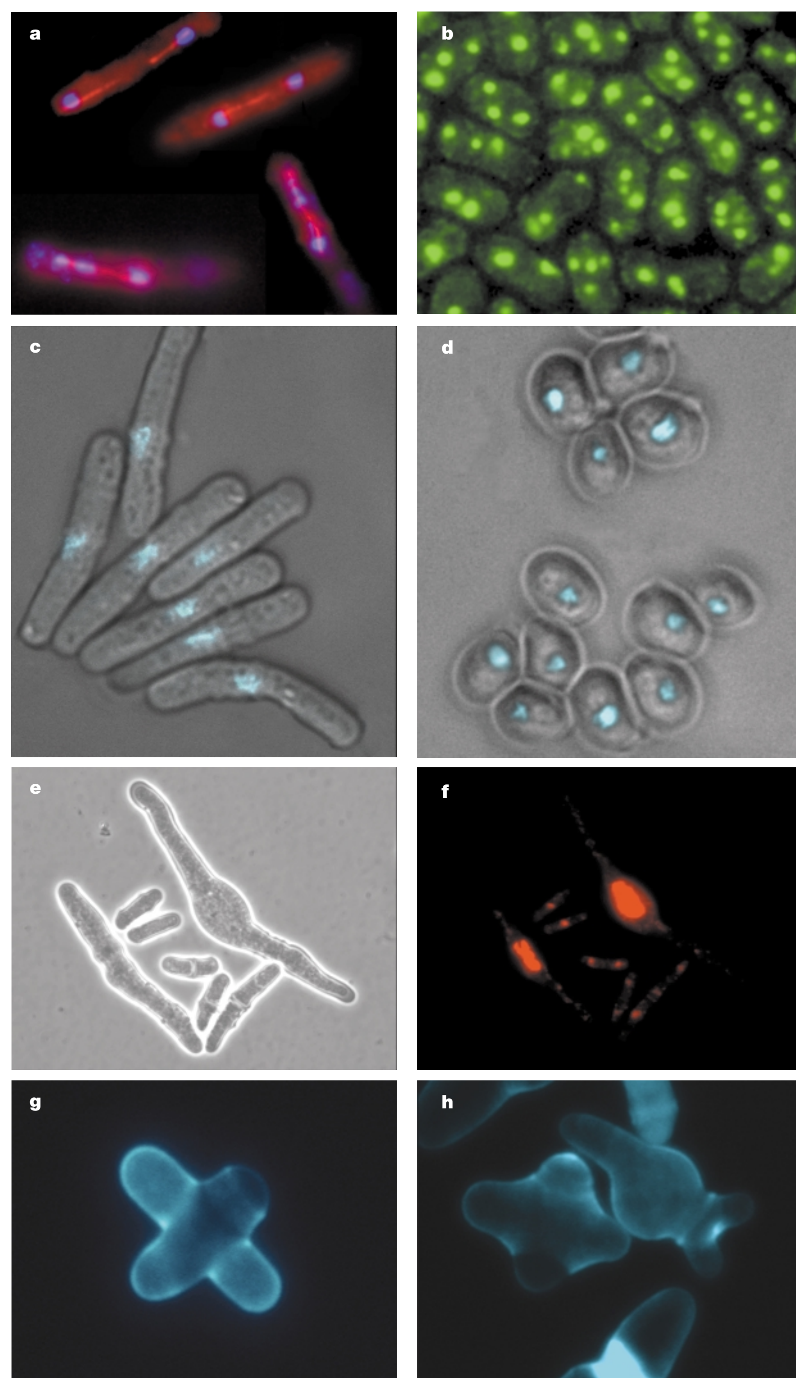

Figure 2 | **Examples of mutant phenotypes in studies of cell-cycle and chromosome dynamics. a**–**c**, **e**–**g** | *Saccharomyces pombe*; **d** | *Saccharomyces cerevisiae*. **a** | Abnormal mitosis in fission yeast. The top two wild-type cells show a normal mitotic spindle (anti-tubulin, red) and proper segregating anaphase nuclei (DAPI, which stains DNA, blue). The bottom two mutant cells show a fragmentation phenotype of irregularly segregating DNA along the spindle. **b** | Cells entering meiosis from the haploid state in a *pat1* mutant. DAPI is false-coloured green. Note the three or four irregularly segregating nuclei per cell body, after a lethal attempt to go through MI and MII divisions. **c** | *cell division cycle 2* (*cdc2*, which encodes a cyclin-dependent kinase (CDK)) mutants elongate without dividing. Composite image of differential interference contrast (DIC) and fluorescence (DAPI). **d** | *cdc28* (CDK) mutants arrest without budding. **e**–**f** | Normal yeast cells (smaller cells) are shown next to re-replication mutants in yeast that overexpress *cdc18*. Mutants undergo several rounds of DNA replication in the absence of mitosis (**e**, phase contrast; **f**, propidium iodide-stained DNA in red). **g** | Cells deleted for *tip1*, with mispositioned growth sites. **h** | Overexpression of an unknown gene that affects cytokinesis, as well as the size, number and positioning of growth sites. (Panels **e**–**f** courtesy of Hideo Nishitani, Japan; panels **g**–**h** courtesy of Damian Brunner, EMBL, Germany.)

However, the difficulty of working with lethal, loss-of-function mutations in essential genes leads investigators to seek more modest point mutations that change, but do not abolish, gene function. A broad spectrum of these mutations can be generated using different chemical mutagens or irradiation. Geneticists are particularly fond of conditional mutants, in which the encoded gene product is functional under one condition, but not under another. A classic condition is temperature. Temperature- or thermosensitive (ts) mutant proteins function at low temperature, but are inactivated or unfolded at high temperature. By contrast, cold-sensitive (cs) mutant proteins are presumed to affect protein–protein interaction surfaces at the low (restrictive) temperature. In these screens, cells are propagated at the permissive temperature and are then screened at the restrictive temperature for the phenotype of interest. These are identified by REPLICA PLATING, and looking for colonies that can grow under permissive conditions but not under restrictive ones. In fission yeast, the difference can be enhanced using phloxin B, a vital dye that stains sick colonies dark pink or red. The cells are then examined further for the desired phenotype, which could be a particular cell or colony morphology, or nuclear or spindle defect. Some examples are shown in FIG. 2.

For example, cells that have *ts* mutations in the cell-division-cycle machinery can grow but cannot divide at the restrictive temperature, leading to mutant cells with a distinct morphology, which indicates an arrest at a particular point in the cell cycle (*cell division cycle*, or *cdc*, mutants). In fission yeast, *cdc* mutants are typically elongated; in budding yeast, they have a distinct bud morphology that corresponds to their position in the cell cycle. These characteristics were exploited in a series of classic papers by Lee Hartwell in budding yeast[20], followed by Paul Nurse in fission yeast[21], and many of the *cdc* genes isolated were subsequently shown to be conserved in all eukaryotes (reviewed in REF. 22). Another condition might be imposed using specific drugs. For example, drugs that disrupt normal cell-cycle events, such as hydroxyurea (which blocks DNA replication) or benomyl (which disrupts microtubule function), provide other ways of challenging normal cell-cycle dynamics in a mutant cell. Some mutants that are defective in the DNA replication checkpoint in fission yeast were identified because of their inability to form colonies on plates that contain low doses of hydroxyurea when wild-type cells were able to grow[23]. The first isolation of spindle-checkpoint mutants in budding yeast relied on a change in cell morphology in response to drug treatment: cells that arrest the cell cycle normally in response to benomyl remain as single cells, whereas mutants with defective checkpoints attempt to divide, forming a mini-colony of dying cells[24,25]. Finally, different nutrient media can also provide conditional selection. For example, strains that are deficient in galactose metabolism will still be able to grow on a medium that contains glucose.

It is important to note that a conditional mutant is mutant at all times, not simply under restrictive conditions. Under permissive conditions, the activity of

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HAPLOINSUFFICIENCY
A gene dosage effect that occurs when a diploid requires both functional copies of a gene for a wild-type phenotype. An organism that is heterozygous for a haploinsufficient locus does not have a wild-type phenotype.

the mutant gene might be near to wild type, or it might be attenuated sufficiently to observe a phenotype such as chromosome loss. Mutants with attenuated activities under permissive conditions can be particularly useful in synthetic enhancement screens (see below). The phenotype of viable cells with partial activity can also be exploited in direct genetic screens. For example, cells with defects in essential genes required for chromosome maintenance or transmission are often identified by assays that monitor relatively minor defects in plasmid stability under permissive conditions. A useful tool now in wide use is a colour marker that allows colonies containing the plasmid to be scored by the naked eye[26,27] (BOX 1).

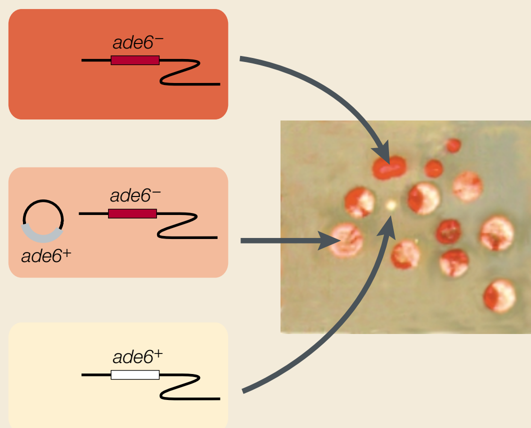
The use of selection, as opposed to screening, is particularly powerful. Whereas a screen requires a strategy to examine large numbers of mutants and then find among them the few with the desired phenotype, a selection establishes conditions in which only the mutants of interest survive. If one considers the difference between screening for mutants that are sensitive to a drug such as hydroxyurea, versus selecting for mutants that are resistant to the drug, it is easy to see that in the latter case only the mutants of interest will be recovered, saving the investigator significant effort.

Screens for dominant, or gain-of-function, mutations are also quite powerful. Sometimes simply increasing the dosage of a gene can result in a mutant phenotype[28]. Overexpression screens are typically carried out using a HIGH-COPY LIBRARY, in which a cDNA is expressed under a controllable promoter, but can also be carried out using a genomic library, by relying on the increased copy number of the EPISOME relative to the chromosome. The particular advantage of these dosage-sensitivity screens is that the gene of interest is essentially cloned already, and can be identified simply by isolating the plasmid from the yeast cell, partially sequencing it and comparing it to genome databases. The phenotypes screened or selected for by overexpression studies are as varied as those used for loss-of-function screening. In a recent example, a screen of budding yeast genes that confer a cell-cycle-related phenotype when overproduced, such as arrest at a particular point of the division cycle, identified several new genes with no previous link to cell-cycle progression[29]. This method can also be used to analyse the function of a previously cloned gene, by mutagenizing it *in vitro* and screening for variants that cause a DOMINANT-NEGATIVE phenotype[30].

Screening for mutants is a statistical effort. Numerous labs can repeat the same screen and come up with a different spectrum of mutants for each iteration. Generally, a screen is said to approach saturation when mutations in the same genes are isolated repeatedly. However, variations of methods, mutagens, plasmid libraries and even investigators continue to provide fruitful outcomes in these analyses.

Box 1 | **Plasmid dynamics**

Plasmid screening
Cells that contain a plasmid can be screened using colour markers[12,26,27]. A yeast cell that lacks the *Saccharomyces cerevisiae ADE2* (or *Schizosaccharomyces pombe ade6*$^+$) gene accumulates a red intermediate in the adenine biosynthesis pathway (see the illustration). If the wild-type copy of the corresponding *ade* gene is placed on a non-essential plasmid or a minichromosome, it complements the chromosomal *ade* mutation and prevents accumulation of the red compound. The colonies that contain the wild-type *ade* gene will therefore be white or pale pink, whereas cells that lose the plasmid will form dark red colonies. Plasmid loss can be monitored by determining whether white colonies produce red papillae or red sectors, and whether individual cells produce red, white, or sectored colonies (see illustration). An increased rate of sectoring can indicate defects in plasmid maintenance. Alternatively, cells will form white or pale pink colonies without sectors if they acquire some other mutation that requires the plasmid for viability. A variation on the theme uses a tRNA suppressor encoded by the plasmid and a nonsense allele in the *ade* gene. The tRNA suppressor incorporates an amino acid at the *ade* nonsense codon. In this case, the degree of colour is an indicator of plasmid dosage: higher copy number or more stable forms of the plasmid result in white cells, low copy number or unstable plasmids result in pink cells and loss of the plasmid results in red colonies.



Plasmid selection
Cells that contain a plasmid are typically selected using nutritional markers; for example, by putting a *LEU2* gene required for leucine biosynthesis on a plasmid and transforming it into a *leu2* mutant yeast strain (see TABLE 2 for some commonly used markers). However, some markers can be used for negative (counter-) selection, to select cells that have lost the plasmid. For example, the *S. cerevisiae CAN1* (*S. pombe can1*$^+$) gene, which encodes an arginine/histidine permease, renders cells sensitive to the toxic arginine analogue, canavanine. Mutant cells are resistant to the drug, because they cannot import it. Therefore, *can1*$^-$ cells that have lost a plasmid containing the *CAN1* marker will be able to grow on canavanine-containing plates (canavanine resistant), whereas cells that contain the plasmid will be canavanine sensitive. The most useful marker of all is the *S. cerevisiae URA3* gene (*S. pombe ura4*$^+$), which has both positive and negative selection properties. *S. cerevisiae ura3* or *S. pombe ura4* mutants require the gene to grow in the absence of uracil, but when the gene is present, they become sensitive to the toxic analogue 5-FOA (5′-fluoro-orotic acid). So, cells transformed with plasmids that contain the wild-type genes and a *ura* marker can be isolated by selecting for growth without uracil, and plasmid-free cells can be recovered by growth in 5-FOA. The *S. cerevisiae TRP1* gene can also be used for counterselection with FAA (5′-fluoroanthranilic acid).

Defining genetic networks

In well-studied systems such as the yeasts, there are few processes that have not yet been targeted for genetic analysis, so for many processes, some genes have already been identified. Mutants in these provide a way to bootstrap through a genetic network and identify other genes in the same pathway, or in different pathways that affect the process of interest. These methods rely on SYNTHETIC INTERACTIONS between different mutations, and are perhaps the most potent weapons in the current genetics arsenal[31,32].

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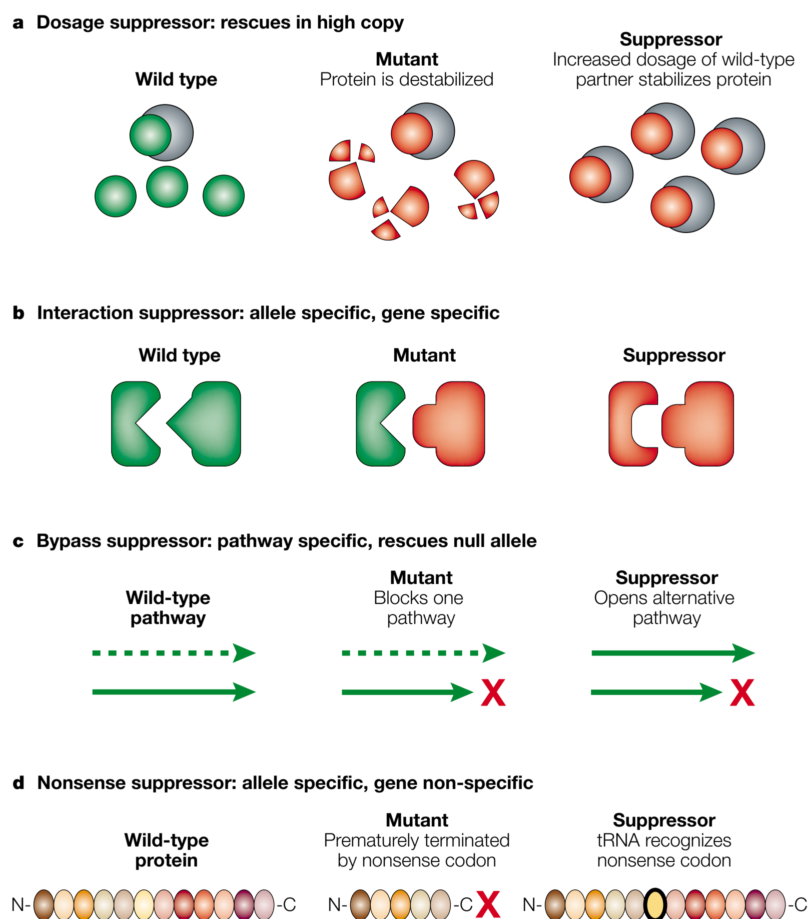

Figure 3 | **Suppressor mechanisms.** Depending on the allele and gene specificity associated with suppressors, mechanisms can be inferred, as shown. **a** | Dosage suppressors encode proteins that stabilize the mutant product when they are expressed at high levels. **b** | An interaction suppressor restores the interaction between the mutant product and its partner(s). **c** | A bypass suppressor activates an alternative pathway to the wild-type pathway. **d** | A nonsense suppressor encodes a tRNA molecule that recognizes a premature termination codon and inserts an amino acid at that position.

REPLICA PLATING
A classic method to duplicate the colonies on an agar plate by stamping them on sterile velvets or filters, and then applying these copies to new (replica) plates. The replica plates can then be used to test the colonies for growth on different nutrient media or at different temperatures.

HIGH-COPY LIBRARY
Most plasmid episomes in yeast are present at greater than one copy per cell, leading to an increased dosage of any gene(s) carried by the plasmid. The high copy dosage effect can be enhanced if the cells are transformed with a library that contains cDNAs expressed by strong promoters.

Two broad approaches exist for working a way through the network. The first is to use suppression analysis: trying to rescue the phenotype of the original mutation by an additional mutation, or by increased gene dosage[31]. The second is to search for synthetic enhancement: trying to exacerbate the phenotype of the original mutation by a mutation in a second gene, or by increased gene dosage, perhaps to the point of death (synthetic lethality)[32].

Suppressor analysis. Suppressor analysis is particularly attractive because it generally uses selection, rather than screening. A hypothetical mutant *yfg1* (*your favourite gene 1*) that is unable to grow under particular conditions is subject either to mutagenesis, or to transformation with a high-copy plasmid library. Survivors are selected directly in restrictive conditions. Subsequent genetic and molecular analysis reveals whether the suppressor simply reverted the original lesion in *YFG1*, or whether it defines an independent locus *SUP1*. However, although conceptually straightforward, the practical application of suppressor analysis is not always easy, and not all *yfg1* mutants will be tractable for suppression.

How does suppression happen? There are several mechanisms for second-site suppressors[31] (FIG. 3). The most informative is the interaction suppressor. Imagine two proteins that interact physically. Mutation of one might disrupt the interaction, but a compensatory mutation of its partner will restore the association and rescue the mutant phenotype. This change is gene specific and allele specific, as not all mutations in the first gene can be rescued by the same compensatory mutation in the second, and a null mutation will not be rescued. This 'lock-and-key' model offers a strong prediction for direct physical interaction that can be readily tested by molecular or cytological assays, such as co-immunoprecipitation or localization. Using this sort of analysis, direct interactions between the fission yeast Cdc2 kinase and the Cdc13 cyclin were predicted[33], subsequently allowing definition of Cdc2 as the cyclin-dependent kinase (CDK) of this organism. A variation of the lock-and-key interpretation was provided in studies of budding yeast actin and its associated proteins, in which suppressor mutations seemed to increase the affinity of the interacting proteins for one another by introducing additional contact surfaces[34].

Another helpful example is a bypass suppressor, in which an alternative pathway can be activated. For example, the Cdc7 kinase in budding yeast is required to initiate DNA replication, probably by modifying the minichromosome maintenance (MCM) protein complex. The MCM complex consists of six highly conserved proteins that are essential for the initiation and progression of DNA synthesis (reviewed in REF. 35). A specific mutation in *MCM5* called *bob1* bypasses the requirement for Cdc7 activity. This is a gene-specific, but allele-nonspecific suppressor, as *mcm5-bob1* suppresses the lethal phenotype of the *cdc7* null as well as the conditional alleles. The *bob1* mutation is thought to induce a conformational change in the MCM complex that would normally require modification by the Cdc7 kinase (REF. 36).

Not all second-site suppressors are relevant to the study of a particular process. For example, if a gene has a mutation that results in a termination codon, it is possible to isolate a rescuing mutation in a tRNA that now recognizes the termination codon and inserts an amino acid at that position. This informational suppressor is not specific to the original gene that was mutated, but will rescue any gene with the same termination codon. It is an allele-specific, but gene-nonspecific suppressor. These are relatively rare in most yeast screens, simply because the typical mutations used for suppressor analysis are temperature sensitive and these are less likely to result from premature termination.

The identity of the genes involved in second-site suppression must be established molecularly by cloning the corresponding genes (FIG. 4). Cloning by complementation (the standard yeast approach) requires a selectable phenotype. For this reason, suppressors are often screened for additional phenotypes; for example, one might suppress a *ts* mutant and screen the rescuers for a

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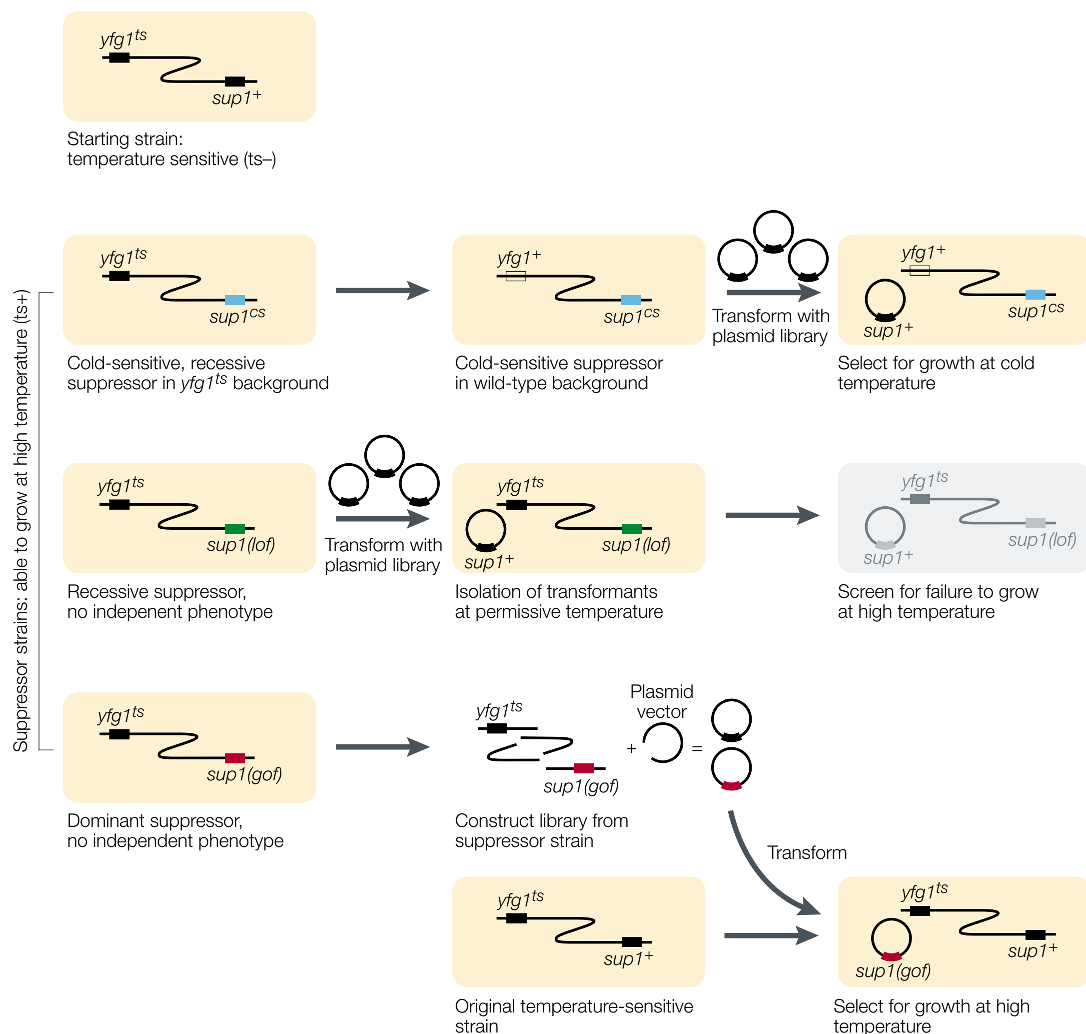

Figure 4 | **Cloning suppressors.** Cloning of suppressors is significantly easier if they confer an independent phenotype (for example, cold sensitivity). However, strategies exist for cloning suppressors without a separate phenotype, as described in the text and shown here for the fission yeast *Schizosaccharomyces pombe*. lof, loss-of-function mutation; gof, gain-of-function mutation; *yfg1*, *your favourite gene1*. See text for details.

EPISOME
An independent DNA element, such as a plasmid, that can replicate extrachromosomally or that can be maintained by integrating into the genome of the host.

DOMINANT NEGATIVE
A mutant allele that interferes with the function of its wild-type version.

SYNTHETIC INTERACTIONS
These occur when a double mutant has a phenotype different from either single mutant parent. For suppressors (synthetic viable), the double mutant is viable when at least one of the single mutants is not. For synthetic lethal mutants, the double mutant is inviable under conditions in which both parents are viable.

new, *cs* phenotype. The suppressor gene would then be identified by transforming a plasmid library into the *cs* strain and complementing it for growth at the restrictive temperature (a direct selection, FIG. 4a). However, not all proteins are susceptible to *cs* mutations.

It is possible, although time-consuming, to clone a suppressor even if it lacks an independent phenotype. If the suppressor mutation is recessive, then it can be cloned in a screen for loss of suppression. In this strategy, the plasmid library is transformed into the suppressed strain that carries the original mutation (*yfg1 sup1*), and the transformants are screened for loss of the suppression phenotype. For example, if the original mutation *yfg1* was temperature sensitive (FIG. 4b), the suppressed strain *yfg1 sup1* would be temperature resistant. But restoring the wild-type copy of the suppressor gene $sup1^{+}$ would again confer temperature sensitivity. This could be determined using replica plating — an entirely feasible, if somewhat laborious approach. Matters become more complicated if the suppressor is dominant, representing a gain-of-function mutation. Under these circumstances, the only options are either to construct a plasmid library from the suppressor strain, transform it into the original mutant *yfg1* and screen for suppression (FIG. 4c), or to genetically map the mutation, and attempt to identify the gene using the complete genome sequence and physical map. This could take months.

The potential for difficulty in cloning second-site suppressors makes high-copy, or dosage suppression, an attractive approach, because the rescuing gene is already cloned. In this strategy, the mutant strain is transformed with a high-copy library, preferably a cDNA library under a controllable promoter. The transformants are screened or selected for survivors under conditions in which the promoter is induced. One likely category of survivors will contain the wild-type version of the mutant gene under study. However, other genes might also be isolated. These dosage suppressors might result from direct interactions that stabilize the mutant protein. Alternatively, increasing the

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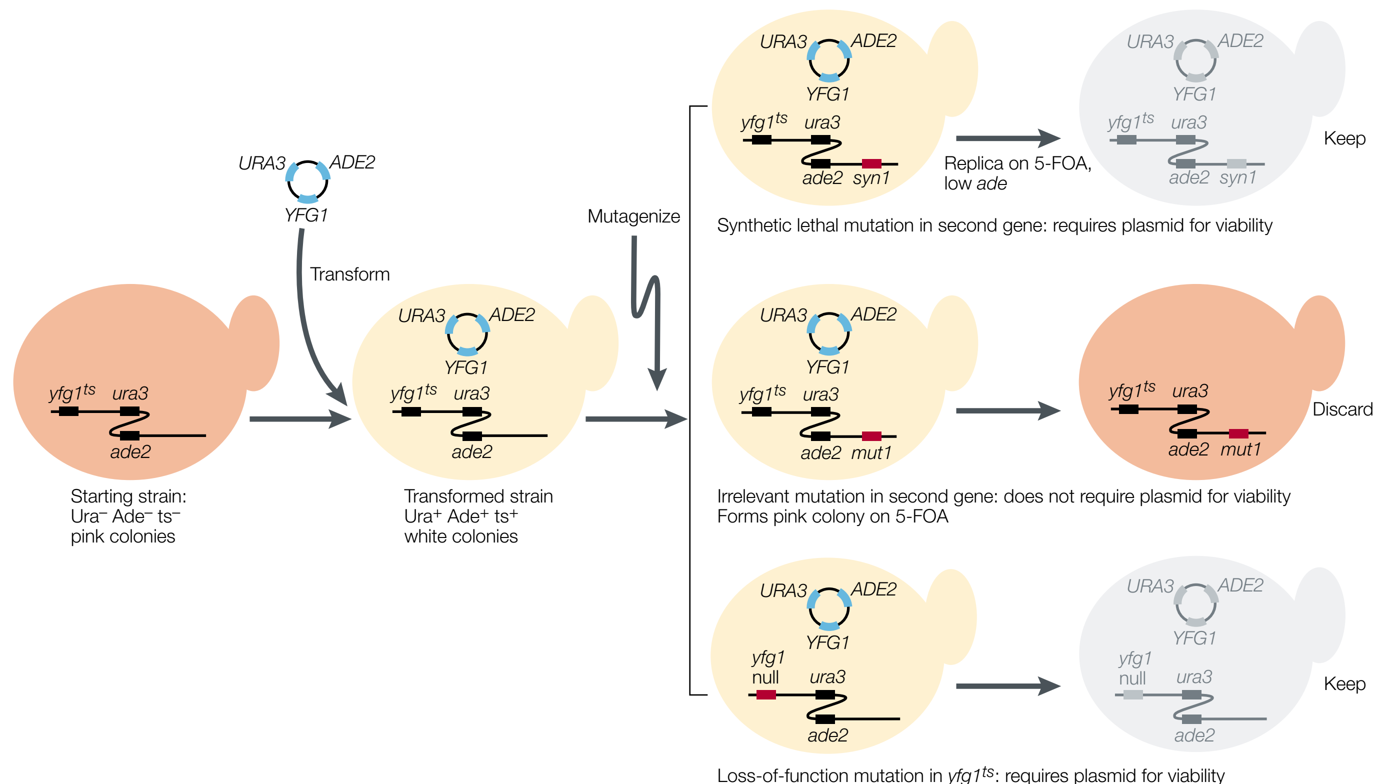

Figure 5 | **Synthetic lethal screens.** Synthetic lethal screens are powerful tools for identifying additional genes in a pathway of interest. The challenge of the screen is to design starting strains that allow rapid determination of whether the new mutation occurs in a different gene, and whether it has an independent phenotype. The figure shows the search for mutations that are synthetically lethal with *yfg1* (*your favourite gene 1*) in the budding yeast *Saccharomyces cerevisiae*. A strain with a chromosomal mutation in *yfg1*, and with *ade2* and *ura3* markers, forms pink colonies. It is rescued by the wild-type *YFG1* that is present on a selectable plasmid marked with *ADE2* and *URA3*, forming white colonies. The plasmid-transformed strain is mutagenized and the surviving cells are scored for those that cannot survive in the absence of the plasmid (and therefore require *YFG1* for viability). These will include strains with null mutations either in the *yfg1* gene itself or in an unrelated gene that is synthetically lethal with *yfg1*. Cells that do not require the plasmid will form pink colonies and will be discarded. 5-FOA, 5′-fluoro-orotic acid; ts, temperature sensitive.

dosage of a downstream factor might bypass the requirement for the original mutated gene. These possibilities could be distinguished genetically, by determining whether the mutation is allele specific or can rescue the null allele, or biochemically, by determining whether the proteins encoded by the genes can physically interact with one another. Suppressing plasmids are readily recovered from the transformed yeast, and the identity of the open reading frames can be rapidly determined by generating a short sequence from the clone and comparing this to the completed genome sequence databases.

One powerful version of plasmid-based suppression is the identification of functional homologues from other organisms. cDNA libraries that contain mammalian genes in yeast vectors can be used to determine whether, for example, a human gene can complement a yeast mutant. The human version of the fission yeast *cdc2*$^{+}$ gene that encodes a CDK kinase was identified in just this way[37]. Banks of *ts* yeast strains have been constructed to exploit this approach (for example, REF. 38). However, despite obvious structural conservation between many genes involved in basic cell function, successful cross-complementation is relatively rare. It might be that precise protein interactions have changed through evolution, or additional components of the system are more variable than the one under study. So, failure of a metazoan gene to complement a yeast mutant does not mean that they are not functional homologues.

Synthetic lethality. Synthetic lethality is the opposite of suppression[32]. Instead of restoring function to the original mutant, the synthetic lethal screen aims to further attenuate function, to the point of death. This strategy can be particularly useful for uncovering redundant pathways or direct protein interactions, depending on the nature of the starting mutation. The hallmark of this approach is that only the double mutant has the phenotype: either single mutant is functional (this explains the 'synthetic' in synthetic lethality).

Consider a disruption allele that has no strong phenotype, but for other reasons the gene is assumed to affect the process of interest. Is there a redundant gene or pathway that keeps the cells functioning in the absence of the original gene? If there is a single additional gene that is redundant, then mutating it in a background that already lacks the first gene will confer the desired phenotype. Only the doubly mutant strain would have the phenotype; either mutant alone would be normal. This can be considered as the parallel pathway model: the cells are

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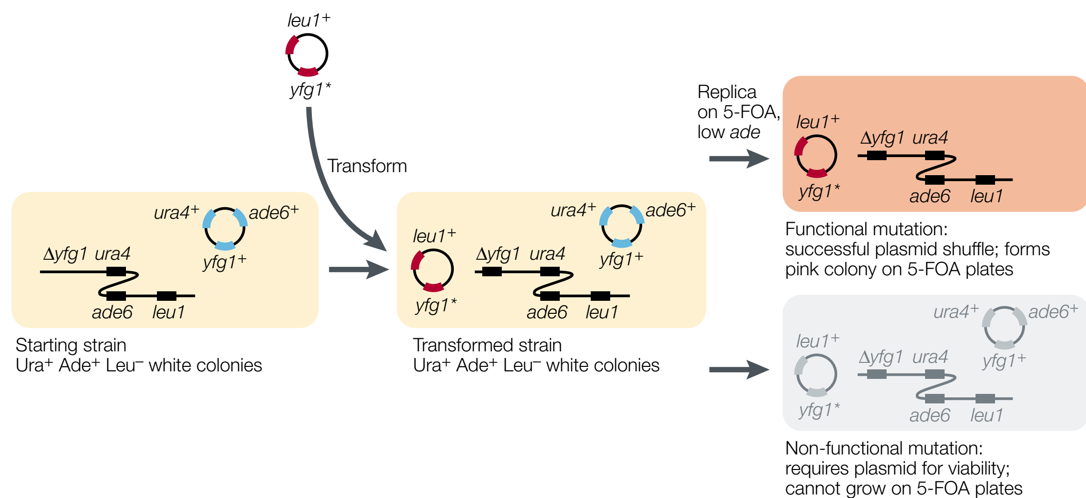

Figure 6 | **Plasmid shuffle.** The plasmid shuffle technique can be used to analyse individual protein function or isolate conditioned alleles. Using a balance between selective and counter-selective pressure, cells can be isolated that either contain a plasmid or are cured of it. Selection can be conferred by a nutritional marker, or by an additional gene on the plasmid that might be required for viability. Using colour-based assays (see illustration in BOX 1), an additional visual screen might be incorporated to allow rapid assessment of plasmid presence. In this example, a null mutation in chromosomal *yfg1* (*your favourite gene 1*) is rescued by the wild-type version (*yfg1*$^+$) that is present on a selectable plasmid in the fission yeast *Schizosaccharomyces pombe*. This strain is transformed with a library of plasmids that contain mutant variants of *yfg1* (*yfg1**). If the *yfg1*$^+$ plasmid can be shuffled out of the transformed cells (here, by selecting against the presence of plasmid-borne *ura4* and *ade6*), then this is a sign that the *yfg1** allele can rescue the lethality of the chromosomal *yfg1* mutation; the *yfg1** allele can then be isolated and studied further. 5-FOA, 5′-fluoro-orotic acid.

viable as long as one pathway is intact. An example of this is the overlapping activity of the Cln1, Cln2 and Cln3 cyclins that control the G1 phase of the cell cycle in budding yeast: any one is sufficient for viability and severe phenotypes are only observed if all are deleted[39].

Conversely, using synthetic lethality as a tool to probe direct interactions requires beginning the screen with alleles that attenuate, but do not abolish, the function of a protein. Consider *ts* mutants that are grown at the permissive temperature. They are viable but, in many cases, the protein function is attenuated even at the permissive temperature. Mutation of another gene in the pathway can interact genetically to abolish function: the cells can tolerate any single insult to the pathway of interest, but cannot tolerate more than one. This can be considered as the protein complex, or single pathway, model. For example, a network of genetic interactions between a collection of budding yeast replication mutants, including suppression and synthetic lethality, provided the first evidence that the proteins they encode form functional complexes *in vivo*[40]. The complication of the synthetic lethal approach is that if the process under study is essential, one must use a screen rather than a selection. The investigator is looking for conditions in which a viable cell will die.

To isolate synthetic mutants, one begins with a strain that contains the original mutation and a plasmid with the wild-type version of the gene. The cells are mutagenized and then screened for loss of the plasmid on the appropriate counter-selection agent (BOX 1 and FIG. 5) under permissive conditions, to isolate strains that have lost the plasmid, and with it, the wild-type gene *YFG1*. The desired mutants will never survive on the counter-selection agent, because they have a mutation that requires the presence of the wild-type copy of the gene for viability. So, the investigator isolates the mutants that are sensitive to the counter-selection agent. This strategy can be improved by including a colour marker to facilitate rapid identification of colonies that have lost the plasmid (BOXES 1 and 2).

If the starting strain contained a null mutant in the original gene that was caused by deletion of that gene, then any new mutation that results in synthetic lethality must occur in another locus. If the starting strain contained a conditional mutant, then the new mutation could also occur in the original gene and render it completely inactive, or it could represent a mutation in a second gene that produces a synthetic lethal phenotype. These can be distinguished genetically in crosses to appropriately marked strains, to determine whether the new mutation is linked to the original mutant, whether it has a phenotype by itself and whether the effect results from a single genetic lesion. The desired phenotype must result from the interaction between the two mutations *yfg1* and *syn1*.

Again, cloning of the genes that correspond to synthetic lethal mutants can be a challenge. In some cases, the *syn1* mutant causes an independent phenotype by itself, such as heat or cold sensitivity, or drug sensitivity. If this phenotype is recessive, the corresponding gene can be cloned by complementation of the *syn1* phenotype. Often, however, there is no obvious independent phenotype and other means must be used. These depend on whether the *syn1* mutation is dominant or recessive. For example, if the haploid strain *yfg1*^{ts} *syn1* is inviable, but the diploid strain *yfg1*^{ts}/*yfg1*^{ts}

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Box 2 | Genetic screens: a case study

An excellent example of a synthetic lethal screen and plasmid shuffle analysis was published recently[47]. *Saccharomyces cerevisiae* *SGS1* encodes a helicase that is required for genome stability and recovery from replication insults, but is not essential for viability. S. J. Brill and colleagues[47] isolated mutants in six genes that are required for viability in a Δ*sgs1* background. The authors used a variation on the colour assay described in BOX 1, by using a double *ade* mutant. The starter strain was *ade2 ade3*, which is unable to grow without adenine. This strain forms white colonies because the *ade3* mutation is epistatic to *ade2* — *ade3* lies upstream in the metabolic pathway and prevents formation of the red pigment that normally accumulates in *ade2* mutants. With wild-type *ADE3* on a starting plasmid, which also contains *SGS1* and *URA3*, transformed colonies will accumulate the red pigment. Cells were mutagenized and plated to look for colonies that were solidly red without sectors, indicating that they required the plasmid for viability. These clones were unable to form colonies on 5′-flouro-orotic acid (5-FOA) plates. Six complementation groups were isolated that depended on the plasmid for viability; because several alleles were isolated for each group, the authors concluded that their screen was approaching saturation. The mutants were called *slx1–6*. Characterization of these new mutants indicated that they formed three phenotypic classes, with two *slx* mutants in each.

Cloning of the wild-type *SLX* genes used a plasmid shuffle method (FIG. 6). Each Δ*sgs1 slx* strain, containing a plasmid with *SGS1, ADE3,* and *URA3* markers, was transformed with a plasmid library that contains the *LEU2* marker. Upon plating on 5-FOA, clones that no longer required the original *SGS1 URA3 ADE3* plasmid for viability were selected. These now relied instead on the *LEU2* plasmid, which contained either the wild-type *SGS1* or the wild-type *SLX* gene. *SGS1*-containing plasmids were identified on the basis of their ability to rescue the lethality of all six Δ*sgs1 slx* mutant strains. Isolation of the *SLX* plasmids allowed identification of the open reading frames (which were for the most part previously uncharacterized), construction of targeted gene disruptions and molecular experiments. Intriguingly, the two *SLX* genes in each phenotypic class seem to interact with each other in a complex.

This example shows how classical yeast genetics methods can be used to identify genes with no previously known function, to place them in existing networks and to predict molecular interactions.

syn1/SYN1 is viable, then the synthetic lethal mutation *syn1* is recessive. If this is the case, the investigator begins to clone *SYN1* using *yfg1*^{ts} *syn1* cells kept alive by *YFG1* on the original plasmid, and transforms them with a plasmid library that contains wild-type genes. Upon plating on the counter-selection agent, the original plasmid is driven out and only those cells that contain a library plasmid with *YFG1* or *SYN1* are able to grow. This is a form of the plasmid shuffle screen (FIG. 6), described in more detail below. An example of a synthetic lethal screen that used this cloning strategy is described in BOX 2.

As is the case for suppressors, synthetic phenotypes can also be generated by overproduction of wild-type genes from a library in a mutant background[41]. This synthetic dosage lethality phenotype might reflect direct interactions, or downstream effectors, and the plasmids are easily recovered and their inserts identified by sequencing.

More commonly, synthetic dosage lethality is used to probe the response of the cell to a known mutant protein expressed in high dosage. Overexpression of a mutant form of *yfg1* might be tolerated by wild-type cells, but not by mutant cells. This can be tested on a case-by-case basis using previously isolated mutant strains, or can be the basis of a screen by identifying mutants that are sensitive to overproduction of the defective protein, when wild-type cells are resistant. For example, expression of a mutant version of fission yeast Mcm2 that lacks its nuclear localization sequence (NLS) is tolerated in wild-type cells, but is lethal in *mcm2*^{ts} mutants even at the permissive temperature. The NLS mutant out-competes the attenuated *ts* protein and sequesters the other members of the MCM complex in the cytoplasm. Only the wild-type Mcm2 is sufficiently robust to withstand this lethal trap[42].

Plasmid-based screens and functional analysis

Both of the yeast species are easily transformed by extrachromosomal plasmids that contain a selectable marker and an origin of replication. By combining forward selection for transformants, and counter-selection to isolate cells that are cured of the plasmid, the investigator gains considerable control in manipulating gene expression and plasmid dosage.

Plasmids can be used as markers for chromosomal dynamics, including DNA replication (mediated by the plasmid origin of replication) and segregation. For budding yeast, the centromere is small enough to be encompassed on an average plasmid; for fission yeast, the much larger centromere can be contained on a large minichromosome, which must be transferred genetically from strain to strain[43]. The presence or absence of a plasmid in a cell can be determined by direct selection, or by a colony colour-based visual screen (BOX 1). The latter screen is readily done and allows the investigator to determine whether all the cells in a colony have lost the plasmid, or whether they are being lost at a rapid rate, by determining whether colony colour is uniform or sectored.

Perhaps the most useful plasmid-screening method is the analysis of individual protein function using the plasmid shuffle[44] (FIG. 6). This method starts with a null allele of Δ*yfg1* in the chromosome, kept alive by wild-type *yfg1*$^{+}$ on a counter-selectable plasmid. A library of *yfg1** mutants (consisting of mutagenized genes) on a compatible plasmid with a different marker is constructed by *in vitro* mutagenesis, for example with hydroxylamine. The method can be enhanced by including a colour marker on the counter-selectable plasmid (BOX 1). The starting strain is transformed with the *yfg1** library and plated on the counter-selection agent to see whether the wild-type plasmid can be shuffled out, its function provided by one of the *yfg1** plasmids. If it can, then the colonies kept alive by *yfg1** can be screened to see if they have any associated phenotypes (for example, drug resistance or temperature sensitivity). Ultimately, the *yfg1** allele can be recovered and used to replace the endogenous chromosomal copy for additional study. This method is commonly used to isolate *ts* alleles and replace them in the chromosome (for example, REF. 45).

Conclusion and perspectives

With the completion of the genome sequences of both yeast species, and the classical genetics tools described here, it is clear that the yeasts are entering a new golden age[46]. Their limited complement of genes

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Links

DATABASE LINKS Cdc2 | Cdc13 | actin | Cdc7 | *MCM5* | Cln1 | Cln2 | Cln3 | Mcm2 | *ADE2* | *ade6*$^+$ | *LEU2* | *CAN1* | *can1*$^+$ | *URA3* | *ura4*$^+$ | *TRP1* | *SGS1* | *ade3* | *slx1–6* | *pat1* | *cdc28* | *cdc18* | *tip1*

YEAST PHYLOGENY Ascomycota | Fungal phylogeny

GENOME DATABASES *Saccharomyces* Genome Database (SGD) | Sanger Centre *S. pombe* database | Proteome (YPD and pombePD)

PROTOCOLS *S. cerevisiae* | *S. pombe*

OTHER YEAST RESOURCES Yeast virtual library | Gene conversion table | *Saccharomyces* genome deletion project | TRIPLES

LABS AND INVESTIGATORS *S. cerevisiae* labs | *S. pombe* labs | Susan Forsburg's lab

COMMUNITY INFORMATION *S. cerevisiae* | *S. pombe*

provides biologists with streamlined systems for understanding fundamental principles in eukaryotic cell biology. Remarkably, the classical methods discussed here are still timely and in wide use; modest refinements are made, but the principles remain the same. These methods help to meet the continuing challenge of the genomic era to identify the function of individual genes. The insights from the yeasts therefore continue to inform experiments in more complex cells, and cell biologists can only benefit from adding these versatile and tractable cells to their toolkits.

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An excellent example of a synthetic lethal screen and plasmid shuffle analysis.

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