Yeast as a Model Genetic Organism

Joshua Chang Mell, *University of California at Davis, Davis, California, USA*
Sean M Burgess, *University of California at Davis, Davis, California, USA*

The budding yeast is an ideal experimental organism for genetic research. The yeast shares a common life cycle and cellular architecture with higher eukaryotes, and as a microorganism it is easily propagated and manipulated in the laboratory.

Introductory article

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Introduction

The budding yeast *Saccharomyces cerevisiae* has emerged as a versatile and robust model system of eukaryotic genetics. Mutant screening and segregation analysis are simpler and easier to perform in yeast than in multicellular organisms; and fundamental eukaryotic biology, such as cell cycle control and alternation of generations, is well-conserved throughout eukaryotic taxa. Yeast has a high endogenous rate of homologous recombination, and a host of extrachromosomal DNA elements can stably transform yeast cells. Thus the analysis and cloning of genes in this organism is significantly easier than in more complex eukaryotes.

How Yeasts Became Popular Model Systems

Humans have cultivated yeast since the dawn of agriculture to make beer, bread, and wine. As a domesticated microorganism and sexual eukaryote, the budding yeast *Saccharomyces cerevisiae* is the most commonly used industrial yeast and has emerged as a remarkably tractable eukaryotic model system. Other widely studied unicellular fungi, particularly the fission yeast *Schizosaccaromyces pombe* and the orange bread mould *Neurospora crassa*, share many of the traits that make *S. cerevisiae* attractive to investigate and are useful for analysing numerous genetic phenomena with no parallel in the budding yeast.

Yeasts belong to the kingdom of fungi, along with moulds, smuts, and mushrooms. They share a common cellular architecture and rudimentary life cycle with multicellullar eukaryotes such as plants and animals. As nonpathogenic, nonmotile microorganisms, yeasts are easily propagated and manipulated in the laboratory. The long history of yeast research has led to a wealth of tools and information, utilized not only by yeast researchers but also by the field of genetics as a whole.

The budding yeast found an early role in metabolic research owing to its association with the fermentation industry. Early geneticists soon found a variety of unicellular fungi useful in demonstrating the underlying genetic control of metabolism, and thus established techniques to order genes along chromosomes and into pathways. Mutations in metabolic genes soon became useful as selectable markers for genetics, acting as indicators of a strain's genotype. This is a common outcome of yeast research; as scientists have pursued specific biological questions, they have simultaneously developed methodology applicable to a wide range of questions. This is illustrated by the discovery of the genetic elements important for chromosome segregation, along with the invention of yeast plasmids and artificial chromosomes.

An important theme of yeast biology is the use and study of homologous recombination, a process by which a broken piece of DNA uses a homologous DNA template as a substrate for repair. Yeast use this process to fix DNA damage, to switch mating types, and to segregate homologous chromosomes during meiosis. Researchers also use this pathway for genetic mapping and integrative transformation of DNA into specific locations of the genome.

Much of what we have learned about cell and molecular biology has come from research on yeast. The genes and mechanisms involved in many cellular processes are highly conserved across eukaryotic taxa, so by studying the yeast we learn about the fundamental biology of all eukaryotes.

The Budding Yeast as a Typical Eukaryote

Like all eukaryotes, yeast cells have numerous membrane-bound organelles, including a nucleus, endosymbiotic mitochondria, the peroxisome, and the organelles of the secretory pathway. The budding yeast carries its genome of nearly 6000 genes in 12 megabase pairs of DNA on 16 linear chromosomes in the nucleus. The genome is very compact for a eukaryote: the number and size of genes are relatively small and the density of genes is relatively high.

Yeast genes have few introns, and intergenic regions are very short. Genetic redundancy in the yeast genome is low, facilitating the analysis of gene function.

As microorganisms, yeasts are grown in batch liquid culture and isolated as colonies derived from single cells on solid media. The generation time is very short (about 90 min), so large populations of individuals can rapidly be grown and analysed. This is crucial in addressing many problems in genetics that require the observation of rare events, such as genetic mutations.

This single-celled eukaryote, though substantially simpler than multicellular organisms, uses variations of the same mechanisms found in higher eukaryotes to make developmental decisions and differentiate into a variety of cell types. Indeed, many of the genes in the yeast are related evolutionarily and functionally to genes in higher eukaryotes.

The life cycle of *Saccharomyces cerevisiae*

The life cycle of the budding yeast is rudimentarily similar to that of any sexual eukaryote, alternating between haploid and diploid states, containing one and two sets of chromosomal complements, respectively (**Figure 1**). Cells divide by budding; a mother cell buds to produce a genetically identical daughter cell. Before the daughter is released, copies of each chromosome (called sister chromatids) segregate by mitosis. In a haploid, there is one of each chromosome in the complement. When two haploid cells mate and fuse, they yield a diploid cell, which contains two of each chromosome. These pairs are called homologous chromosomes. A diploid can either grow by budding or undergo meiosis, a double round of cell division yielding four haploid spores held together in an ascus (or tetrad). Spores can be individually isolated and propagated as haploid spore clones or mated to one another to form a diploid. As in other eukaryotes, yeast cells age. A mother cell produces a limited number of daughter cells. Life span in yeast is under the control of a genetic programme that shares features in common with multicellular animals.

The vegetative cell cycle

As the budding yeast progresses through the cell cycle, its morphology changes (**Figure 1**). When the yeast appears as a round, unbudded cell, it is in the G_1 phase of the cell cycle. The chromosomes are diffuse and indistinguishable in the nucleus. A small bud emerges from one end of the cell when the cells are in S phase. During this stage, the chromosomes are duplicated, yielding pairs of sister chromatids. In the G_2 phase, the bud grows, and the nucleus is found adjacent to the bud. The pairs of sister chromatids remain attached to each other and are still diffuse in the nucleus.

During mitosis, chromosomes condense about twofold, and sister chromatids are segregated between the mother and bud. Chromosome condensation in the yeast is orders of magnitude less than in higher eukaryotes. The cohesion

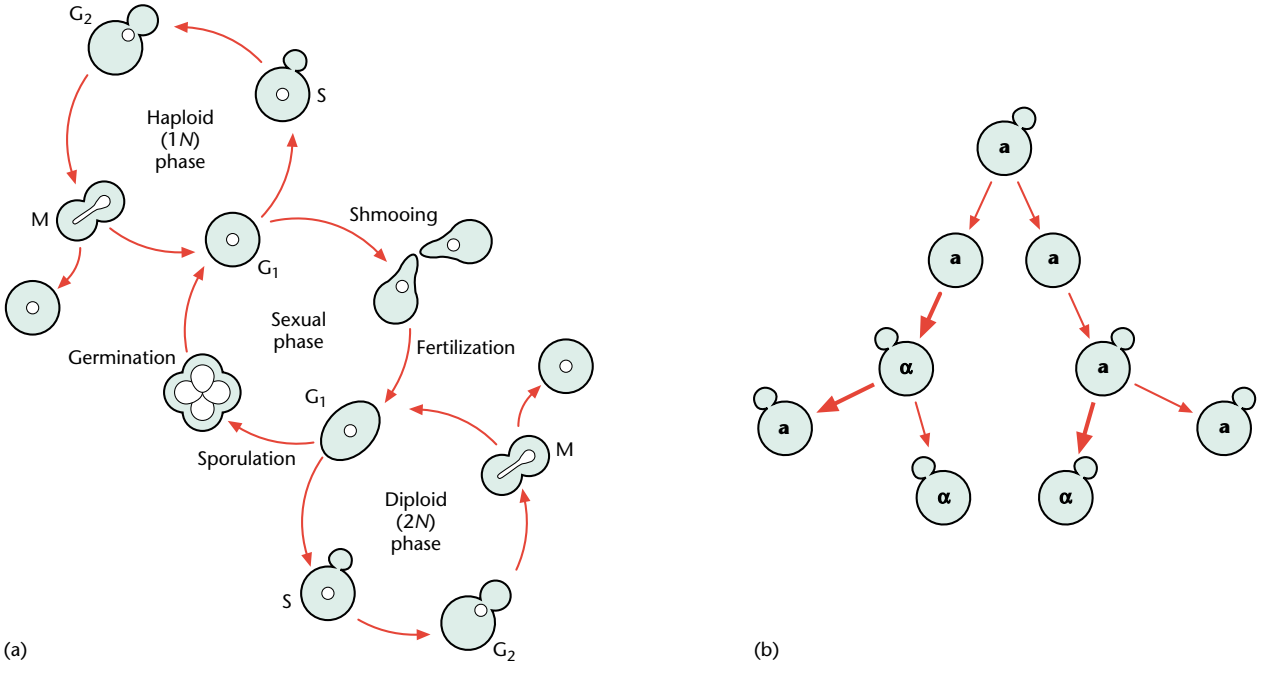

Figure 1 The life cycle of *Saccharomyces cerevisiae*. (a) Alternation of generations and vegetative growth. (b) Mating type switching in homothallic (Ho^+) haploids. Mother cells switch in G_1.

between sister chromatids, established in S-phase, is severed before the spindle apparatus pulls the chromatids apart. Finally, cytokinesis leaves genetically identical nuclei in two G_1 cells, one mother and one daughter cell. Unlike in mitosis in plants and animals, there is no nuclear envelope breakdown or metaphase plate during yeast cell division.

Budding morphology is instrumental in analysing genes involved in cell cycle regulation. Mutations in *CDC* (cell division cycle) genes arrest at different stages in the cell cycle. These genes have been found to control the progression through each of these steps. Yeast geneticists also exploit metabolic marker genes on artificial chromosomes to identify and analyse the role of different genes in properly segregating chromosomes. When tester chromosomes marked with *ADE2* missegregate during cell divisions in an *ade2Δ* genetic background, red clonal sectors arise in colonies, thus evaluating the rate of chromosome loss in different mutant strains.

Mating and homothally

Yeast haploids only mate with haploids of the opposite mating type. The budding yeast has two mating types, termed **a** and **α**. A yeast cell of one mating type produces a pheromone detected by a cell of the opposite mating type, which stimulates fertilization. The cells first grow projections towards each other in a process called shmooing. The cells then fuse to form a diploid cell, which is **a**/**α** and cannot mate. The newly formed zygote can divide mitotically to generate diploid colonies or cultures. When a diploid undergoes meiosis to generate four haploid progeny, two will be **a** and two will be **α** (2:2 segregation).

In the infancy of yeast research, crosses were complicated by homothally, a trait in which a haploid cell switches its mating type between **a** and **α** in alternating mitotic divisions. Thus the yeast is self-fertile, able to mate with clonal offspring to form diploids. The mother cell switches during G_1 after budding, so it and its subsequent daughter have the opposite mating type as the previous daughter and its progeny bud. The mating type of a haploid cell is determined by whether **a** or **α** information is expressed at the *MAT* locus in the middle of chromosome III. The cell possesses the information for both **a** and **α** at two transcriptionally silent loci (*HML* and *HMR*) at opposite ends of the chromosome. When a cell switches, DNA encoding the opposite type is copied from the silent locus by homologous recombination, and the old mating type information at *MAT* is degraded. A DNA double-strand break made by HO endonuclease at the *MAT* locus induces this specific recombination event during G_1 in mother cells. Most laboratory yeast strains are heterothallic, bearing a mutation in the HO endonuclease. This allows researchers to maintain stable haploid cell cultures, unless they are studying the switching process itself.

Mating type switching in yeast is a major paradigm of research. The asymmetrical switching pattern is reminiscent of cellular differentiation in multicellular eukaryotes, offering the yeast as a simplistic stem cell model. Additionally, the mechanism of mating type switching is intensively investigated as a model for homologous recombination. Finally, the silent mating type loci offer a simple model of gene regulation by chromatin.

Sporulation and meiosis

When diploid budding yeast are starved of nutrients, they can undergo meiosis to generate four haploid spores (**Figures 1** and **2**). Spores are more resistant to the environment than are vegetatively dividing cells. The four spores of a single meiosis are held together in an ascus, or tetrad, surrounded by a thick wall. When haploids are released from the ascus, they are free to divide by mitosis or to mate to form a new diploid. In contrast, higher eukaryotes mostly grow by somatic (mitotic) cell division, and the germline – containing the haploid-generating cells – is constitutively segregated into specific tissues, rather than being a response to nutritional cues.

Two closed nuclear divisions characterize meiosis in yeast. The first, meiosis I, is a reductional division, in which the homologous chromosomes physically associate along their lengths before segregating to daughter nuclei. This differs from the equational division of mitosis or meiosis II, since homologous chromosomes are segregated instead of sister chromatids. Thus the number of chromosomes is halved in the daughter nuclei and the parental contributions are mixed together through independent assortment of the individual chromosomes. The paired homologues engage in crossing-over by homologous recombination (**Figure 2**). This mechanism is specifically co-opted for the developmental programme of meiosis to ensure that homologous chromosomes are properly segregated in the first meiotic division and that recombinant chromosomes are generated. Meiosis-specific genes ensure that recombination occurs between homologous chromosomes, instead of sister chromatids. After homologous chromosomes pair, synapsis occurs, in which a proteinaceous structure called the synaptonemal complex (SC) forms between them. Crossovers mature in this structure.

The second round of meiotic segregation, the equational division, is analogous to mitosis, with sister chromatids segregating to daughter nuclei. Meiosis is completed with the formation of a four-haploid-spore tetrad (see **Figure 2**). Some yeasts, such as *Neurospora* undergo a further mitotic division after meiosis to yield an octad. Such a mitotic division also often occurs in higher eukaryotes, particularly in plants, though only one of these eight cells is a fertile oocyte.

Meiosis is remarkably conserved across sexual eukaryotic taxa. However, some variations exist in the basic

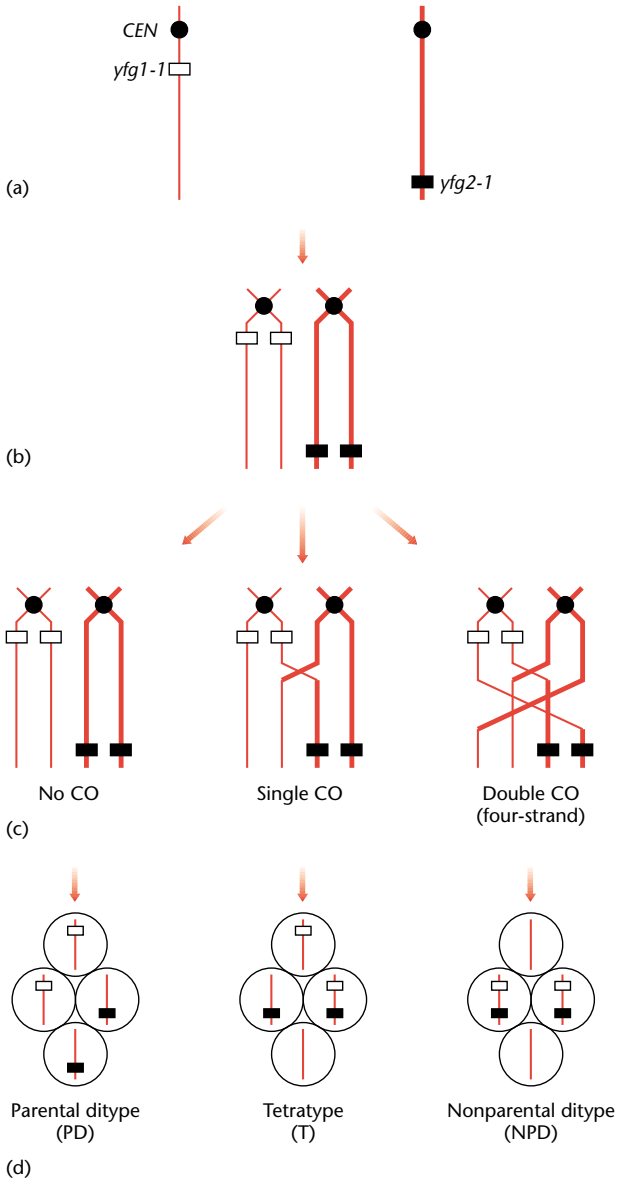

Figure 2 Meiotic segregation in the budding yeast. (a) Cross between two mutants, *yfg1* × *yfg2*. The white and black boxes represent the mutant alleles of *yfg1* and *yfg2*, respectively. The black circle represents the centromere. (b) Schematic of chromosomes after DNA replication and the pairing of homologous chromosomes. (c) Schematic of three possible chromosome configurations after recombination and synapsis. CO stands for crossover. (d) Tetrad classes resulting from a given crossover event are represented with the genotype indicated in the spore. Not shown: two-strand double crossovers result in PD; three-strand double crossovers result in T.

mechanisms. For example in the budding yeast, synapsis depends upon recombination between homologous chromosomes, while fly and worm are the opposite, requiring synapsis to undergo crossing over. In this respect, mouse and plants appear to behave more similarly to yeast than fly or worm. Yeast meiosis is perhaps one of the most important reasons the budding yeast became such a widely studied model organism for eukaryotes: it allows researchers to easily control crosses between strains, and it illustrates that this simple microorganism is similar in fundamental ways to complex multicellular organisms.

Classical Genetic Analysis in Yeast

Mutagenesis and genetic screens

Saccharomyces cerevisiae is a convenient organism for identifying genes by mutagenesis, having a short generation time and a small genome. Mutations in DNA can result in a loss or gain of gene function. The phenotype conferred by a genetic mutation points to the function of that gene in the cell. Unlike higher eukaryotes, heterothallic yeast strains (those containing a mutation in the HO gene) can be grown in stable haploid or diploid states, thus greatly simplifying the identification and analysis of mutations. Many plants and animals are obligate diploids, and many of these also have an obligate haploid phase to generate new individuals. As such, it takes at least two generations to see phenotypes arise from homozygous recessive mutations induced in the parental generation. In the haploid phase, yeast has one copy of each chromosome, so only one allele is responsible for a Mendelian trait. Thus the phenotypes of recessive alleles are immediately evident during a mutant hunt. Additionally, since haploids can be propagated and gametes can be specifically chosen after phenotypic evaluation, crosses in the yeast can be precisely controlled. Nomenclature of budding yeast is presented in **Table 1**.

To find mutations affecting a particular process, a culture of haploid yeast is mutagenized with chemicals or radiation and then screened or selected for a mutant phenotype. For example, if a researcher wanted to identify genes affecting uracil biosynthesis, a culture of haploid cells would be mutagenized and plated to rich media to

Table 1 Budding yeast genetic nomenclature for *YFG1* ('your favourite gene')

YFG1	Wild-type allele
yfg1-1	Recessive mutant allele
YFG1-2	Dominant mutant allele
yfg1Δ	Deletion allele of *YFG1*
yfg1::URA3	Insertional disruption of *YFG1* with *URA3*
Yfg1$^+$	Phenotype of *YFG1*
Yfg1$^-$	Phenotype of mutant
Yfg1p	Protein product of *YFG1*
[*YFG1*]	Cytoplasmic genetic factor

generate clonal colonies from viable mutagenized yeast cells. These colonies are replica-plated to minimal media lacking uracil, so that the replica colonies with mutations in genes involved in uracil biosynthesis will be unable to grow. By comparing growth on selective and nonselective media, Ura^- colonies are identified and propagated for further analysis.

There are innumerable schemes for screening for mutant phenotypes in the yeast. For example, temperature-sensitive mutants are identified by slow growth on replica plates held at a high temperature. Such conditional alleles are vital for identifying and analysing genes essential for survival, so mutant strains can be maintained at the permissive condition. Often it is useful to conduct secondary screens to find mutants with specific phenotypes. For example, to isolate *CDC* genes, which are often essential for growth, an initially screened set of temperature-sensitive yeast strains is examined for cell cycle defects at the nonpermissive temperature.

Once a set of mutant strains has been identified and isolated, they can be further characterized by crosses and segregation analysis. These classical genetic tests map the location of a gene, determine the number of genes involved in a process, and order genes in pathways. After establishing the basic character of the mutations, modern methods can be used to clone and characterize the genes involved in the process of interest at the molecular level.

Dominance and complementation tests

When a mutant of one mating type is crossed to wild-type yeast of the opposite mating type, the phenotype of the resulting diploid indicates whether the mutant allele is dominant or recessive. For example, if a wild type *MAT**a*** haploid were crossed to a *MATα ura3* mutant haploid, unable to grow in the absence of uracil (Ura^-), the resulting diploid would have a phenotype of Ura^+, able to grow in media lacking uracil. Thus the mutant *ura3* allele is recessive to the wild-type *URA3* allele.

When two mutant haploids have the same phenotype, for example Ura^-, the mutations may or may not be in the same gene. If both mutant alleles are shown to be recessive, the phenotype of the diploid indicates whether or not the two alleles reside at the same locus. This is called a complementation test. If the diploid is Ura^+, the mutations complement and probably reside at different loci. If the diploid is Ura^-, the mutations do not complement and most likely reside in the same gene. One must interpret the results of a complementation test carefully. Mutations in different parts of a single multifunctional gene may complement, whereas mutations in different genes with physically interacting products may not complement. Although these scenarios are rare, they are testable by segregation analysis, discussed below. The complementation test allows a researcher to determine how many genes affect a particular phenotype. Further phenotypic analysis of double mutants, called epistasis analysis, orders genes into pathways that control a process.

Tetrad analysis

Since all four meiotic products of yeast are viable, mapping of genes in yeast is powerful and straightforward. Individual spores from a single tetrad are separated into rows on solid media by microdissection. The haploid colonies (or spore clones) that arise are replica-plated to different types of media to evaluate the phenotype (and thus genotype) of individual spore isolates for segregating genetic markers. When a mutant with a single underlying chromosomal mutation is crossed to wild type, the phenotypes will segregate 2:2. This simple common test reveals whether a particular mutant phenotype is caused by a single locus.

When two markers are followed in a cross, both will show 2:2 segregation; but when they are evaluated together, three classes of tetrad can result, called parental ditype (PD), nonparental ditype (NPD), and tetratype (T). These are illustrated in **Figure 2**. The relative frequency of the different classes of tetrads is an indication of the genetic linkage of the two markers under consideration (**Table 2**). When genes are unlinked, segregating independently, the frequencies of PD and NPD are equal. When they are linked, the frequency of PD is substantially higher than that of NPD. Tetratypes arise by crossover events between the two markers. When two markers are unlinked to their centromeres or each other, the frequency of tetratypes is much higher than that of the ditype classes. If two markers are unlinked but very close to their respective centromeres, tetratypes only arise from crossovers between marker and centromere. Thus the frequency of tetratypes (called second division segregation) is much lower for centromere-linked genes.

The major advantage of tetrad analysis is that few tetrads need to be evaluated to generate accurate map distances. The other distinct difference is that the genetic position of centromeres can be evaluated. Mapping in the yeast is further facilitated by the high rate of endogenous homologous recombination; thus genetic distances are long relative to physical distances. On average, there is 1 centiMorgan (1% crossovers over an interval) for each 3 kb of budding yeast DNA. Higher eukaryotes have vastly

Table 2 Results of a two-factor genetic cross

All PD	Allelic markers
4 T = 1 PD = 1 NPD	Unlinked markers
PD > T ⩾ NPD	Linked markers
PD = NPD ⩾ T	Unlinked markers, linked to CEN

less efficient meiotic recombination, with 1 centiMorgan being equivalent to hundreds or even thousands of kilobases.

Non-Mendelian segregation

Sometimes, a phenotype may not segregate 2:2 in a cross between a mutant and wild type. This can arise for a number of reasons, some because of second-site nuclear mutations and others because of cytoplasmic genetic factors. The latter class of factors is characterized by mitotic segregation and apparent 4:0 or 0:4 segregation. Analysis of *kar* mutants (karyogamy defects) tests for cytoplasmic inheritance. When *kar* mutants are crossed, the nuclei do not fuse. New buds randomly inherit a copy of one of the two haploid nuclei (so nuclear markers will segregate randomly in buds dissected from *kar* mutant zygotes), but cytoplasmic markers are inherited by all buds.

The mitochondrial organelle, descended from a bacterial progenitor, is inherited cytoplasmically. Unlike most eukaryotes, yeast respires facultatively; it can live entirely by fermentation, so it can still be propagated without functional mitochondria. These cells are called *petite* or *rho*$^-$. If such yeast is crossed to wild type with functional mitochondria, all the spores from a tetrad will be able to respire; the phenotype segregates 4:0. The cytoplasm is mixed between all four haploid nuclei, so all the spores get mitochondria. The mitochondrion has its own small DNA genome with a host of genes involved in respiration and protein synthesis. In a given yeast cell, there are a few dozen copies of this genome, but, when the cell buds, only one or two of these genomes are chosen to be replicated into the bud. Thus, a cross between a wild-type strain and a strain with a mutation in the mitochondrial DNA shows apparent 0:4 or 4:0 segregation, depending on which mitochondrial genome is replicated for segregation. The mitochondrial genomes within a yeast cell are able to recombine with each other, facilitating the genetic mapping of this cytoplasmic DNA.

There are a number of other non-Mendelian inherited factors, including prions (made entirely of protein), RNA viruses, and DNA plasmids (discussed further below). Also, organelles cannot be formed *de novo*, except for the peroxisome, so new buds must inherit each organelle, as well as the full chromosomal complement. Within the nucleus, on the chromosomes, there are also mobile genetic elements called Ty. These DNA sequences encode the genes to make duplicate copies of themselves at low frequency for insertion at other genomic locations through an RNA intermediate.

Some yeasts do not have a characterized sexual cycle. These include many wild species of *Saccharomyces* as well as the human pathogen responsible for yeast infections, *Candida albicans*. In such yeasts, the parasexual cycle can be exploited for genetic mapping. Artificially created diploids are unstable and divide with high rates of chromosome loss (aneuploidy) and mitotic recombination. Once enough divisions have ensued, the resulting haploids will be stable and recombinant with respect to their parents and can thus be analysed genetically. Use of the parasexual process is not as powerful as use of organisms with stable diploid phases and easily isolated meiotic products, but these methods do allow for genetic analysis of organisms that cannot be induced to undergo sexual differentiation.

Modern Genetic Analysis in Yeast

Overview of chromosomal elements

The development of powerful molecular tools for cloning and analysing genes in yeast has been coupled with isolation of the determinants required to repair broken DNA and segregate chromosomes during mitosis. The linear chromosomes of eukaryotes require at least three DNA elements to be transmitted with high fidelity. Origins of replication are required for initiating DNA synthesis; centromeres are necessary for the spindle apparatus to recognize and segregate chromosomes; telomeres are needed to maintain the ends of linear DNA molecules through S-phases. The isolation of these elements has enabled researchers to create powerful plasmids and artificial chromosomes for a variety of uses, as well as unlocking the mechanisms and genes involved in the replication and segregation of chromosomes.

The budding yeast naturally possesses a plasmid called the 2-micron circle. This plasmid encodes the DNA elements and protein coding genes required for its high copy maintenance and segregation but has little apparent function for the yeast cell itself. This DNA was isolated and fused with *Escherichia coli* plasmids (containing bacterial origins of replication and selectable markers) to yield shuttle vectors, able to be transformed into and propagated by both yeast and bacteria. 2-Micron-based vectors are useful expression plasmids and were the starting point for engineering extrachromosomal elements based on the endogenous chromosome maintenance machinery.

Many extrachromosomal elements have been developed: from stable to unstable, from high copy to low copy, able to accommodate a variety of insert sizes, and utilized for a variety of purposes. A yeast selectable marker, usually the wild-type allele of a metabolic gene (such as *URA3*), is nearly always included on engineered extrachromosomal elements. This vector is then transformed into an appropriate auxotrophic strain of yeast (such as *ura3Δ*), so that yeast stably transformed with plasmid can be selected on deficient media.

The minimal component that a circular DNA requires to be maintained in yeast is an origin of replication. The

125 bp *ARS* (autonomously replicated sequence), as the name implies, ensures that the eukaryotic cellular machinery will replicate the plasmid during S-phase. However, a plasmid with only an *ARS* will randomly segregate during cell division; the plasmid is lost at a high rate through cell division, even though it is faithfully replicated. Thus, in order to maintain an *ARS* plasmid in yeast, selection for the marker gene must be maintained, and the number of such plasmids present in each cell is highly variable.

The sequence in budding yeast specifying a centromere, called *CEN*, is unusually compact among eukaryotes. This short stretch of nucleotides is sufficient and necessary for ensuring accurate segregation of a DNA molecule during mitotic division. Thus a plasmid bearing *CEN* and *ARS* will be replicated and segregated at low copy through cell divisions. These plasmids are lost at a low rate, even without selection, and the number of copies per cell remains fairly constant.

Circular DNA can be fairly large, but to accommodate truly large inserts, yeast artificial chromosomes (YACs) were developed. The added complication of maintaining a linear DNA in a cell, like a chromosome, is that the ends will degrade slowly through cell divisions, owing to the asymmetry of DNA replication. Eukaryotes have repeated DNA structures called telomeres (or *TEL*) at chromosome ends and numerous *trans*-acting genes encoding the machinery for their maintenance. Thus, to clone and characterize yeast *TEL*, DNA fragments were found that, when attached to the free ends of linearized plasmid, could be stably transformed into yeast. This not only allowed researchers to discover the DNA sequence that encodes the telomere but enabled the construction of artificial chromosomes. Genomic YAC libraries are crucial for ordering large DNA fragments along chromosomes, so that YACs have been instrumental in genome projects for all organisms.

Cloning by complementation

Yeast plasmids are extremely useful for cloning genes. In order to clone the gene affected by a recessive mutation, a wild-type genomic library (a mixture of plasmid vectors bearing heterogeneous genomic inserts) is transformed into the mutant yeast to screen for a plasmid that complements the mutation (i.e. alleviates the mutant phenotype). Plasmids recovered from rescued yeast may contain the wild-type yeast gene. If the mutant allele of interest is dominant, a genomic library is prepared from the mutant and transformed into wild type to screen for a transformant with the mutant phenotype.

Plasmid DNA prepared from individual yeast transformants is subjected to sequence analysis to determine the genomic library sequence that complemented the phenotype. The cloned sequence may not be the gene originally mutated, however. For example, it could be a gene that suppresses the mutant phenotype when in high copy. The next step is to construct null alleles of the genes isolated on plasmids at their endogenous location (discussed below). By crossing these new strains to the original mutant, segregation analysis determines whether the original mutant and the cloned sequence are allelic.

Another technology, similar to a complementation test, developed in the yeast is used to analyse protein–protein interactions. The yeast two-hybrid assay utilizes multiple plasmids bearing genes for fusion proteins, which will activate a reporter gene if the fusion proteins (bait and prey) physically interact. This assay has found general application in the cloning of genes that interact with a protein of interest from any organism.

Homologous recombination and integrative transformation

As already mentioned, the budding yeast has a high rate of homologous recombination to repair DNA double-strand breaks. This not only facilitates genetic mapping but also allows researchers to precisely engineer yeast strains by targeted integrative transformation. Free DNA ends are not well-tolerated in yeast, except for *TEL* sequences. Other free ends undergo homologous recombination. If an insert in a plasmid vector is cut and transformed into yeast, the two free ends will invade and recombine with endogenous sequence homology and the entire construct will be integrated into the chromosomal site (**Figure 3a,b**).

Integrative vectors have been developed that can be propagated in bacteria and possess yeast selectable markers, but no *CEN* or *TEL*. Such a vector can be linearized with restriction enzymes and used to test a gene cloned by plasmid complementation (as discussed above). Integration of the vector into the clone's endogenous site generates a marked null allele. When crossed to the original mutant, the marker and mutant phenotypes will segregate 2:2 if the cloned plasmid did indeed bear the wild-type allele of the original mutant. The phenotype of the yeast with the null allele determines whether the original mutant was a loss-of-function allele or not.

Cloning by gap repair

Another way in which yeast researchers have exploited homologous recombination is to clone the sequences of many different alleles of the same gene with gapped plasmid constructs, bearing homology to the flanks of the target gene (**Figure 3c**). The gap is repaired by gene conversion from the chromosomal DNA. Only recircularized plasmids can be stably maintained through replication, so plasmids that were repaired can be isolated and the gap-repaired region can be sequenced to determine the specific lesions in a set of alleles from a complementation

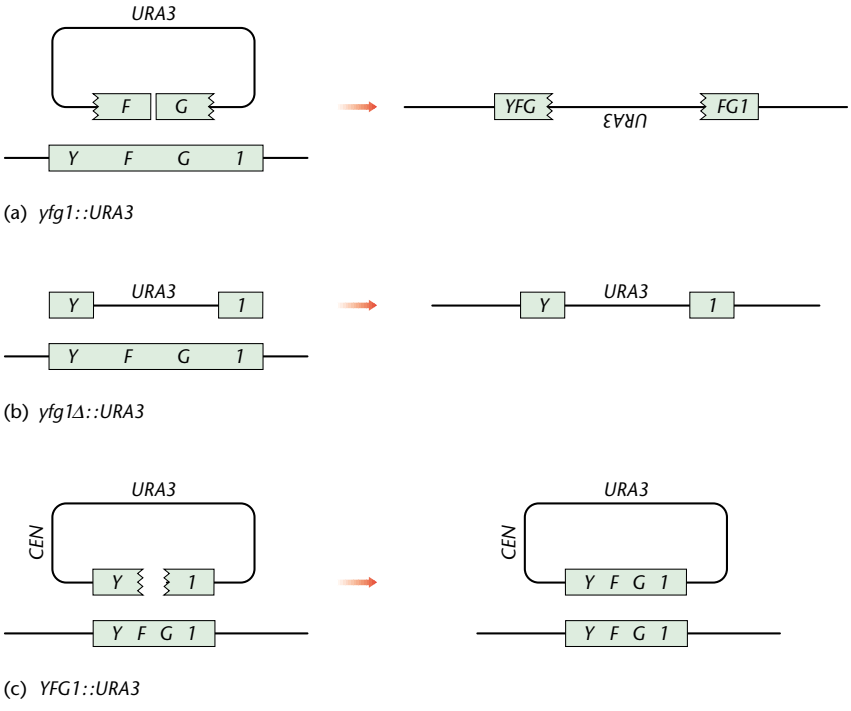

Figure 3 Schematic of targeted transformation by homologous recombination. (a) Plasmid integration. (b) Allele replacement. (c) Plasmid gap repair by homologous recombination.

group. Gap repair has been instrumental in and was discovered while defining the mechanism of homologous recombination.

Yeast Resources

The long history of yeast research has led to an enormous set of resources that are available to the scientific community. The genome is completely sequenced; a complete set of nonessential gene knockouts has been constructed; open reading frame (ORF) and genomic arrays are cheaply available; and robust and detailed databases on the budding yeast have been developed and are available online. These resources and the massive amount of accumulated data on the budding yeast have made it a central component of modern biological research.

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