The new yeast genetics

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Gene cloning and yeast DNA transformation techniques have greatly enhanced the power of classical yeast genetics. It is now possible to isolate any classically defined gene, to alter the yeast genome at will by replacing normal chromosomal sequences with mutated derivatives produced in vitro, *and to create DNA molecules that behave as autonomous replicons or minichromosomes. These unique features of the new yeast genetics have been used to study many problems in eukaryotic molecular biology.*

IN the past seven years, the genetics of the yeast *Saccharomyces cerevisiae* has been transformed from a classical field to a molecular one. The distinction does not concern the rationale for using genetic analysis to study biological problems, but rather procedural matters which nevertheless have profoundly influenced our knowledge of yeast cells. The manipulations of classical genetics involve the intact organisms. Mutations are identified by selecting for organisms having new properties and then characterized by analysing the progeny of sexual matings. In contrast, the distinguishing feature of the new yeast genetics is that the manipulations are performed directly on the genetic material itself. A region of the yeast genome is isolated by molecular cloning and mutations are generated by enzymatic or chemical treatment of the DNA. The phenotypic consequences of these mutations are then assessed on introduction of the mutant DNAs back into yeast cells.

The revolution in yeast genetics quickly followed the advent of recombinant DNA technology. Classically defined yeast genes were isolated as cloned DNA by virtue of their ability to be functionally expressed in *Escherichia coli* such that they complemented defects in a specific bacterial gene[1–3]. Then, such cloned DNA segments were introduced into yeast cells, where they caused heritable changes; specifically they complemented defects in the analogous yeast genes[4]. The combined effect of these experiments and subsequent developments is the ability to manipulate the yeast genome by using artificial vectors of genetic information, previously impossible due to the lack of naturally occurring viruses that infect yeast. Of particular importance is the fact that cloned DNAs can be introduced exactly at their normal chromosomal locations or as part of artificially constructed chromosomes that behave strikingly like real ones. These unique features make the sophistication of yeast genetics considerably greater than that of other eukaryotic organisms, and in fact, the equal of prokaryotic organisms such as *E. coli*. Indeed, almost all the techniques of the new yeast genetics are analogous to those that have been used in *E. coli* for many years.

It is important to stress that although yeast genetics has been 'prokaryoticized', the organism itself is a eukaryote. The genetic material is confined to the nucleus in the form of chromatin, and it is organized on many chromosomes, each of which possess a centromere, two telomeres, and multiple sites of DNA replication. The transcription and translation processes have characteristic eukaryotic features (for example, three RNA polymerases, 5′ capped-3′ poly(A) mRNA which sometimes arises from spliced initial transcripts, no operons, translation beginning from the first AUG codon). Yeast cells contain distinctly eukaryotic proteins (histones, actin, tubulin, peptide hormones) as well as macromolecular assemblies or organelles that resemble those of higher eukaryotes, for example a nuclear membrane, mitochondria, 80S ribosomes, rough endoplasmic reticulum, Golgi apparatus and lysosomes. The biology of yeast involves the fusion of haploid germ cells of opposite mating type to form diploid zygotes that, in appropriate conditions, undergo a fairly typical meiosis. It is a particular advantage that both haploid and diploid cells can be propagated vegetatively in defined medium. As such, they represent a form of cell culture in which the karyotype and other characteristics are maintained through many generations with sufficient fidelity to reproduce sexually. Thus, yeast is useful for studying many basic questions in eukaryotic biology, including gene expression, DNA replication, recombination, transposition, chromosome segregation, chromatin structure, secretion, the cell cycle, and the control of cell type (for general reviews see refs 5–12).

Here I shall first describe the principles of yeast molecular genetics with particular emphasis on those aspects that offer unique approaches to biological problems; then I will discuss how the methodology has contributed to our present knowledge of genes and chromosomes. Given the wide range of subjects being studied and the increasing population of yeast workers (since 1975, it has doubled approximately every 2 years), it is obviously impossible to review all recent developments.

Transformation and its genetic consequences

The transformation event is defined operationally as the selected and genotypic change of a yeast cell dependent on a particular DNA molecule. Experimentally, DNA containing a wild-type marker gene (M^+) is introduced into a strain that harbours a mutant copy (m^-) and M^+ transformants are selected from the original population. Typically, the m^- strain lacks an enzyme necessary for growth in certain conditions (for example, in medium lacking a particular amino acid), and the M^+ DNA encodes the enzyme. In essentially all experiments, M^+ transforming DNA molecules also contain sequences that permit replication in *E. coli* cells (as plasmids or phages) as well as genetic markers that allow for selection in *E. coli*. For reasons of technical ease, the DNA is usually isolated from *E. coli* cells.

In the standard transformation protocol[4], spheroplasts from an m^- strain are prepared by enzymatically digesting the outer cell wall. These spheroplasts are incubated with M^+ DNA in the presence of polyethylene glycol and calcium chloride, then plated in selective conditions that also allow regeneration of the cell wall.

What happens to DNA molecules on introduction into yeast cells? The answer depends on the particular DNA sequences present on the transforming DNA (Fig. 1). Different modes of yeast transformation have been described[4,13–16]; their properties are listed in Table 1 and are summarized below. Taken together, they constitute the basis for the new genetics.

Although most DNAs are unable to replicate autonomously in yeast cells, molecules containing segments of yeast DNA can be propagated if they recombine with homologous sequences in the host genome[4,14]. A molecule containing two separate segments of yeast DNA can integrate at either genomic site. The transformants usually contain one copy of the transforming DNA per cell although multiple copies can integrate in tandem.

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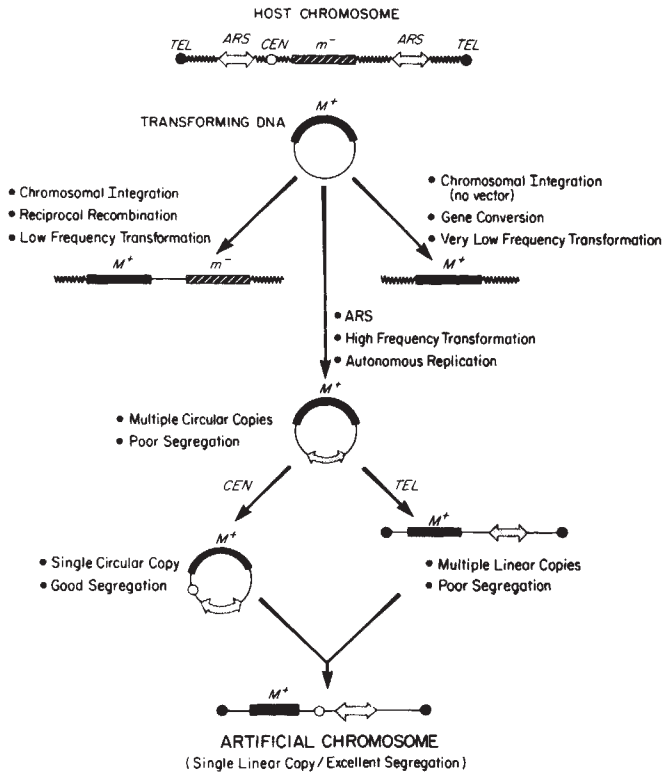

Fig. 1 Modes of yeast transformation/construction of artificial chromosomes. The top line represents a typical host chromosome with the following genetic elements: telomere (*TEL*, ●); centromere (*CEN*, ○); multiple replication origins (*ARS*, double-headed arrow); mutant sequences corresponding to the vector gene (m^-, striped bar); and other sequences (wavy line). The circular transforming DNA beneath the host chromosome consists of an M^+ marker (solid bar) and vector sequences (solid line). When introduced into yeast cells, this molecule integrates into the chromosome either by reciprocal recombination or by gene conversion (only the chromosomal region near the *m* locus is shown). Addition of cloned *ARS*, *CEN* or *TEL* elements produces molecules with various properties; when combined, they result in an artificially created chromosome (bottom of figure). See text for details.

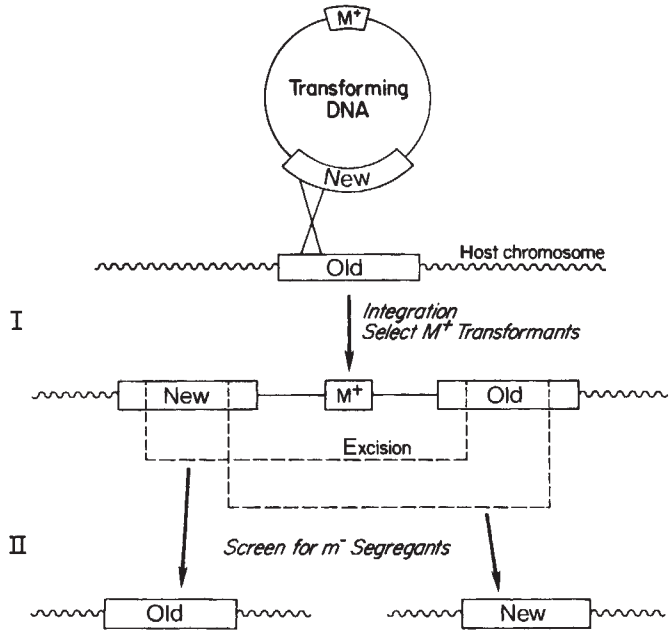

Fig. 2 Gene replacement is accomplished in two steps: integration then excision. The top part of the figure shows an homologous recombination event between a circular transforming DNA molecule and the normal linear chromosome (wavy line). The transforming DNA contains the M^+ vector marker and a 'new' cloned allele, while the original chromosome contains the 'old' allele. Integration results in a chromosome containing both alleles separated by the M^+ vector (middle of figure). The desired integration event can be obtained in several ways. Integrating vectors are generally used although these are inefficient because of their low transformation frequency and because integration can also occur at the normal chromosomal site of the M^+ marker[30]. As described in the text, appropriate cleavage of the transforming DNA increases this transformation frequency and directs the site of integration[17,107]; however, problems can arise because of multiple integration events. Alternatively, replicating vectors can be used to increase the transformation frequency and integration events identified by selecting for mitotically stable colonies[19]. The lower part of the figure shows the excision step. Two classes of segregants are obtained depending on whether the cross-over occurs to the right or to the left of the cloned mutant allele. m^- Segregants are generally identified by replica plating following growth in non-selective medium[30]. The low frequency of excision (see Table 1) can be improved by selectively killing the M^+ population by inositol-less death[120]. The two classes of segregants can be distinguished by virtue of phenotype or DNA structure. A method that allows for the direct selection of gene replacement events has been described recently[34]. Using standard methods in a *cyh2r* (cycloheximide-resistant) strain, the wild-type *CYH2* gene[121] was placed in the middle of the *his3* gene at the normal *his3* locus. The resulting strain is sensitive to cycloheximide because drug sensitivity (*CHY2*^{2}) is dominant to resistance (*cyh2r*). Thus, gene replacement at the *his3* locus can be achieved by selecting for segregants that are resistant to cycloheximide.

Generally, the integrated DNA is transmitted for many generations; occasionally, however, it is excised by homologous recombination (Fig. 2). In some cases, transformants do not contain any copies of the transforming DNA[4]; that is, the original m^- allele is replaced by the M^+ copy from the transforming DNA. These are formally equivalent to two recombination events within the homologous sequences, but they probably arise by gene conversion. Because mitotic recombination is a relatively rare event, transformation via chromosomal integration occurs at low frequency (~1–10 colonies per microgram of transforming DNA).

Certain DNA sequences allow molecules to replicate autonomously in yeast cells and consequently to transform yeast at high frequency (1,000 to 10,000 colonies per microgram of DNA). These autonomously replicating sequences (*ars*) are derived from the endogenous yeast plasmid[13,14,17] or from yeast chromosomal DNA[14,18,19]. While *ars*-containing molecules replicate efficiently, they do not segregate properly during mitosis or meiosis. Although they are present on average in multiple copies (from 3 to 30 per cell depending on the molecule), many cells do not have any[14,19]. However, autonomously replicating DNA molecules can be faithfully transmitted from mother to daughter cells if they contain yeast centromeric sequences (*CEN*) in addition to *ars* elements[15,20]. Furthermore, such molecules are stably maintained at one copy per cell and they usually segregate properly during meiosis. Thus, they behave as circular minichromosomes.

When linear molecules are introduced into yeast cells, they either recombine with the genome or circularize and subsequently replicate. However, molecules can replicate in the linear form if, in addition to an *ars* element, they possess specialized end sequences such as those normally found at the ends of the linear *Tetrahymena* ribosomal DNA palindrome or at the telomeres of yeast chromosomes[16].

Isolation of structural genes

The ability of yeast replicating vectors to transform both yeast and *E. coli* at high frequency and therefore to be readily interchanged between these two organisms makes it possible to clone essentially any gene for which phenotypically distinct mutations

Table 1 Properties of yeast transformation

	Chromosomal integration	Gene conversion	Episomal replicator	Chromosomal replicator	Mini-chromosome	Linear DNAs
Vector	I	I	E	R	C	L
Transformation frequency	10	1	10,000	10,000	10,000	10,000
Autonomous replication	None	None	Circular	Circular	Circular	Linear
Copies per cell	1	1	5–40	3–30	1	5–30
Vector sequences	Yes	No	Yes	Yes	Yes	Yes
Integration frequency	1	1	Variable	10^{-5}	10^{-7}	NT
Required elements	Yeast DNA	Yeast DNA	2μ *ARS*	*ARS*	*ARS* *CEN*	*ARS* *TEL*
Mitotic loss	0.1%	0	30%	30%	1%	30%
Meiotic loss	1–10%	0	90%	90%	30%	90%

Each column represents a particular mode of yeast transformation. To use their distinct properties, yeast shuttle vectors were developed; for simplicity they are categorized as I, E, R. C and L. It should be noted that these distinctions, being somewhat arbitrary, break down in complex situations. The transformation frequency is measured in colonies per μg of transforming DNA. In cases involving high rates of mitotic loss, the number of copies per cell represents an average. Except for I vectors, the integration frequency is calculated as events per generation; for I vectors, the frequency of 1 indicates that all transformation events require integration. (Chromosomal integration of E vectors is discussed in refs 14, 94 and 95.) Mitotic loss is measured on a per generation basis. Meiotic loss is determined by tetrad analysis and thus the values also reflect mitotic instability. *CEN*, centromeric DNA; *TEL*, telomeric DNA; *NT*, not tested.

are available. In other eukaryotic organisms, it is usually difficult to isolate a gene by virtue of its mutant phenotypes, and many interesting genes are defined solely in this manner. It has been estimated[21] that over 100 previously defined yeast genes have been isolated by these methods (some early examples may be found in refs 14, 22–27).

The essence of the method is that defects caused by particular mutations can be 'cured' by introducing the cloned wild-type gene. Such genetic complementation is applicable for recessive mutations, by far the majority class. In practice, DNA segments representing the entire wild-type yeast genome are cloned into yeast replicating vectors, introduced into *E. coli*, and propagated *en masse*. The resulting hybrid DNA molecules are isolated and then introduced into an appropriate yeast strain—for example, one that fails to grow at high temperature due to a defective protein. Simultaneous selection for transformants that contain the vector marker and that grow at high temperature presumptively identifies the gene of interest. For more subtle phenotypes, transformed cells are first selected by virtue of the vector marker and then screened by whatever method serves to distinguish the phenotype of the recessive allele from its wild-type counterpart. Having obtained the desired complementation event, DNA is prepared from the relevant yeast transformant and introduced back into *E. coli*. The specific hybrid DNA when re-isolated from *E. coli* should complement the recessive mutation and hence should contain the cloned gene of interest. Given a reasonably complete collection of hybrid molecules, the entire procedure requires ~1–10 μg of transforming DNA and takes about 2 or 3 weeks.

It is important to note that isolating DNA sequences by this procedure does not constitute proof that the desired gene has been cloned. Such proof may be established either by demonstrating that the identical DNA fragment from a mutant strain (isolated by nucleic acid hybridization to the presumptive cloned gene) is unable to complement the recessive mutation[3], or by introducing the cloned DNA segment into the genome by homologous recombination and genetically mapping the integrated copy[4].

To isolate the gene corresponding to a dominant mutation, the procedure is reversed. The hybrid pool is constructed by using genomic DNA from the strain containing the dominant mutation, and it is then introduced into a strain that contains the wild-type version. Transformants receiving the dominant allele are distinguished from the original host.

In some cases, genes can be cloned by indirect complementation (suppression). For example, the prediction that overproduction of the α mating type pheromone could suppress the phenotype of mutations in another gene (*bar*1) was used successfully to isolate the pheromone gene on a multicopy replicating vector[28].

Gene replacement

A critical feature of the new genetics is the ability to replace normal yeast chromosonal sequences with cloned DNAs. Given the awesome array of enzymatic and chemical tricks for mutating DNA, the yeast genome can be altered at will.

The gene replacement procedure (sometimes called transplacement) is analogous to an old *E. coli* technique, homogenotization[29], in that it depends on homologous recombination between transforming DNA and the host genome[4,14]. A simple scheme, taken from the first example[30], is shown in Fig. 2. An M^+ integrating vector containing a new cloned allele is integrated into the genome of an m^- strain at the chromosomal site specified by the original allele. In the relevant transformant, the new (transforming) and the old (host) alleles are separated by the M^+ vector sequence. Then, strains in which the integrated DNA has been excised are identified by screening for m^- segregants. Two classes of segregants are observed, both of which lack all vector sequences. One contains the original allele, while the other, more interesting, class represents replacement of the original host allele by the cloned one.

This two-step procedure is generally applicable, because its only requirements are that homologous recombination can occur on both sides of the lesion in the transforming DNA and that the transforming allele can be distinguished from the host allele in some manner. Because the desired recombination events are selected by unrelated genetic markers, these genomic changes can be made even if they produce subtle or no discernible phenotypes. Furthermore, by using diploid strains, it is possible to perform gene replacement on one chromosome while the essential function is supplied by the wild-type version on the homologue. By observing the properties of haploid cells that arise from such diploid strains, on meiosis, it has been proven that histones, β-tubulin and actin are essential for viability, and temperature-sensitive (*ts*) alleles have been created for some of them (refs 31–33, and subsequent work). A recent modification of this method facilitates gene replacement[34]: instead of screening for rare segregants and then distinguishing between the two classes, a scheme has been devised that allows for the direct and independent selection of gene replacement events at a given locus (see Fig. 2 legend).

In comparing the molecular genetics with the classical variety, two additional points are worth noting. First, mutagenesis of DNA *in vitro* confines the sequence alteration to a small target. Previously, many mutations have been induced by harsh treatments such as X rays, UV light or chemicals. Because the entire organism is subjected to these mutagens, the strains that arise frequently contain multiple mutations. To isolate a desired mutation away from the others requires many backcrosses, a tedious exercise, and in addition it is difficult to know whether such

secondary mutations are present. Second, strain constructions can be performed without mating. As a result, it is easy to generate sets of isogeneic strains that differ only at defined loci. This is in marked contrast to progeny derived from sexual crosses which have 50% of each parental genome. Also, constructions involving strains having mating or sporulation defects or with non-mendelian factors (the endogenous yeast plasmid, mitochondria or killer RNAs) do not constitute a problem.

Gene regulation

Although prokaryotic promoter and regulatory sequences were defined with classically isolated mutations, similar attempts in yeast have proved unsuccessful. In contrast, analyses of mutations produced by *in vitro* methods have enhanced our understanding considerably.

Given that yeast genes, unlike many *E. coli* counterparts, are not clustered in single transcription units (operons), it is difficult to distinguish undesired mutations in the structural gene from those affecting either its expression or regulation. The vast majority of mutations map in the structural gene simply because it is much larger than the control region. However, by *in vitro* manipulations, it is easy to alter DNA sequences in a highly localized manner. Another consideration that exacerbates the problem is that point mutations (by far the predominant class produced *in vivo*) frequently eliminate protein function, whereas their effects on promoter efficiency are less marked; thus many mutations 'escape the genetic screen'. On the other hand, the recent advances have depended on deletion, substitution and insertion mutations, all of which have more drastic effects on gene expression.

Furthermore, *in vitro* methods lend themselves to systematic changes in DNA sequence, in marked contrast to *in vivo* methods which rely on providence. Because test tube mutations can be obtained without regard to phenotype, mutations can be created without the inherent bias of a particular genetic selection or screen. Of particular benefit is the ability to produce mutations that behave indistinguishably from the wild-type gene. These are invaluable for determining which parts of the genetic material are not important for a given function; such information is precluded by classical genetic approaches. When analysed in conjunction with mutations that do cause functional changes, a much clearer view of a eukaryotic control region emerges.

Having obtained a set of *in vitro*-generated mutations, the next step is to assay their phenotype. The principle here is similar to experiments in which mutant DNAs are assessed following microinjection into frog oocytes or following transformation into mammalian cells. The difference between the new yeast genetics and these other approaches is that the DNAs are introduced back into the intact organism from which they were derived. Moreover, as DNAs are reintroduced in defined ways with respect to copy number and chromosomal location, the end results of these manipulations are equivalent to real genetics. These properties have some important consequences in studies of gene regulation. First, mutant genes are assayed in conditions in which all normal forms of regulation are occurring. Second, by using appropriate yeast strains, it is possible to determine how individual proteins (which are altered or removed by mutation) influence the expression of a given gene. Third, the absolute levels of products specified by mutant genes are directly comparable with their wild-type counterparts and with the levels of other genes. In other systems, levels of gene expression can be determined only relative to an arbitrarily defined DNA molecule.

Given the versatility of yeast transformation, mutant phenotypes can be analysed in several ways. The gene replacement procedure discussed previously constitutes the best possible *in vivo* assay for mutations produced *in vitro* and it has been used for studies of the histidine biosynthetic genes *his3* and *his4* (refs 35–37). Another approach—that used for the cytochrome *c* (*cyc1*)[38,39], alcohol dehydrogenase (*adr1*)[40], and mating type genes[41]—is to clone mutant DNAs into a yeast replicating vector and then select for transformants by using the vector marker. Once again, the phenotype is determined independently of the transformation event itself. Although the autonomously replicating molecules are not identical to real yeast chromosomes, this procedure has several advantages compared with gene replacement. It is considerably simpler than gene replacement because: (1) replicating vectors transform yeast cells at high frequency; (2) the desired strains are obtained in a single step; and (3) complications due to recombination with the host genome are avoided. Furthermore, with the appropriate vector choice, it is possible to introduce the mutated derivatives in multiple or single copies per cell. Multiple-copy vectors allow for gene dosage studies that are difficult to perform by classical genetics. In either case, the resulting strains contain both the cloned mutant allele and the original host allele. By altering the host allele such that its gene product is distinguishable from the incoming allele (say, by a small deletion internally within the structural gene), such strains provide an excellent basis for *cis–trans* tests, analogous to *E. coli* strains containing F′ factors or specialized transducing phages[42].

Gene fusion, an approach used for nearly 20 yr to study prokaryotic gene regulation[43], has been adapted to yeast cells. Generally, the regulatory region under study is fused to a structural gene which can be assayed easily for genetic and biochemical function, for example the *E. coli* β-galactosidase or the herpes thymidine kinase genes[44–46]. More importantly, complicated regulatory circuits can be dissected into individual components because the gene product that is assayed does not affect the regulation being analysed. For example, fusions of the (*cyc1*) regulatory region to the β-galactosidase structural sequences indicate that haem has an important role in gene control[39]. By standard methods, this would have been difficult to elucidate because haem is also necessary for cytochrome *c* activity and presumably for its intracellular stability.

Even with the differences in approaches and genes, several general conclusions have emerged. A feature of yeast RNA polymerase II promoters is that DNA sequences located relatively far from the site of transcriptional initiation are critical for function[35,37–40]. The actual distance ranges between 120 and 350 base pairs (bp) depending on the gene. Yeast promoters, like those of higher eukaryotes, also contain TATA box sequences and several studies have indicated their importance in gene expression[35,38–40,47]. Further analysis suggests that for a given gene, the upstream promoter element can be moved with respect to the TATA box and mRNA start point with little phenotypic consequence[39,47]. These properties differ radically from those of a prokaryotic promoter (reviewed in refs 48, 49), but they resemble those of viral enhancer sequences[50–52]. That the upstream sequences confer their effects over a long and variable distance has suggested to many that this may involve chromatin structure. The gene replacement procedure has been used to examine the chromatin structure of *his3* mutant genes in their normal chromosomal location; the upstream element is necessary for preferential cleavage of the TATA box by micrococcal nuclease and the effect is not due simply to transcription of the gene[53].

In regard to proper control of expression in response to specific physiological cues, many genes have been shown to be regulated at the level of transcription, and several regulatory sites have been defined. Mutant studies of *his3* and *his4* have identified a particular sequence that is essential for proper regulation, but which is not critical for expression *per se*[36,37]. This short sequence is repeated several times at the 5′ ends of these and other genes controlled by the state of amino acid biosynthesis, for example *trp5* and *his1*[54,55]. For genes controlled by the source of carbon in the medium (*adr1*, *cyc1*, *gal7*), the regulatory sites differ in sequence. However, all are located far upstream from the structural gene and they are critical for expression in addition to regulation[38,40,56]. Chromatin studies of the *adr1* regulatory site indicate that the structure, as tested by DNaseI hypersensitivity, correlates with its function[57]. By all these criteria, these regulatory sites resemble enhancer sequences which are effective only in certain cell lines[58]. Regula-

tion of the mating type genes occurs by a completely different mechanism. Classical genetic experiments strongly suggested that the expressed copy at the mating type locus was similar to the silent copies located elsewhere[7]. Analysis of the cloned genes[59,60] indicates that they are identical, thus leading to the idea that an unusual, long-range position effect is involved in regulating gene expression. Deletion analysis defines two regulatory sequences, each located ~1,000 bp past the 3′ end of the mating type genes and only at the chromosomal sites specified by the silent copies[41]. These sites, in combination with regulatory genes defined previously[61–63], affect chromatin structure in terms of nuclease sensitivity and supercoiling such that gene expression from the silent copies is repressed[41,64].

In addition to regulatory sites, the control of gene expression also involves special regulatory proteins, most of which have been identified by classically isolated mutations. By the methods described earlier, genes encoding these presumptive regulatory proteins have been cloned, thus permitting new genetic studies[59,60,65–68]. Of more importance, the cloned genes should prove invaluable in identifying, possibly overproducing, and hopefully purifying the gene products. Some of these, when overproduced, may be detrimental for cell growth. A potential way to avoid this problem is to fuse the structural gene of interest to an easily manipulated regulatory region, for example that specifying galactose control[56]. Thus, by using unrelated physiological means (growth on galactose in this case), synthesis of a desired gene product could be induced just before collecting the cells. Thus, in principle, it should be possible in the future to correlate biochemical and genetic functions of regulatory proteins and their target sites.

A recent application of the new genetics has led to intriguing results concerning the splicing of intervening sequences from mRNA precursors. Using native genes or fusions in which β-galactosidase activity depends on accurate splicing, a conserved sequence has been found within the excised region which appears necessary for the processing reaction[69,70]. The key sequence resembles that of U1 RNA, a small nuclear transcript found in higher eukaryotes that is believed to be important in splicing[71]. Thus, the proposed structure between U1 RNA and the splice junction in the precursor[72,73] may be achieved in yeast by an intramolecular equivalent.

Chromosomes

Eukaryotic genomes encode other essential genetic information besides structural genes. Yeast transformation provides functional assays for isolating some of these elements. Presumptive chromosomal DNA replication origins allow hybrid molecules to transform yeast at high frequency, functional centromeres cause replicating molecules to segregate properly through mitosis and meiosis, and telomeres permit the replication of linear DNAs. Therefore, these previously elusive genetic elements can now be treated just like structural genes.

DNA replication: The issue of whether eukaryotic chromosomes contain specific origins of DNA replication has provoked interest and controversy[74,75]. The ability of *ars* elements to permit autonomous replication of hybrid molecules strongly suggests that they serve as chromosomal origins; several observations support this view. The number of *ars* elements calculated by genetic experiments[76,77] agrees well with the number of replication units as determined by fibre autoradiography[78]. Regions of the yeast genome encoding a given *ars* element are replicated at characteristic times during the S phase of the cell cycle, and this specificity is maintained on autonomously replicating molecules[79]. Finally, *in vitro* replication systems initiate DNA synthesis at *ars* elements[80–82], and these have been used to purify the protein specified by the *cdc8* gene[83,84].

ars elements contain conserved features within an extremely A+T-rich region[85]. Their precise organization throughout the genome is not critical for viability as they may be inserted in new locations or even deleted from their normal one[19]. In two cases, it seems that these presumptive chromosomal origins are used to regulate certain genes. The histone H2b gene is normally transcribed at a particular time during the cell cycle[86]. By deletion analysis of appropriate gene fusions, the sequence responsible for this control maps far past the 3′ end of the gene and coincides with an *ars* element[87]. In the second case, the regulatory sites that control the position effect on the mating-type genes (see previous section) both coincide with *ars* elements[41,85]. Such observations suggest that regulation of certain genes may be due to structural changes that arise in conditions when DNA synthesis is initiated from the relevant *ars* element.

Yeast transformation techniques have been invaluable for studies on the endogenous yeast plasmid because the naturally occurring molecule confers no detectable phenotype on its host. The plasmid replicon may be analogous to chromosomal ones in that the same genes are important for replication[88] and because each plasmid DNA molecule duplicates only once per cell cycle[89]. By using various hybrid constructs[90], the yeast plasmid *ars* element has been mapped to the origin of DNA replication determined by electron microscopic techniques[91], and genes responsible for copy number control and site-specific recombination within the plasmid have been identified[90,92]. Although the yeast plasmid does not normally become integrated into the genome[93], hybrid molecules containing chromosomal sequences may do so by homologous recombination[14,94,95]. Because the resultant chromosomes are frequently lost entirely, it is possible by using appropriately marked diploid strains to determine which chromosome contains the plasmid sequences[96]. Thus, yeast DNA segments cloned in plasmid vectors can be mapped independently of their phenotype. In fact, many genes are more easily mapped by cloning them and using this chromosome loss method than they are by classical tetrad analysis.

Chromosome segregation: It has long been assumed that centromeres and telomeres have critical roles in ensuring that each daughter cell receives the proper chromosomal complement. The properties of cloned centromeric and telomeric sequences provide direct evidence for some of these roles and they have been used to define the important functional aspects. Recently, artificial linear minichromosomes have been constructed by combining *ars* elements, centromeric and telomeric DNA sequences in the same molecule (ref. 97; see Fig. 2). Their stability, though impressive, is still considerably less than for real chromosomes; this may be due to their small size[97,98].

Centromeric sequences are probably the primary determinant of proper chromosome segregation during mitotic and meiotic growth because autonomously replicating molecules containing a single *CEN* sequence segregate properly[15,20]. However, when molecules containing two *CEN* sequences are introduced into yeast, the resulting colonies are heterogeneous for a mixed and continuously changing population of rearranged molecules[99]. Such behaviour is reminiscent of 'breakage/fusion/bridge' cycles in maize[100], and it may result from mechanical breakage of individual DNA molecules attempting to segregate simultaneously to opposite poles. Given that centromeres in higher organisms are cytologically visible, it is perhaps surprising that yeast *CEN* function is confined to a very small region of DNA (<500 bp). Centromeres III and XI each possess three distinct sequence elements that are positioned in an almost identical spatial arrangement within the functional region[101–103]. Centromeric sequences do not seem to be part of normal nucleosomal arrays *in vitro*[104]; it has been suggested that this results from their interaction with the spindle apparatus. In this regard, a protein fraction that binds specifically to centromeric DNA may prove informative[103].

Studies of telomeric sequences indicate that they are necessary and sufficient for propagating linear molecules, and that they occur at the ends of most if not all yeast chromosomes[16]. Formation of the hairpin structures at chromosome ends depends specifically on telomeric sequences; artifically constructed hairpins are not sufficient to constitute functional telomeres[105]. The fact that the ends of the *Tetrahymena* ribosomal DNA palindrome maintain their unusual gapped structure

at ends of replicating linear molecules in yeast suggests that telomere function is strongly conserved evolutionarily between kingdoms[105,106].

Preliminary studies using diploid strains containing two homologous, but genetically distinguishable minichromosomes have addressed a long-standing problem in genetics and physical chemistry—how do the duplex DNAs of homologous chromosomes pair during meiosis. It seems that circular and linear minichromosomes segregate preferentially to opposite poles, but that they segregate randomly with respect to the real chromosomes that contain the identical centromeres[15,97]. This suggests that pairing may result from homology *per se* and not from a centromere and/or telomere organizing structure. In a more direct test of this hypothesis, the centromere of chromosome III was replaced by the centromere of chromosome XI; this novel chromosome pairs only with the native third chromosome, and it is equally stable during mitosis[122].

Recombination: The frequency of transformation by non-replicating molecules can be increased dramatically by cleaving the DNA at sites that are homologous to yeast chromosomal DNA[15,107]. The DNA ends are highly recombinogenic, presumably because they represent an intermediate in the normal process of repairing double stranded breaks[107]. These events occur equally efficiently even when linear DNA substrates contain double-stranded gaps with respect to the native chromosome; furthermore, the gapped region on the transforming DNA is correctly repaired from the host copy. This reaction depends on expected recombination genes and it is associated with gene conversion and reciprocal recombination events near the site of the double-stranded break[107,108]. The results agree well with experiments that demonstrate the formation of double-stranded breaks during the transportation event that results in yeast mating type interconversion[109]. These considerations have led to the proposal that gene conversion in *S. cerevisiae* may be initiated by double-stranded breaks and subsequent DNA repair[110]. This model is an alternative to classical models that postulate invasion of duplex DNA by single-stranded DNA[111–113]. Distinguishing between these models will probably depend on biochemical experiments[110]; in this regard, a recently developed *in vitro* recombination system may prove useful[114].

Other experiments that take advantage of the ability to place homologous sequences at different chromosomal locations, have revealed additional features of gene conversion[115,116]. First, it has been shown that recombination can occur between homologous sequences even if they are located on separate chromosomes. In the examples involving gene conversion between different copies of the yeast transposable element Ty*1*, gene expression is frequently affected (ref. 116; for a recent review of the properties of this transposable element see ref. 11). This property has been used to generate specific reciprocal translocations[117]. Second, and more surprisingly, interchromosomal gene conversion occurs at the normal frequency even if the recombination breakpoints within homologous regions are separated by a large substitution of non-homologous DNA[115].

The fate of classical yeast genetics

The invention of the automobile quickly relegated transportation by horse and buggy to a sentimental pastime. However, a similar fate is unlikely to be in store for classical yeast genetics. The major reason for this is that the new methods, while providing powerful tools for analysing previously defined genes and DNA sequences, are less useful for probing the unknown. Before genes can be cloned and manipulated, they must be identified. With the rare exceptions of genes that are known by virtue of their gene products, most are initially defined by mutations that confer particular phenotypes. Such mutations are more likely to be uncovered by a broad mutagenesis and screening procedure. Moreover, it is certainly easier to 'produce phenotypes' by altering wild-type sequences than by cloning wild-type genes, and the initial characterization of phenotype is frequently the way in which new biological phenomena are discovered. For example, yeast mutants defective in various stages of secretion have been used to understand various biochemical aspects of the overall process[118,119]. It is hard to see how such mutations could have been found with the new yeast genetics. Another general approach for identifying new genes relies on mutations that suppress the phenotypes of other mutations. Suppression is frequently indicative of interaction (direct or indirect) between the gene products defined by the original and the suppressor mutation. The most revealing interactions are usually not specifically predictable, thus making the general search mode a more useful one. In addition, it should be noted that powerful genetic selections (capable of detecting 1 variant per 10^{11} cells) are available for yeast, and these are often required to isolate suppressor mutations.

Thus, the new yeast genetics does not simply replace the standard approach, but rather is integrated with it.

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ARTICLES

Structure of the cosmic microwave background

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We predict the structure expected in the cosmic microwave background over large angular scales in realistic anisotropic and inhomogeneous universes. In a homogeneous anisotropic universe we find that there exist two possibilities, a quadrupole or a hotspot pattern with and without dipole contributions. A hotspot allows a direct observational test of an open universe. We show how infinite wavelength inhomogeneous perturbations of the Friedman universe can be represented as ensembles of different homogeneous universes.

THE cosmic microwave background radiation has propagated towards us through space and time carrying with it a wealth of information about the geometrical structure of the Universe and the distribution of matter within it. Small deviations from the perfect homogeneity and isotropy of the idealized Friedman cosmological models will be manifested as an angular variation of the radiation temperature over the sky[1–3]. At present, one positive claim[4,5] and two strong upper limits[6,7] exist for the magnitude of any quadrupole component in the angular temperature distribution, $T(\theta)$. The upper limits correspond to $\Delta T/T \leqslant 10^{-4}$ over $>10°$ and are derived from 50% and 80% sky coverage respectively.

We aim to predict some general features of the universal microwave background radiation. We consider the temperature profiles obtained in representative anisotropic and inhomogeneous cosmological models and show that in general there can arise two distinct sky patterns.

We investigate the 'hotspot' phenomenon first noticed by Novikov[8] and examine its effect in inhomogeneous cosmological models. An observation of this effect offers the possibility of determining the total density of the Universe without the uncertainties of non-luminous matter, unknown elementary particles[9] or the calibration of the cosmic distance scale. We provide a physical interpretation of the hotspot and delineate the set of cosmological models in which it can and cannot occur. We also show how the sky pattern can be calculated for inhomogeneous universes by a particular superposition of sky maps for different homogeneous universes.

Homogeneous quadrupoles

The simplest, astronomically relevant cosmological models with non-isothermal microwave background temperatures are the homogeneous and anistropic universes. They are finite in number. Their spatial geometries are described by the classification of homogeneous spaces first performed by Bianchi (see ref. 10); the one exceptional homogeneous cosmology that does not fall within the Bianchi class is the Kantowski–Sachs closed universe[11–13].

The simplest of the Bianchi models is the type I axisymmetric case and the metric is[14] (general relativistic conventions follow ref. 15):

$$ds^2 = dt^2 - X^2(t)\{dx^2 + dy^2\} - Y^2(t)\,dz^2 \qquad (1)$$

This provides a concrete example of the typical quadrupole temperature pattern found also in Bianchi types VII_0, VIII and

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