biology. A young biologist embarking on a career at the present time could perhaps look forward to a lifetime of research in the mainstream of biology by choosing to study the evolution of the microbial cell.

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Yeast: An Experimental Organism for Modern Biology

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The yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* have become popular and successful model systems for understanding eukaryotic biology at the cellular and molecular levels. The reasons for this success are experimental tractability, especially in applying classical and molecular genetic methods to associate genes with proteins and functions within the cell.

THE IDEA THAT A REVOLUTION IS OCCURRING IN BIOLOGICAL research has already achieved the status of cliché. Nonetheless, it is true that much of what can now be done experimentally could only be dreamed of as recently as 15 years ago. The agencies of this revolution are a set of new experimental tools. Foremost among these tools is, of course, the basic "recombinant DNA technology" itself: the ability to isolate individual genes from any organism and to determine their nucleotide sequences, thereby providing the amino acid sequence of any protein product. This prime tool has spawned a large number of generally useful technologies including the use of the cloned gene analytically to study the pattern of normal expression or to follow inheritance of the gene or its neighbors on the chromosome, the use of the cloned gene to produce essentially unlimited quantities of protein for study and for use as reagents, and, not least, the use of cloned genes to produce useful therapeutic agents.

Recombinant DNA technology grew directly out of classical molecular genetics, a field that concentrated on studies of bacteria (especially *Escherichia coli*) and their bacteriophages. The bacterial systems provided not only the materials for recombinant DNA technology (such as plasmid and phage vectors, suitable hosts, and

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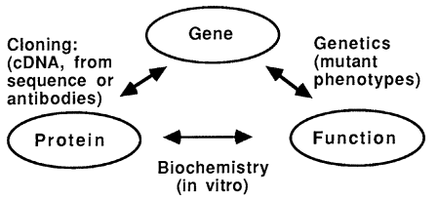

Fig. 1. Recombinant DNA technology allows yeast biologists readily to associate genes, the proteins they specify, and the biological functions the proteins perform. For given mutations, the gene can be cloned by complementation and the protein discovered by using the gene's DNA sequence. For a given protein, the gene can be cloned and the function of the protein analyzed by the production of mutations in vitro and replacement of the normal gene with these mutants.

Table 1. Degree of identity in amino acid sequence between corresponding proteins of yeast (*Saccharomyces cerevisiae*) and humans (*Homo sapiens*). The data were generated from sequences in Genbank and other published sources.

Yeast or human protein	Identity (%) in amino acid sequence	Reference
Ubiquitin	96	(*27*)
Actin	89	(*28*)
β-tubulin	75	(*29*)
HMGCoA reductase	66	(*30*)
Cytochrome c	63	(*31*)
Citrate synthetase	62	(*32*)
RAS1/N-ras; RAS2/K-ras	60	(*33, 34*)
Glucose transporter	25	(*35*)

expression systems) but also, and more important, the intellectual basis (including the very idea of plasmid and replication origin, the concept of promoter, and the notion as well as the identity of the signals for beginning and ending transcription and translation). Bacteria paved the way because they had many advantages: they grow rapidly; they are easy to manipulate both biochemically and genetically; and they share fundamental properties with all other organisms (including DNA genes, messenger RNA and ribosome-based protein synthesis, and metabolic economy based on adenosine triphosphate and nicotine-adenine dinucleotide). Moreover, they were excellent model systems for the invention and maturation of experimental designs and methods that proved also to be applicable to other systems.

There are, however, limitations to the application of both the facts and the methods of classical bacterial molecular genetics to eukaryotes. The biology of eukaryotic cells is significantly different from that of bacteria in fundamental ways, including the rules for transmission of genetic material, the type and function of subcellular organelles such as the mitochondria, and in basic aspects of metabolism and regulation. Even more obvious is the fact that multicellular eukaryotic organisms consist of many types of specialized cells. The connection between the DNA and the phenotype is therefore more complicated than it is in bacteria. The methods of bacterial molecular genetics, even in their recombinant DNA incarnation, are not easily applied to eukaryotic cells derived from multicellular organisms. Profound problems inherent in the differentiation process, slow growth, large DNA contents, and poorly tractable genetics all make it difficult to apply the paradigms of bacterial genetics to higher eukaryotes directly.

The solution to this problem, of course, has been the development of experimental systems based on eukaryotic microorganisms. The most highly developed of these is *Saccharomyces cerevisiae* (*1*), a free-living yeast with excellent classical genetics and a fast growth rate (roughly half as fast as that of *E. coli*). It is this yeast that is used to make bread and a variety of alcoholic beverages (including beer and wine) throughout the world. Another evolutionarily unrelated yeast, *Schizosaccharomyces pombe*, shares most of the experimental advantages (*2*) but has developed less rapidly as a model system. The two yeasts sometimes have different advantages: one might choose *S. pombe* for its relatively large chromosomes or *S. cerevisiae* for the fact that its mitochondrial genome is dispensable and thus genetically more tractable. Comparisons between the two are very useful, and many laboratories have taken to using both systems.

These yeasts have proved to be good model systems; they are experimentally tractable yet at the same time typical enough so that lessons learned in the model have a good likelihood of still being true in many other organisms. Being microorganisms, they share with bacteria the simplicity and rapidity of growth and the suitability for biochemical and genetic methods that allows application of the full range of molecular genetic technology. Being eukaryotes, they share with their multicellular cousins many fundamental properties of cell biology (such as cytoskeletal organization, subcellular organelles, secretion systems, receptor and second messenger arrangements, metabolic regulation, and chromosome mechanics). In the discussion that follows, we give some examples, often from our own work; these are meant as illustrations only. No attempt has been made to review the literature or to ascertain priority for any observations or ideas.

We think that the power of yeast molecular biology as a model for all eukaryotic biology derives from the facility with which the relation between gene structure and protein function can be established. As shown in Fig. 1, the application of the recombinant DNA technology allows one to associate a protein with its function in a number of ways, provided one can both clone a gene having only a mutation (that is, find a gene knowing only the mutant defect) and produce a mutation starting with the cloned gene (that is, determine the function by examining the consequence of the loss of a gene's activity). Two types of manipulation are involved: (i) the insertion of mutations made in vitro into the genome in their correct chromosomal context and (ii) the cloning, on a routine basis, of both the wild-type and mutant alleles of genes identified through mutations in vivo. Both of these kinds of manipulations are easily done in yeast because DNA introduced into yeast by transformation (*3*) behaves in a well-understood way.

Homologous Recombination

Yeast vector systems are of three generic types (*4*). When introduced into *S. cerevisiae* they allow the propagation of the cloned DNA in three different forms: as low-copy, autonomously replicating, stable, properly segregated plasmids [such vectors carry a yeast centromere (*5*) and are called YCp, for yeast centromere plasmid]; as high-copy, autonomously replicating, unstable, irregularly segregated plasmid [such vectors carry a replication origin from the yeast 2-μm plasmid (*6*) and are called YEp, for yeast episomal plasmid]; and finally as segments of DNA integrated by homologous recombination into the yeast genome (such vectors are called YIp, for yeast integrating plasmid). All the yeast vectors are also "shuttle vectors" that allow propagation and large-scale preparation of their DNA in *E. coli*.

The variety of options made possible by this array of vectors is surely a big advantage. It is now common for yeast workers to test the behavior of each new gene or mutation at both low copy and high copy. Informal standardization of vector design has made interchange of vector type (in vitro or in vivo) very easy. Quite frequently it turns out that overproduction of a normal gene or mutant allele has interesting consequences, even lethality (*7, 8*); in other cases many genes can suppress a mutation at high copy while only the correct gene does so at low copy (*9*).

It is the option of integration into the genome by homologous recombination that provides the yeast systems with their greatest advantage, for this is the feature that allows the movement of genes and mutations into and out of the yeast genome. The uses of integration begin with simple integration (*3*) of a YIp plasmid by homology in the cloned segment (Fig. 2a). Integration can be directed by the cleavage of the plasmid (*10*) and results in a duplication of the cloned gene and the concomitant addition of the vector sequences, including one or more selectable markers. The integration thus marks the locus of the cloned gene and is often used to map the locus. However, if the cloned copy contains a mutation, subsequent excision of the plasmid at a point other than the integration point will result in the placement of the mutation onto the genome (sometimes called "transplacement") in its correct position (*11*), lacking any vector sequences, just as if the mutation had been made in vivo. This kind of "perfect construction" is routinely possible only in yeast and bacteria. Yet this is the only way that one can truly test the consequences of mutations made in vitro.

Many generally useful variations of this use of integrating plasmids have been devised. Two of these allow the recovery of mutations made in vivo onto vector plasmids that can then be propagated in *E. coli*. By integrating a YIp plasmid carrying the wild-type gene into a strain bearing the mutant allele, one can construct a heterogenotic duplication (Fig. 2a). If one then uses a suitable restriction enzyme (shown by the arrows) that cuts only outside the duplication, one can recover the mutation by circularizing the cut DNA and selecting in *E. coli* for the vector marker (*12, 13*). An alternative method (Fig. 2b) is to introduce a linear plasmid bearing a deletion of the region of the mutation. Yeast repair systems use the homology to repair the gap, resulting in a plasmid that has a copy of the mutant allele and that again can be selected for in *E. coli* (*14*). This normal repair reaction is the basis for using cloned genes to carry out fine-structure mapping of mutations directly in yeast (*15*).

Finally, homologous recombination can be used to prepare null mutations (including insertions, deletions, and "integrative disruptions") in yeast. Several ways of doing this have been devised. One is simply introduction of a deletion allele by the method (*11*) of Fig. 2a. Another is a replacement strategy by which an insertion (with or without concomitant deletion) in the gene containing a selectable marker is constructed; this construction is then used to replace the normal gene by transformation with linear DNA (*16*) (Fig. 2c). The third method, integrative disruption (*17*), consists of the integration of a YIp vector carrying an internal fragment of the gene (*15*); this results, after homologous recombination, in a partial duplication that splits the gene into two inactive parts (Fig. 2d). All of these systems have variations useful in different contexts. All share the enormous advantage that one can examine a null phenotype for any cloned gene by a straightforward procedure.

The biology of yeast is particularly helpful here, allowing the recovery and detection of recessive lethal mutations. Introduction of the null mutation in a diploid strain allows subsequent meiosis to yield two normal progeny and two mutants (associated with vector markers). If these fail to grow, then the mutation is lethal. If they are viable they can be studied for any phenotype they might display. If null mutations are lethal, the function can be inferred from conditional-lethal mutations. Several procedures have been developed to

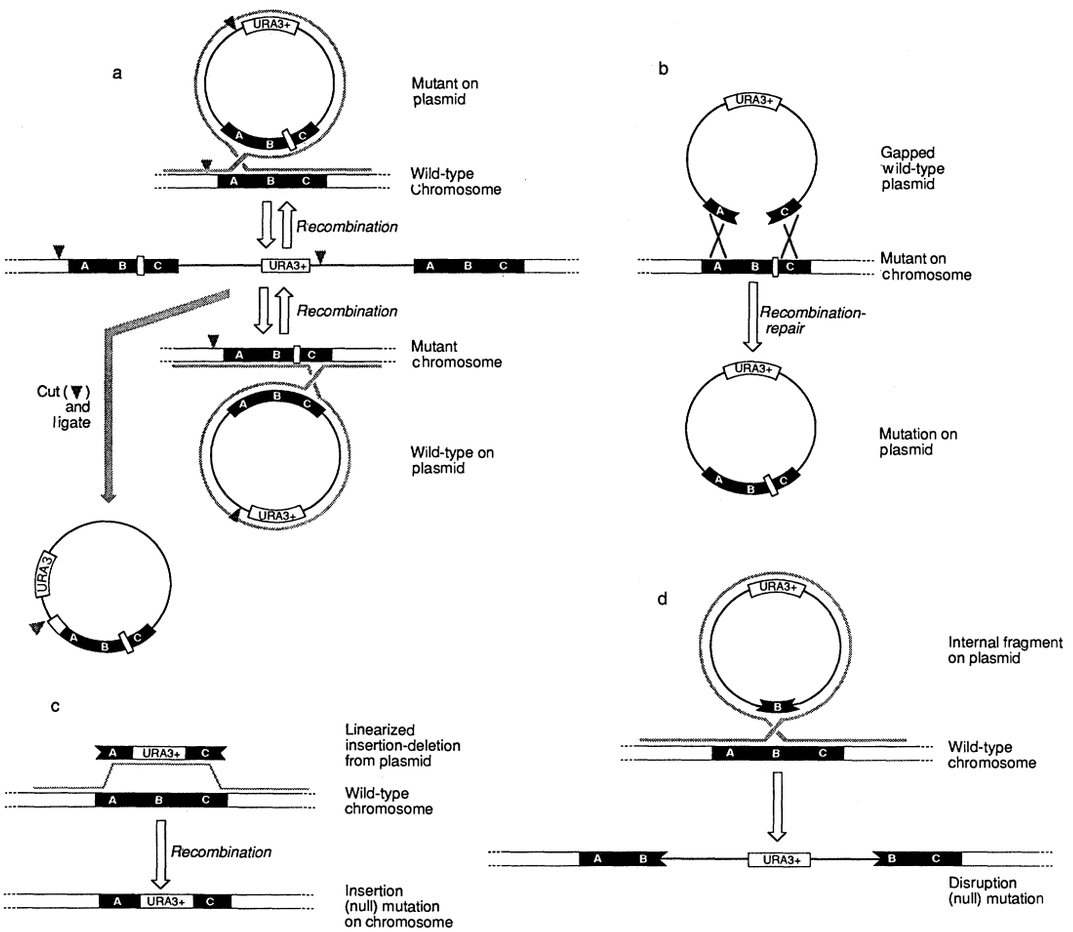

Fig. 2. The use of homologous recombination to transfer mutations into and out of the normal locus on a yeast chromosome. (**a**) Integration of a cloned gene (A-B-C) by homologous recombination into the mutant locus results in a heterogenotic duplication. The same duplication can be produced by homologous recombination of a mutant plasmid into the normal locus, as shown below. Depending on the position of the crossover event, excision of the plasmid by homologous recombination from the duplication can result in either a mutant or a wild-type gene at the locus. If one digests the DNA containing the duplication with a suitable restriction endonuclease (shown to the left) and ligates the fragments, one can obtain the mutation by selection for vector markers in *Escherichia coli* (*12*). (**b**) A mutation on a yeast chromosome can be recovered by recombination-repair after transformation with a suitably gapped plasmid carrying the wild-type gene as shown (*13*). (**c**) Gene disruption can be accomplished by integration of a linear fragment containing an insertion or deletion containing a selectable marker (*16*). (**d**) Integrative gene disruption occurs when an internal fragment of a gene integrates by homologous recombination into the intact locus, splitting the gene into two partially duplicated but incomplete parts (*17*), one missing the amino-terminal coding region and the other missing the carboxyl terminal.

screen for such mutations after in vitro mutagenesis of the cloned gene. Thus virtually any gene (*15, 18*), vital or not, can be tested for function.

Some confusion has occasionally arisen about the interpretation of mutant studies, especially when the results conflict with prior assumptions or biochemical evidence (*19–21*). Like any genetic result, apparent lethality or lack thereof must be interpreted with caution because it may depend on the particular circumstances used, or it might indicate the presence of a complicating factor. Failure to find lethality in a gene assumed (or even known) to carry out a vital function may simply indicate the presence of a second gene specifying the same protein or a different one with overlapping function. Likewise, finding of unexpected lethality may result from an unanticipated second function of a gene (for example, in spore germination) or the absence of a normally compensating function. Nevertheless, it sometimes happens that apparently essential proteins are dispensable for growth and vice versa; such results, even if unexpected, are still essentially the only way to test what is really required by the living cell.

The cloning of a gene in yeast thus allows not only the standard recombinant analysis (sequence of the protein, analysis of regulation, production of the protein product, raising of antibodies) but also the immediate opportunity to study both point mutations and known null mutations. We argue, in fact, that proteins discovered elsewhere but present in yeast may best be studied first in yeast, for the access to genetic analysis of function in yeast is so much better. Indeed, many laboratories have taken this road, and major efforts to understand cytoskeletal proteins (such as actin and tubulin) (*22, 23*), proteins involved in secretion (such as clathrin) (*23, 19–21*) and cell cycle regulation (such as the RAS proteins) (*24*) are only the earliest beginnings of the use of yeast to make progress in understanding proteins common to all eukaryotes.

Saccharomyces cerevisiae: A Surprisingly Typical Eukaryote

One of the most surprising generalities to emerge from the widespread application of recombinant technology to the entire gamut of organisms is that most eukaryotic proteins are extremely well conserved in amino acid sequence. This conservation extends to *S. cerevisiae* (Table 1). Conservation is most extreme in ubiquitin and the cytoskeletal elements, but is still substantial (about 60% identity) for a great variety of enzymes and regulatory functions. Thus, it is not surprising that receptors in yeast strongly resemble generic receptors in other organisms, as do cytoskeletal elements and enzymes of similar function. This generality strongly supports the notion that there has been functional conservation to at least the same degree. This argument has already been buttressed in several cases by the demonstration of actual appropriate function of mammalian genes in yeast cells (for example, mammalian *ras* genes complement yeast *ras* mutants) (*24*) or vice versa [for example, assembly of hybrid tubulins in animal cells) (*25*)]. Recently a human gene was cloned directly by complementation of a *cdc2* mutation in *S. pombe* (*26*). We think that conservation most strongly validates the use of the yeasts as models for the primary deduction of functional and mechanistic aspects of proteins and protein systems shared by eukaryotes.

Finally, it is probably necessary to say that just as one cannot uncritically make conclusions from lethality in yeast, so must one be cautious about concluding too much about the function of proteins in animals based only on studies of their homologs in yeast. We believe that the facility of gene manipulation will continue to allow molecular genetic studies in yeast to lead the way to understanding gene function in more complex organisms. Nevertheless, every major conclusion will have eventually to be tested directly in higher organisms. The point of any model system is to make these final tests possible, not to make them unnecessary.

The Community of Yeast Biologists

An important ingredient in the success of yeast studies as a scientific field is the attractiveness of the yeast community itself. Newcomers find themselves in an atmosphere that encourages cooperation. In keeping with a set of traditions that began with the phage group founded by Delbruck, Luria, and Hershey, not only are published strains and materials generally made available, but many (if not quite all) laboratories in the field routinely exchange strains, protocols, and ideas long before publication.

In conclusion, we believe that the yeasts are a nearly ideal model system for eukaryotic biology at the cellular and molecular level. The main reason is experimental tractability, especially in associating genes with proteins and functions in the cell, but the open and cooperative traditions of the community also play an important role.

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Xenopus laevis in Developmental and Molecular Biology

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***Xenopus laevis* is a prime system for the study of embryogenesis in vertebrates. Both prelocalized information in the egg and inductive interactions between cells contribute to the ordered increase in complexity during development. Embryonic induction, discovered in amphibians, is being studied intensely in *Xenopus*; recent work suggests a role for growth factors in this process. Contributions of the *Xenopus* system to the analysis of ribosomal and 5S RNA genes, and the diverse and highly productive applications of the oocyte injection technology, are also summarized.**

PERHAPS THE BEST KNOWN EXPERIMENT IN EMBRYOLOGY IS the Spemann and Mangold experiment on embryonic induction, defining what these researchers called the "organizer" (*1, 2*). Induction is widespread and fundamentally important phenomenon in biology; in its broadest terms it describes any interaction between cells or groups of cells that affects differentiation. As such, induction also occurs in adult organisms, but the term is usually used in the context of embryogenesis, when the processes that generate new tissues and cell types are most active. How different tissues with their great morphological and functional diversity are formed from the comparatively simple egg is the basic question of developmental biology. Inductive interactions constitute one of the two general developmental mechanisms—cytoplasmic localization of information in the egg being the other—that are thought to be instrumental in setting up regional differences in the embryo, which result in a complex organized structure. Although induction events occur in the development of all animals, this phenomenon has been studied most extensively in amphibians, the phylogenetic class in which it was discovered. The original work involved newts, but more recently *Xenopus laevis* has become the animal of choice for studies of induction as well as many other aspects of embryogenesis, in particular at the interface of molecular and developmental biology.

The advantages of *Xenopus* as an experimental animal include its easy husbandry, the fact that it is a vertebrate, the accessibility of embryonic material from the earliest stage onward, and the comparatively large size of the egg and embryo that facilitates physical manipulations. These advantages, in spite of the limitation of the almost total inapplicability of classical genetics, have stimulated much research on *Xenopus* over the past three decades. In this article we discuss three areas in which this system has made important contributions: (i) the role of localized cytoplasmic information and of inductive interactions in the establishment of the polarity and initial tissue differentiation in the embryo, and on the nature and molecular basis of embryonic induction; (ii) the study of genes for RNA components of the ribosome and the control of their expression; and (iii) the productive use of the *Xenopus* oocyte as a "super test tube" in a broad range of studies on translation and transcription.

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The Spemann-Mangold Organizer Concept

Amphibian gastrulation begins with cell migrations at the dorsal side of the embryo. Cells moving up along the blastocoel roof form the presumptive dorsal mesoderm (chordamesoderm), and the ectoderm overlying this tissue develops into the central nervous system (CNS). As the earliest externally visible sign of gastrulation the dorsal lip forms at a subequatorial position in conjunction with these migrations, marking the future dorsal side of the embryo. The Spemann-Mangold experiment involved the transplantation of the dorsal lip from one embryo into the ventral side of another, leading to the development of two dorsal axes in the host (*1–3*). Figure 1 illustrates such an experiment: a cross section of a host embryo is shown at the late neurula stage with two neural tubes, two notochords, and duplicated somites. In external morphology a second head or tail is formed with almost complete duplication of the embryo as a "Siamese twin" in certain cases. Most of the tissue in