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GENETIC APPROACHES TO THE ANALYSIS OF MICROBIAL DEVELOPMENT

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CONTENTS

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INTRODUCTION

The subject of this review is a set of genetic tools suitable for the analysis of complex ordered processes in microbial systems. It is not our purpose to argue whether such processes constitute true "development"; it is not even our intention to describe what is known about development in microorganisms. We intend only to present a number of genetic methods that can be applied to the analysis of ordered processes that have one or more of the aspects of development: morphological change, defined sequences of events, formation and dissolution of substantial subcellular assemblies, and directed changes in cell function.

A number of different microbial systems in which genetic analysis is possible display phenomena that have generally been regarded as developmental. We list these, along with references to recent reviews, because we believe that the genetic techniques that we describe below can be applied to these systems now or in the near future, depending upon the sophistication of the genetic systems available for each organism. Probably the most complex developmental process well-studied in microorganisms is the formation of fruiting structures by myxobacteria [e.g. *Myxococcus xanthus* (30)] and by slime molds [e.g. *Dictyostelium discoideum* (43, 52a)]. Sporulation in bacteria [e.g. *Bacillus subtilis* (10)] and yeasts [e.g. *Saccharomyces cerevisiae* (6, 13)] and conidiation by fungi [e.g. *Aspergillus nidulans* (4, 53)] are also developmental processes amenable to genetic analysis. The morphogenesis of bacteriophages (7), although clearly not a metamorphosis at the level of the cell, has been analyzed extensively in a context that could be thought of as developmental. The induction of bacteriophage lysogens has been investigated in detail as an example of a simple developmental switch (19, 29).

For the purposes of this review, which concentrates on genetic methods, we introduce two other microbial processes that have much in common with development. These are the progress of the cell cycle in a yeast, *Saccharomyces cerevisiae* (15, 50) and the replication of DNA in a bacterium, *Salmonella typhimurium* (34, 38, 56, 57). Both of these processes have some aspects of development: the yeast cell cycle features morphological changes, defined sequences of events, and the involvement of extensive subcellular assemblies; the bacterial DNA replication also involves subcellular assemblies and defined sequences of events. Many of the methods and approaches described in this review have been developed for and applied to analysis of these two phenomena.

CLASSICAL GENETIC ANALYSIS OF COMPLEX PROCESSES

Four levels of genetic analysis have been applied to complex processes in microorganisms. These are mutant isolation and characterization, tests of epistasis, determination of order of gene function, and pseudoreversion analysis. The ease with which these ideas can be applied depends upon the power of the genetic system in the organism to be studied, and organisms in which all methods can easily be used are clearly at an advantage.

Mutant Isolation and Characterization

The first step in any genetic analysis is the isolation of mutants that have lost specifically the ability to carry out the process under study. This step is indispensable, and involves the definition of phenotypes that can be distinguished as specific to the process. In the case of sporulation or fruiting, the mutants are viable but fail to form spores and/or fruits. In the case of the cell cycle or DNA replication, the mutants are often necessarily conditional lethals that display distinctive phenotypes under nonpermissive conditions. For yeast cell cycle mutants the phenotype is usually arrest with a characteristic morphology, while for DNA replication mutants the phenotype is loss of the capacity to make DNA while retaining the ability to make RNA and protein. Mutants thus collected can be sorted into loci by genetic mapping and into functional groups (genes) by complementation analysis.

Once the mutants have been isolated and sorted into genes, the usual procedure is to examine the phenotypes of typical representatives of each gene in some detail. In the case of sporulation, fruiting, and cell cycle mutants, the analysis can be on morphological grounds alone. In the case of DNA replication, the analysis has been biochemical and physiological: one can see, for example, whether DNA replication initiates or whether particular protein complexes assayed in vitro are present.

Tests of Epistasis

Knowledge of detailed phenotypes can be used to interpret tests of epistasis. The idea behind this kind of analysis is that a double mutant carrying two mutations that confer distinguishable phenotypes and that affect different points in the same dependent pathway will have a predictable phenotype, i.e. the phenotype of the mutation occurring earlier in the pathway (18). This method requires relatively little of the genetic system other than the capability to construct double mutants. Often tests of epistasis yield information not obtainable by inspection of mutant phenotypes. For example, yeast cell cycle mutants sometimes display an arrest morphology quite different from that characteristic of the point in the cell cycle (called the execution point) at which the mutant gene product is actually required (9).

Tests of epistasis allow the provisional grouping of mutations into a pathway structure: two mutations failing to show epistasis cannot be on the same dependent pathway. A simple example is assembly of phage T4: mutations resulting in defects in head assembly have no effect on tail assembly and vice versa, but mutations defective in DNA replication are epistatic to mutations in both head and tail assembly (11, 12).

Several points can be made concerning tests of epistasis. First, these tests require detailed knowledge of mutant phenotype. Second, double mutants sometimes have phenotypes different in gross or detail from the phenotypes of either single mutant, in a way that precludes interpretation. Third, the pathway structure revealed often results from regulatory rather than morphogenetic effects. The T4 example is such a case: the failure of DNA replication-defective mutants to make tails results from a failure to activate the late genes and not from a direct involvement of replicated DNA in tail morphogenesis (12, 26).

Direct Tests of Order of Function

It is very common, in developmental or morphogenetic pathways, to find many genes with indistinguishable mutant phenotypes. In such cases, simple tests of epistasis cannot be carried out. For this reason, developmental geneticists have sought ways of determining order of function that do not depend upon detailed examination of phenotypes. One such way of determining functional order is the reciprocal shift method, which was first described by Jarvik & Botstein (27) and applied by them to phage morphogenesis. A very similar test based on the same principle was devised independently and applied to the yeast cell cycle by Hereford & Hartwell (18).

The main requirement for application of reciprocal shift methods is a capability to block serially the progress of the pathway(s) under study in two different ways. Blocking can be accomplished by using a double mutant carrying both a heat-sensitive (*ts*) and cold-sensitive (*cs*) mutation (27, 39). The alternative to conditional mutations is use of inhibitors that prevent progress of the pathway(s) under study but do not kill the cells. Examples that have been used in studies of the yeast cell cycle are the α factor, which inhibits *a* cells at the start of the cell cycle, and hydroxyurea, which inhibits DNA synthesis (16, 18).

Table 1 shows the possible results of reciprocal shift experiments using *cs-ts* double mutants defective in the yeast cell division cycle. Incubation of such cells at the high nonpermissive temperature (during which the gene bearing the *ts* mutation fails to function) followed by incubation at the low nonpermissive temperature (during which the gene bearing the *cs* mutation fails to function) results in progress to the next cell cycle if and only if the gene carrying the *cs* mutation can act before the gene carrying the *ts*

Table 1 Results expected in reciprocal shift experiments using *cs-ts cdc* double mutants

Dependency relationship	Result of shift	
	17° → 37°	37° → 17°
Dependent *ts* → *cs* →	+[a]	−[a]
Dependent *cs* → *ts* →	−	+
Independent *ts* → *cs* →	+	+
Interdependent (*cs*, *ts*) →	−	−

[a] A "+" indicates passage to a second cell cycle (i.e. two arrested cells are found) and a "−" indicates arrest in the first cell cycle (i.e. one arrested cell is found). Taken from Moir & Botstein (39).

mutation. Reversing the order of incubation at the two temperatures should have the opposite result if the two genes in which the mutations lie are part of a dependent pathway.

It can be seen from Table 1 that the method not only yields information about the order of gene function when the two genes lie on a dependent pathway, it also tells whether two gene functions are arranged in such a dependent pathway. Application of the reciprocal shift method to a developmental process thus allows the determination of the order of gene function and also allows important inferences to be made about the pathway structure of the process. This is not an idle question, since branched pathway structures have been found in phage morphogenesis (as mentioned above) and also in the yeast cell cycle [(17, 39); see below].

Finally it should be noted that genes that function independently of one another may nonetheless execute their respective functions in a fixed temporal order in a cell. Reciprocal shift experiments can reveal such a relationship whereas simple observations over time cannot.

Pseudoreversion Methods

Many developmental and morphogenetic processes involve not only a complex set of ordered dependent pathways, but also assemblies of many gene products. Sometimes these assemblies can be detected by biochemical or even morphological examination, but often they cannot. Independent genetic approaches to the detection and study of subcellular assemblies are thus of great interest to the developmental biologist. This is so especially because genetic analysis is not subject to the possible artifacts introduced by cell rupture and handling inherent in morphological and biochemical studies.

Most mutants, particularly conditional lethals, readily revert to normal phenotype. Some of the reversion events occur at a site distant from the original mutation; i.e. the reversal of the mutant phenotype is a "pseudoreversion" event. The secondary mutations in pseudorevertants that result in the reversal of mutant phenotype are by definition "suppressor" mutations. The collection of pseudorevertants and the characterization of the suppressor mutations they contain are valuable tools for geneticists interested in complex processes because some kinds of suppression are caused by interactions of specific genes and/or their products. The suppressors of interest are, of course, to be distinguished from informational suppressors, which affect mutations by altering the protein synthetic machinery (e.g. tRNA suppressors of nonsense or frameshift mutations).

Several kinds of compensating change can result in suppression. First, and of some interest, are bypass suppressor mutations, which cause the activation of a new pathway that substitutes for the blocked pathway. Second, and also interesting, are mutations resulting in overproduction of the mutant gene product or assembly. Mutations encoding an inefficient enzyme would likely be suppressible in this fashion. Third, and most useful, are interaction suppressors, which restore function directly to a defective gene product through a compensating change in an interacting gene product. Genetically, these various kinds of suppressors can be distinguished at least partially by the spectrum of mutations suppressed. Bypass suppressors should suppress all mutations in the gene(s) specifying the function(s) bypassed. Thus such suppressors are specific for only one or a small number of genes, but generally are allele-nonspecific. Interaction suppressors, on the other hand, will generally be very specific for a limited subset of mutations in a single gene: they should be highly gene- and allele-specific (27, 40).

Suppressors are of relatively little interest in studies of development unless some way can be found to determine the normal function(s) of the suppressor gene. A simple solution to this problem is to screen among revertants for mutations that, in addition to the suppressor phenotype, have a new phenotype of their own. Jarvik & Botstein (28) first applied this method in their study of phage P22 morphogenesis, finding suppressors that simultaneously suppressed *cs* mutations and conferred upon the phage a new heat-sensitive phenotype. Genetic analysis indicated that the suppressors (now referred to as Sup/Ts) retained their temperature-sensitive phenotype even when separated from the *cs* mutation they suppressed. The new heat-sensitive phenotype turned out to be recessive, and thus the gene in which the Sup/Ts mutation had arisen could be identified by simple complementation analysis.

Thus a general scheme for mapping gene interactions in vivo emerges: interaction suppressors having an additional phenotype are obtained from

a developmental mutant, and the new phenotype serves to identify the suppressor gene. This kind of analysis has been applied to studies of yeast cell division cycle mutants (40), in which it was found that unlinked Sup/Ts mutations often have, at their own new nonpermissive temperature, a cell cycle phenotype indistinguishable from the suppressed mutation. In addition it was observed that the Sup/Ts mutations are generally recessive for temperature-sensitivity but dominant for suppression. As discussed below, recognition that suppressors are commonly dominant (i.e. for suppression) has led to the development of powerful methods for using suppression to study gene interactions, especially in bacteria.

Pseudorevertants selected for having new phenotypes are also useful both for finding new mutations in previously identified genes and for identifying additional genes involved in a particular process. This was certainly so for yeast cell division cycle mutants, where several new *cdc* genes were identified in reversion studies (40). A particularly elegant recent example of the use of pseudoreversion to find new mutations in related genes is the isolation of mutants in the α-tubulin structural gene of *Aspergillus nidulans* as suppressors of mutants in the β-tubulin structural gene (42).

Genes yielding interaction suppressors whose own mutant phenotype is unlike that of the original mutation may also be very significant. One of the nagging problems in developmental genetics has been the possibility that genes intimately involved in a developmental pathway may also have other, possibly essential, functions. Mutations in such genes will be missed by searches for mutations with the developmental phenotype but should appear among suppressors of developmental mutants.

Pseudoreversion analysis makes heavy demands on a genetic system. Demonstration of a suppressor requires separation and reassociation of the suppressor and the original mutation, implying an ability to conduct crosses and observe segregation of markers among progeny. In addition, there must be a relatively simple way of carrying out complementation and/or mapping studies, in order to classify suppressors. These constraints have limited the use of pseudoreversion as a major genetic tool, particularly in bacteria.

IMPACT OF NEW GENETIC TECHNOLOGIES ON ANALYSIS OF COMPLEX PROCESSES

Although the classical methods summarized above clearly have the power, in principle, to allow extensive genetic analysis of developmental pathways, their application has been limited by the difficulties of the genetic manipulations, especially in bacteria. Much of the manipulation (complementation analysis, construction of double mutants, separation of suppressors from the original mutations in pseudorevertants, tests of cross suppression by

crosses back to original mutants, etc) is laborious to the point of infeasibility even in those organisms for which methods are available in principle. Many of the difficulties, however, have recently been ameliorated by the application of new genetic technologies.

Uses of Transposons

The discovery of transposable elements carrying genes conferring antibiotic resistance made possible a large number of new methods of in vivo genetic manipulation in prokaryotes. Many of these have become standard and have been reviewed (32). Probably the most important uses derive from the ability to place transposons (and their associated drug-resistance determinants) within or near genes of interest, making it possible to transduce alleles of these genes simply by selecting the drug resistance. Such linked elements immediately simplify the construction of double mutants. As discussed below, transposon markers can also be used to distinguish among unlinked genes with similar phenotypes. Uncharacterized mutations with the same phenotype can be tested for transductional linkage to the various transposon markers; one thus avoids more laborious conjugational mapping. Many insertions of transposons into the genomes of *E. coli* and *S. typhimurium* have been mapped so that the probability that any new gene is within cotransduction range of a mapped element is high and increasing.

The ability to use cotransduction with transposons to replace virtually all conjugation methods means that organisms without a well-developed genetic system can be developed much more rapidly. All that is needed is a good generalized transducing phage and a way of making transposon insertions. Even long-range mapping of genes separated by a distance beyond cotransduction range can be accomplished using the method of duplication mapping (1, 32), which depends upon transposon insertions to maintain duplications selectively. Such duplications can also be used to carry out complementation analysis and dominance tests: a duplication maintained by transposon selection is made heteroallelic by transduction. These techniques have actually been used to establish a genetic system without conjugation in a developmentally interesting organism. In their study of the complex gliding motility systems of *Myxococcus xanthus* (21, 22), L. Avery and D. Kaiser (personal communication) have carried out complementation analysis using duplications maintained with transposons.

The usefulness of insertions that carry a selectable marker is not limited to transduction systems. In yeast, integrative transformation of recombinant DNA plasmids can be used to place a selectable marker carried by the plasmid vector virtually anywhere in the genome (20). These new markers can be used in ways analogous to the use of transposon insertions in bac-

terial genetics. Of particular importance is the ability to use such inserted markers to map genes whose mutant phenotypes are difficult to score directly.

Recombinant DNA Methods

For most organisms it is now straightforward to make recombinant DNA libraries that are virtually certain to contain every gene of the organism. The cloned DNA fragments can be very large with respect to a single gene or operon, and they can be generated in ways that approximate randomness (31, 37). Therefore a very complete library will contain a gene many times with varying amounts of flanking DNA, insuring that at least sometimes the genes or operons are recovered with their flanking regulatory sites intact. In most microorganisms with developed genetics (e.g. *Escherichia coli, Salmonella typhimurium, Bacillus subtilis, Saccharomyces cerevisiae*) there are relatively simple ways of returning the cloned genes in libraries to the organisms by DNA transformation or phage infection (20, 23, 36, 51). Using mutations that show a defect, it is therefore usually possible to isolate any gene by its ability to complement the defect. By subcloning smaller and smaller fragments, it is also possible to identify structural genes with certainty, and to separate control regions from structural genes.

These possibilities have consequences for complementation analysis. For example, in bacterial systems, complementation using F' episomes (35) was always hampered by the relatively small number of available episomes, their poorly defined extents, their variable stability and copy number, and by the task of constructing F' episomes bearing appropriate mutations. Now complementation analysis can easily be done with well-defined recombinant λ bacteriophages, which, as prophages, can be inherited stably as single copies at the prophage attachment site.

Suppression experiments can also be improved and simplified by the use of recombinant DNA libraries. Since many interaction suppressors are dominant (e.g. 2, 40), a given recombinant DNA phage or plasmid can be mutagenized and tested for its ability to give rise to mutants that suppress when introduced back into cells carrying the target mutation. The identity of the gene on the recombinant phage or plasmid that gives rise to suppressors can then be identified by complementation analysis or by the introduction of second mutations that simultaneously abolish gene function and suppression. This route provides a good alternative to the necessity for finding suppressors with new phenotypes, which, although usually possible to find, are often exceedingly rare.

Additional uses for recombinant DNA methods include localized mutagenesis (24), "reverse genetics," i.e. proceeding from knowledge of a protein via recombinant DNA clone to mutant phenotype (49), and differential

plaque hybridization to identify developmentally regulated genes (52). Examples of these applications are presented below. Even then, the list of uses for recombinant DNA clones is grossly incomplete, and new ideas are regularly being introduced.

COMBINING THE ELEMENTS: AN OVERVIEW

The preceding summary shows that there are now a large number of genetic tools of considerable power available for the study of development in microorganisms. There are, however, still some requirements that must be met by the underlying genetics of the organism under study. Minimally, the organism must be manipulable to the extent that double mutants can easily be constructed: this means a transposon/generalized transduction genetics (attainable with most bacteria) or equivalent (e.g. the meiotic genetics of yeast). The genetics must allow the backcrossing of pseudorevertants to wild type and the recovery of suppressors: this too is easily achieved with transposon/transduction systems since a mutation linked to a transposon can be transduced away from its suppressor by selecting the drug resistance. If the full range of methods is to be used, then a way must be found to return to the organism (by DNA transformation, at least) cloned DNA fragments and to have them maintained through the agency of chromosomal integration or some kind of plasmid system. Finally, to make best use of modern in vitro mutagenesis methods, a way is needed to replace normal alleles of genes with mutant alleles carried on plasmids or phage. These requirements are not so difficult to meet in prokaryotes and lower eukaryotes.

No technology, however, will substitute for the requirement for mutants in the process of interest. Generally these will still have to be isolated in the first instance by direct screening. However, once a few mutants have been obtained, several additional routes, possibly more direct and efficient, become available. Using transposons linked to genes of interest, one can saturate the gene and surrounding region by localized mutagenesis (24, 32) without mutagenesis of the entire genome. One can also clone DNA fragments that complement the mutations of interest, and use these clones to make more mutations, including completely defective alleles (e.g. deletions or nonsense mutations) even when the gene function is an essential one. It is useful, of course, to seek cold-sensitive and heat-sensitive alleles of interesting genes in order to carry out reciprocal shift experiments.

One can then proceed to isolate pseudorevertants, either directly in the genome or in cloned DNA segments. The genes identified as giving rise to suppressors are amenable to further analysis, either because the suppressors have an additional phenotype, or because the suppressors are in the form of recombinant clones.

Finally, if there is suspicion that a particular protein might be involved in the process of interest, one can use recombinant DNA methods to isolate the structural gene(s) encoding this protein and make mutations therein and, using an allele replacement system, make mutations in the genome whose phenotype can be assessed.

In the remainder of the this review, we provide a few specific applications of these methods (taken mainly from work in our own laboratory) to illustrate the interplay of the genetic methods.

SPECIFIC APPLICATIONS TO COMPLEX PROCESSES

Bacterial DNA Replication

Replication of bacterial DNA is estimated to require some 20 gene products, exclusive of those involved in precursor synthesis. The overall process is complex, involving at least four distinct phases (each of which may be enzymatically complex): initiation of a new round of replication; repeated initiation of Okazaki fragments (lagging strand synthesis); elongation; termination and separation of daughter molecules. The second and third steps, especially, have been the subjects of intensive biochemical investigation using viral DNA templates. (For review see 33, 34, 41, 45, 55, 57). DNA replication apparently proceeds by a defined sequence of gene action and is catalyzed by specific protein complexes. From our perspective, then, it may be analyzed genetically like a developmental pathway.

MAPPING OF MUTANTS USING TRANSPOSONS In *E. coli* known replication genes are dispersed around the chromosome (5). Only *dnaA-dnaN* in one case and *dnaJ-dnaK* in another are found to be adjacent (46, 47). The number and distribution of genes makes it tedious to show, by traditional methods, that a new replication mutant is different from anything known. This difficulty has been overcome through the use of transposons as linked genetic markers. In the *Salmonella* system, where this approach was applied systematically, each replication locus is defined by an adjacent Tn*10* insertion, conferring tetracycline resistance (38). Characterization of a new mutation then reduces to a test of transductional linkage with a set of Tn*10* insertions; since linkage can be detected in a spot test, a mutation can be characterized in a one plate experiment. This system permits mutants to be genetically characterized without requiring them to be mapped relative to standard markers on the genetic linkage map. However, the map positions of increasing numbers of transposon insertions are being learned. Association of an uncharacterized mutation with an insertion in this latter group provides information about map position as well as locus definition.

COMPLEMENTATION USING RECOMBINANT DNA In the *Salmonella* system, the ability of a λ transducing phage to complement a replication-defective host was scored as a plaque morphology marker using an unusual λ-sensitive *Salmonella* derivative. Complementing phage made a red plaque while other phage made a white (i.e. colorless) plaque (38). It was explicitly shown that the red plaque response reflects the formation of partial diploids through lysogenization and thus is a true complementation assay. The plaque assay is general in that complementation of any conditional lethal mutation can be scored. The assay has also been used in *E. coli* (3). Briefly, the assay develops in two stages. In the first stage, plaques are allowed to form on an ordinary indicator. In the second stage, the mature plaques are overlaid with soft agar seeded with tester cells (e.g. replication-defective) that are lysogenic for λ. The plates are then incubated at the nonpermissive condition. Phage can diffuse from a plaque into the adjacent overlayer and infect tester cells. If the phage complement the defect in the tester, the infected cells will grow and, because of a dye in the overlayer, turn red.

Allelism of two chromosomal mutations was established using specialized transducing phage and mutant derivatives thereof. Allelic mutations were expected to meet two criteria. First, any λ clone complementing one mutant strain must complement both. Second, any mutant derivative of the λ clone that failed to complement one mutant strain must fail to complement both. Adherence to these criteria was observed among *dnaC* and, separately, among *dnaE* mutants of *Salmonella* (38).

EXTRAGENIC SUPPRESSION USING λ CLONES Access to cloned replication genes combined with the plaque assay described above led to a powerful new way to identify and characterize extragenic suppressor mutations. The approach is illustrated with the example of *dnaB* mutations that suppress *dnaC* (R. Maurer, B. C. Osmond, and D. Botstein, in preparation). Initially it was found that a λ*dnaB*$^+$ transducing phage (identified by its ability to form a red plaque on a *dnaB* mutant host) formed white plaques (indicating noncomplementation) on a *dnaC* mutant host. This was expected because *dnaB* and *dnaC* are different genes that are so widely separated on the chromosome that λ*dnaB*$^+$ cannot carry the *dnaC* gene as well (5). In a mutagenized lysate of λ*dnaB*$^+$, variants appeared that now formed red plaques on a *dnaC* host. Such phage carry suppressors of *dnaC* which may be mutants of *dnaB* or of another gene carried by λ*dnaB*$^+$. Additional observations proved that the suppressors were in fact alleles of *dnaB*. First, the suppressor phage were still able to complement (i.e. form red plaques) on a *dnaB* mutant host. Thus the phage exhibited two phenotypes: complementation of *dnaB,* attributable to the *dnaB* gene, and

suppression of *dnaC*. Second, mutagenesis of the suppressor phage produced derivatives that simultaneously lost both phenotypes. Moreover, one of these derivatives recovered the ability to both complement *dnaB* and suppress *dnaC* when the tests were done in *amber*-suppressing hosts, which demonstrated that a single *amber* mutation can affect both activities. Therefore, suppression of *dnaC* must be attributable to the *dnaB* gene on the phage.

EXTENSION OF THE SUPPRESSOR METHOD The method just described was applied to genomic libraries (cloned in λ) to obtain suppressor mutations anywhere in the genome, rather than in a chosen gene. All that was required was that the library be prepared from a strain bearing some mutation in the gene for which suppressors were sought (the target gene). The mutation in the library did not have to be identical to the target mutation; it had only to be in the same gene. This provision insured that the library contained no phage with complementing activity toward the target mutation. Such a library was mutagenized and screened for variants that formed red plaques on hosts mutant in the target gene. Red plaque isolates were characterized by their plaque color on other mutant hosts.

This method was used to obtain suppressors of *dnaE* (R. Maurer, B. C. Osmond, and D. Botstein, in preparation). A library prepared from a *dnaE* mutant strain of *Salmonella* was mutagenized and screened for variants that formed red plaques on a *dnaE* mutant host. All three isolates that were found also made red plaques on a *dnaQ* mutant host (25), but not on any other strain. This result was most simply interpreted as showing that *dnaQ* can mutate to a form that suppresses *dnaE;* but other interpretations were possible. To confirm the simple interpretation, phage bearing the wild-type *dnaQ* gene were mutagenized to produce mutants that formed red plaques on a *dnaE* mutant host. These suppressor mutants were shown to be alleles of *dnaQ* by comutation of the *dnaQ* complementing and *dnaE* suppressing activities as described above.

TESTING ALLELE SPECIFICITY An important element in the characterization of any suppressor mutation is a determination of its activity toward different mutations in the same target gene. The suppressors carried on λ phage described above were examined for plaque color on several different mutant hosts. This process required only the plating of the various phages bearing suppressors onto the various hosts bearing the target mutations to be tested. No strain constructions were required, as would have been the case if the suppressor and the target mutation had to be recombined together. In these experiments the limitation of the plaque assay—that it is not quantitative—became evident. It was much easier to show suppression

than it was to show lack of suppression. Thus while strong suppressors were easily characterized, the allele specificity of weak suppressors was difficult to establish with certainty. Nevertheless, it was clear that both allele-specific and allele-nonspecific suppressors were observed.

LOCAL MUTAGENESIS AND ALLELE REPLACEMENT The preceding paragraphs illustrate that the use of genes cloned in λ provides great versatility to genetic analysis of bacteria. Most importantly, an unlimited spectrum of mutants can be obtained in the cloned gene. In contrast, the requirement for cell viability restrains the permitted phenotypes of chromosomal mutants. For example, recombinant phage mutants that have lost the ability to complement some particular host typically carry missense mutations, which would confer absolute lethality if present in the chromosome. Such mutants are easily obtained on the phage, but impossible to obtain in the chromosome. In addition, mutagenesis of genes cloned on a phage constitutes a form of local mutagenesis with the attendant advantages of that method (24). In particular, the desired mutants can be induced with a relatively low degree of mutagenesis and can then be studied in unmutagenized cells.

In a sense, the use of genes cloned in λ represents a favorable compromise between the conflicting demands of molecular biology on the one hand and classical genetics on the other. It is clear that having a gene cloned in λ provides an adequate starting point for detailed molecular study of the gene and its product. Equally, the gene or a mutant form of it can easily be reintroduced into the cell for characterization in vivo. It should also be pointed out that allele replacement, that is, substitution of a chromosomal copy of a gene with a different allele introduced on a phage, is also possible in the bacterial system. For example, the *dnaE* mutant strain used to construct a genomic library (see above) actually carried an *amber* mutation in *dnaE.* The origin of this strain is instructive. First, a λ clone carrying the wild-type *dnaE* gene was isolated by its ability to form a red plaque on a *dnaE* mutant host. This phage was mutagenized, and a derivative that now made a white plaque on the same host was obtained. The mutation in this phage was characterized as an *amber* because the phage made a red plaque if the host strain carried an *amber* suppressor. The phage carrying the *dnaEamber* was mutagenized again, this time with a transposon derived from Tn10. A derivative carrying the transposon in the cloned *Salmonella* DNA (but not in *dnaE*) was obtained. Introduction of this λ phage into an *amber*-suppressing *Salmonella,* under conditions in which the phage could not replicate or lysogenize, resulted in the isolation of a tetracycline-resistant transductant, free of λ-specific DNA, which carried the *amber* mutation at the *dnaE* locus (R. Maurer, unpublished). The same sequence

of steps can be applied to any gene as long as the allele in question is compatible with cell viability.

Yeast Cell Cycle

The singular strength of yeast as a genetic system is in the presence of both haploid and diploid vegetative growth states and the easily manipulated conversion between these two states. As a consequence, recessive mutations can be obtained as easily as in bacteria (by using the haploid phase), yet thorough complementation and segregation analysis can also be carried out. Thus yeast is a particularly suitable organism in which to apply certain of the genetic methods for analyzing complex processes.

The cell division cycle of yeast apparently requires over 50 gene products. Many of these genes (*cdc* genes) were identified and their roles in the cell division cycle to some extent elucidated some time ago (for review see 17, 50). Recent work on this subject focuses on the steps of nuclear division, one element in the cell division pathway. The methods used include the isolation of cold-sensitive (*cs*) cell cycle mutants, pseudoreversion studies, and reciprocal shift experiments.

COLD-SENSITIVE CELL CYCLE MUTANTS Among 200,000 mutagenized haploid yeast, 18 mutants were found that were cold-sensitive for growth, exhibited a characteristic arrest morphology, and (after crossing to a wild-type strain) segregated cold sensitivity from diploids 2:2 (as expected for a single nuclear mutation) (40). All 18 mutations were recessive to wild-type as shown by growth of heterozygous diploids at the cold temperature. The mutations were characterized further by their arrest morphology and by complementation analysis. A total of nine complementation groups (one with seven members, another with four, and the rest with one) were observed. Six of the seven mutants that fell into single-member complementation groups arrested at the stage of medial nuclear division. Since a large number of known *cdc* mutants arrest with this characteristic morphology, these new mutants were chosen for further genetic study to see if the pathway of nuclear division could be more fully defined.

First it was asked whether the new *cs* mutants were alleles of known *cdc* genes (defined only by *ts* mutations). Direct tests of complementation between *cs* and *ts* mutants were not possible, and therefore this question had to be approached by genetic linkage studies. Three of the new mutants were crossed to representative *ts* mutations in all known nuclear division *cdc* mutants, and the linkage of *cs* and *ts* was determined. The result was a lack of linkage in every case. Therefore the three genes represented by these three mutants were indeed new.

PSEUDOREVERSION Cold-resistant revertants were isolated from four of the nuclear division *cs* mutants (including the three that were clearly new genes) (40). In contrast to the bacterial experiments described above, where all suppressors could be characterized because they were obtained on phage clones, in these experiments it was anticipated that only those suppressors that conferred a conditional phenotype in their own right would be amenable to analysis. Among all revertants, about 1 in 50 exhibited a temperature-sensitive growth defect. Without exception, in linkage studies the temperature sensitivity and suppression of cold sensitivity were inseparable and therefore apparently the result of a single Sup/Ts mutation.

Studies on the linkage of the Sup/Ts to the target *cs* mutation generally showed a lack of linkage. One *cs* mutation was an exception: all of its suppressors were tightly linked to the *cs,* and further detailed analysis suggested that these were in fact intragenic suppressors. The remaining, unlinked, Sup/Ts mutations were obtained free from the *cs* mutation that generated them and characterized genetically and morphologically. Morphologically, the significant result was that the Sup/Ts mutations conferred a cell cycle phenotype (except for some of the weakest suppressors). In all but one case the defect was in nuclear division; the exception arrested as an unbudded cell. Complementation tests of these new Sup/Ts mutations against representative *ts* mutations in known *cdc* genes showed that the Sup/Ts that arrested as an unbudded cell was an allele of *cdc32,* while the remaining Sup/Ts mutations defined two new genes, *cdc46* and *cdc47.* Interestingly, *cdc46* could mutate to form that suppressed *cs* mutations in either of two *cdc* genes, but a given suppressor mutation was active only on one *cs* or the other. Thus the pattern of suppression was allele specific in so far as it was determined. The results of these pseudoreversion experiments confirm that this type of analysis specifically probes other genes involved in the same specific pathway in the cell.

RECIPROCAL SHIFT EXPERIMENTS With the new cold-sensitive nuclear division mutants described above, and existing heat-sensitive mutants affecting the same pathway, it was feasible to attempt reciprocal shift experiments to elucidate the detailed structure of the nuclear division pathway (39). The general procedure for these experiments was as follows. A *cs-ts* double mutant strain was grown permissively and synchronized at the beginning of the cell cycle by treatment with a hormone, α factor. The cells were then washed free of hormone and incubated at a nonpermissive temperature. (Synchronization with the hormone was convenient as it allowed the cells to arrest after the shortest possible incubation at the nonpermissive temperature.) Arrested cells were isolated on an agar slab, which was placed at another temperature, and the progress of individual cells through another

round of cell division was followed microscopically. The interpretation of these experiments depended on the inclusion of two vital control incubations. One of these was an incubation at the permissive temperature following the first arrest; this control measured the reversibility of the first arrest. The other was a continued incubation at the first nonpermissive temperature. This measured the leakiness of the first block. Ideal mutations would be completely reversible and not at all leaky, but in practice this was not achieved. Numerical corrections to the raw data were made using the results of the control incubations.

In these experiments, two new *cs* nuclear division mutants (genes *cdc44* and *cdc45*) were tested with three *ts* mutants in genes known to affect this pathway: *cdc9, cdc16,* and *cdc14* (17). A significant new result from these tests was that *cdc16* and *cdc45* functioned independently of one another, even though both of these genes function in the same larger pathway (i.e. after DNA replication but before the action of *cdc14*). The interpretation of this result was that the nuclear division pathway includes an internal branch point, with *cdc45* acting on one branch and *cdc16* acting on the other. The branches rejoin before the action of *cdc14. Cdc9* and *cdc44* could not be unambiguously placed in this scheme (see Figure 1). According

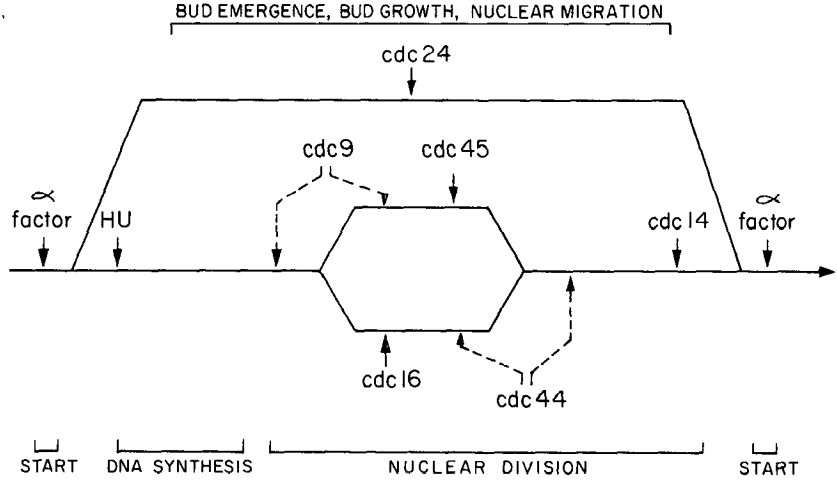

Figure 1 Schematic diagram of part of the yeast cell division cycle pathway deduced from reciprocal shift experiments. The order of gene functions on the dependent pathways is shown by the arrows; dotted arrows indicate alternative possible positions for a gene function. The distances between arrows are arbitrary; the pathway is not drawn to any scale. HU and α indicate blocks imposed by hydroxyurea and the hormone, α factor. The branching of the pathway indicates independent function of the gene functions associated with the two branches; execution of functions on one branch is not prerequisite to functions on the other. For simplicity, some *cdc* genes have been omitted. Adapted from Moir & Botstein (39) and Hartwell et al (17).

to Hartwell's earlier formulation of the cell division pathway, the steps in nuclear division were themselves a branch, separate from the steps of budding (17). The new information suggests that the cell division pathway is more complex, consisting of a nested series of branched dependent pathways.

Optimal application of the reciprocal shift method demands the availability of both *cs* and *ts* mutations in each *cdc* gene. Otherwise, for example, a gene defined only by a *ts* mutation can be tested only with those genes defined by *cs* mutations. Since at the present time almost all *cdc* genes are defined by only one type of mutation, this is a serious limitation. Fortunately, methods are being developed for mutagenizing cloned genes from yeast and introducing the resulting mutations back into yeast cells (48). An example of such a method is discussed in the next section. Clearly, this will be the preferred method for generating the necessary additional *cdc* mutations.

Yeast Actin Mutants

The ability to reintroduce cloned yeast genes into yeast via plasmid transformation (20) makes it possible to study a yeast gene by reversing the customary sequence. Techniques to do this were elaborated with the yeast actin gene, which was discovered and cloned by homology to actin genes of other eukaryotes (14, 44). No identified actin mutants existed in yeast, and thus the directed formation of such mutants illustrates the feasibility of "reverse" genetics.

NULL MUTANTS IN ACTIN The necessary first step was to establish a phenotype for actin mutants (D. Shortle, J. E. Haber, and D. Botstein, submitted for publication). To accomplish this, one of the two actin genes present in a diploid cell was disrupted to produce a null allele (Figure 2*a*), and the null phenotype was then observed in haploid spore clones derived from this strain. The technique for disrupting the actin gene was as follows. A fragment of the gene internal to the coding sequence was subcloned into a plasmid vector. The vector chosen carries a gene (URA3) selectable in appropriate mutant yeast, and of equal importance, the vector forms stable transformants only by integrative recombination between homologous sequences present in the plasmid and in the genome. Thus Ura^+ transformants obtained with the actin plasmid included some that integrated at the URA3 locus and others that integrated at the actin locus; these were distinguished by appropriate gel-transfer hybridization experiments. Integration of the plasmid at the actin locus necessarily disrupted the actin gene. This can be understood if the internal actin fragment present in the plasmid is

thought of as a double deletion mutant of the actin gene. Recombination between the double deletion and the intact actin gene produces two actin genes (separated by plasmid DNA), each a single deletion mutant. Tetrad analysis of the integrants at the actin locus showed that the actin null mutant behaved as a recessive lethal, at least as regards germination of spores and probably for vegetative growth of cells as well.

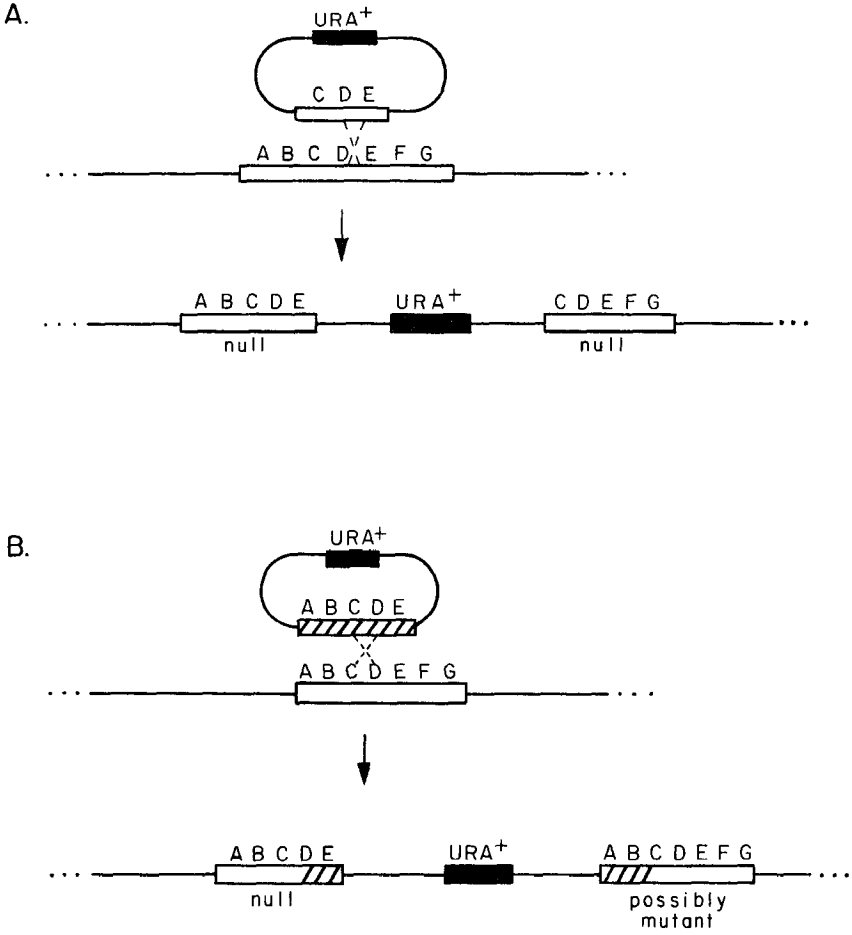

Figure 2 Directed mutagenesis of the yeast actin gene. (*a*) Sequence disruption. Recombination between an internal fragment of actin (CDE) and the intact gene (A-G) produces two null alleles (A-E and C-G). (*b*) Sequence alteration. Recombination between a mutagenized actin fragment (A-E, hatched) and the intact gene produces one null allele (A-E) and one full-length allele (A-G) containing mutagenized sequences (hatched). In both parts of the figure the crossover position shown is arbitrary.

CONDITIONAL LETHAL MUTANT IN ACTIN An adaptation of the disruption procedure led to the isolation of a temperature-sensitive mutation in the actin gene (D. Shortle and D. Botstein, in preparation). In this case a plasmid was constructed that carried a copy of actin deleted only at one end. It will be readily appreciated that integration of such a plasmid at the actin locus produces two copies of actin, one full length and the other deleted at one end. The full-length copy necessarily includes some material contributed by the plasmid; how much depends on the site of crossing over (Figure 2*b*). To generate mutant actins, the plasmid actin gene was subjected to mutagenesis in vitro (49) prior to transformation. A haploid strain transformed with mutagenized plasmid gave rise to a temperature-sensitive transformant. The mutation in this strain mapped at the same locus as the null mutation described above. In addition, preliminary complementation tests indicated that the same function was affected by the null mutation and the temperature-sensitive mutation. These results confirmed that actin is required for vegetative growth of yeast and created the opportunity to study the essential role of this gene.

Developmentally Regulated Genes in Aspergillus nidulans

Another approach is exemplified by the study of conidiation (asexual spore formation) in the fungus *Aspergillus nidulans.* This process can be synchronously induced in vegetative cells, and it has been found that induction brings about the elaboration of hundreds of messenger RNA species that are absent from or present at low levels in vegetative cells (4, 53). The differential production of these RNA species in conidiating, as compared to vegetative, cells formed the basis for identifying clones from a genomic library carrying developmentally regulated genes

Zimmerman et al (58) prepared a labelled cDNA probe from poly(A)$^+$ RNA of conidiating cells. This DNA was enriched for development-specific sequences by repeated hybridizations with poly(A)$^+$ RNA from vegetative cells followed by hydroxyapatite chromotography. Finally, the surviving unhybridized cDNA was hybridized with poly(A)$^+$ RNA from conidiating cells and unhybridized sequences were discarded. This highly enriched probe was used to identify almost 400 clones containing developmentally regulated sequences.

Particular identified clones were then used to study the expression in vivo of the corresponding genes (54). Questions addressed included the level of expression in vegetative cells and the timing of increasing expression during conidiation; the effect of mutations that block conidiation at various stages (8); and the structural organization of the genes and transcripts. At present it is not possible to reintroduce cloned genes in *Aspergillus;* therefore it is not possible to seek the identity of the cloned genes by complementation of

developmental mutants. It is to be hoped that such information can be obtained in the future. This drawback notwithstanding, clearly a wealth of information about development can be obtained from the cloned genes.

CONCLUSION

In the ways we have described it seems possible now to discover much about the genes controlling a developmental or morphogenetic event, the order in which these genes act, the arrangement of the gene functions into dependent pathways, and the assembly of the gene products into functional subcellular assemblies.

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Literature Cited

1. Anderson, R. P., Roth, J. R. 1977. Tandem genetic duplications in phage and bacteria. *Ann. Rev. Microbiol.* 31:473–505
2. Atlung, T. 1981. Analysis of seven *dnaA* suppressor loci in *Escherichia coli.* in *The Initiation of DNA Replication,* ICN-UCLA Symp. Mol. Cell. Biol., ed. D. S. Ray, C. F. Fox, 12:297–314. New York: Academic. 628 pp.
3. Auerbach, J. I., Howard-Flanders, P. 1981. Identification of wild-type or mutant alleles of bacterial genes cloned on a bacteriophage Lambda vector: isolation of *uvrC*(Am) and other mutants. *J. Bacteriol.* 146:713–17
4. Axelrod, D. E., Gealt, M. A., Pastushok, M. 1973. Gene control of developmental competence in *Aspergillus nidulans. Dev. Biol.* 34:9–15
5. Bachmann, B. J., Low, K. B. 1980. Linkage map of *Escherichia coli* K-12, edition 6. *Microbiol. Rev.* 44:1–56
6. Baker, B. S., Carpenter, A. T. C., Esposito, M. S., Esposito, R. E., Sandler, L. 1976. The genetic control of meiosis. *Ann. Rev. Genet.* 10:53–134
7. Casjens, S., King, J. 1975. Virus assembly. *Ann. Rev. Biochem.* 44:555–611
8. Clutterbuck, A. J. 1969. A mutational analysis of conidial development in *Aspergillus nidulans. Genetics* 63:317–27
9. Culotti, J., Hartwell, L. H. 1971. Genetic control of the cell division cycle in yeast. III. Seven genes controlling nuclear division. *Exp. Cell Res.* 67:389–401
10. Doi, R. H. 1977. Genetic control of sporulation. *Ann. Rev. Genet.* 11:29–48
11. Edgar, R. S., Lielausis, I. 1968. Some steps in the assembly of bacteriophage T4. *J. Mol. Biol.* 32:263–76
12. Epstein, R. H., Bolle, A., Steinberg, C. M., Kellenberger, E., Boy de la Tour, E., Chevalley, R., Edgar, R. S., Susman, M., Denhardt, G. H., Lielausis, A. 1963. Physiological studies of conditional lethal mutants of bacteriophage T4D. *Cold Spring Harbor. Symp. Quant. Biol.* 28:375–94
13. Esposito, M. S., Esposito, R. E. 1978. Aspects of the genetic control of meiosis and ascospore development inferred from the study of *spo* (sporulation-deficient) mutants of *Saccharomyces cerevisiae. Biol. Cellulaire* 33:93–102
14. Gallwitz, D., Seidel, R. 1980. Molecular cloning of the actin gene from yeast *Saccharomyces cerevisiae. Nucleic Acids Res.* 8:1043–59
15. Hartwell, L. H. 1974. *Saccharomyces cerevisiae* cell cycle. *Bacteriol. Rev.* 38:164–98
16. Hartwell, L. H. 1976. Sequential function of gene products relative to DNA synthesis in the yeast cell cycle. *J. Mol. Biol.* 104:803–17

17. Hartwell, L. H., Culotti, J., Pringle, J. R., Reid, B. J. 1974. Genetic control of the cell division cycle in yeast. *Science* 183:46–51
18. Hereford, L. M., Hartwell, L. H. 1974. Sequential gene function in the initiation of *S. cerevisiae* DNA synthesis. *J. Mol. Biol.* 84:445–61
19. Herskowitz, I., Hagen, D. 1980. The lysis-lysogeny decision of phage λ: explicit programming and responsiveness. *Ann. Rev. Genet.* 14:399–445
20. Hinnen, A., Hicks, J. B., Fink, G. R. 1978. Transformation of yeast. *Proc. Natl. Acad. Sci. USA* 75:1929–33
21. Hodgkin, J., Kaiser, D. 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): genes controlling movement of single cells. *Mol. Gen. Genet.* 171:167–76
22. Hodgkin, J., Kaiser, D. 1979. Genetics of gliding motility in *Myxococcus xanthus* (Myxobacterales): two gene systems control movement. *Mol. Gen. Genet.* 171:177–91
23. Hohn, B., Murray, K. 1977. Packaging recombinant DNA molecules into bacteriophage particles *in vitro*. *Proc. Natl. Acad. Sci. USA* 74:3259–63
24. Hong, J.-S., Ames, B. N. 1971. Localized mutagenesis of any specific small region of the bacterial chromosome. *Proc. Natl. Acad. Sci. USA* 68:3158–62
25. Horiuchi, T., Maki, H., Sekiguchi, M. 1978. A new conditional lethal mutator (*dnaQ49*) in *Escherichia coli* K-12. *Mol. Gen. Genet.* 163:277–83
26. Hosoda, J., Levinthal, C. 1968. Protein synthesis by *Escherichia coli* infected with bacteriophage T4D. *Virology* 34:709–27
27. Jarvik, J., Botstein, D. 1973. A genetic method for determining the order of events in a biological pathway. *Proc. Natl. Acad. Sci. USA* 70:2046–50
28. Jarvik, J., Botstein, D. 1975. Conditional-lethal mutations that suppress genetic defects in morphogenesis by altering structural proteins. *Proc. Natl. Acad. Sci. USA* 72:2738–42
29. Johnson, A. D., Poteete, A. R., Lauer, G., Sauer, R. T., Ackers, G. K., Ptashne, M. 1981. λ repressor and cro— components of an efficient molecular switch. *Nature* 294:217–23
30. Kaiser, D., Manoil, C., Dworkin, M. 1979. Myxobacteria: Cell interactions, genetics, and development. *Ann. Rev. Microbiol.* 33:595–639
31. Karn, J., Brenner, S., Barnett, L., Cesareni, G. 1980. Novel bacteriophage λ cloning vector. *Proc. Natl. Acad. Sci. USA* 77:5172–76
32. Kleckner, N., Roth, J., Botstein, D. 1977. Genetic engineering *in vivo* using translocatable drug resistance elements: new methods in bacterial genetics. *J. Mol. Biol.* 116:125–159
33. Kolter, R., Helinski, D. R. 1979. Regulation of initiation of DNA replication. *Ann. Rev. Genet.* 13:355–91
34. Kornberg, A. 1980. *DNA Replication.* San Francisco: Freeman. 724 pp.
35. Low, K. B. 1972. *Escherichia coli* K-12 F-prime factors, old and new. *Bacteriol. Rev.* 36:587–607
36. Mandel, M., Higa, A. 1970. Calcium-dependent bacteriophage DNA infection. *J. Mol. Biol.* 53:159–62
37. Maniatis, T., Hardison, R. C., Lacy, E., Lauer, J., O'Connell, C., Quon, D., Sim, G. K., Efstratiadis, A. 1978. The isolation of structural genes from libraries of eucaryotic DNA. *Cell* 15:687–701
38. Maurer, R., Osmond, B. C., Botstein, D. 1981. Genetic analysis of DNA replication in *Salmonella typhimurium.* See Ref. 2, pp. 375–86
39. Moir, D., Botstein, D. 1982. Determination of the order of gene function in the yeast nuclear division pathway using *cs* and *ts* mutants. *Genetics.* In press
40. Moir, D., Stewart, S. E., Osmond, B. C., Bostein, D. 1982. Cold-sensitive cell-division-cycle mutants of yeast: Isolation, properties, and pseudoreversion studies. *Genetics.* In press
41. Molineux, I., Kohiyama, M., eds. 1978. *DNA Synthesis, Present and Future.* New York: Plenum. 1161 pp.
42. Morris, N. R., Lai, M. H., Oakley, C. E. 1979. Identification of a gene for α-tubulin in *Aspergillus nidulans. Cell* 16:437–42
43. Newell, P. C. 1978. Genetics of the cellular slime molds. *Ann. Rev. Genet.* 12:69–93
44. Ng, R., Abelson, J. 1980. Isolation and sequence of the gene for actin in *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA* 77:3912–16
45. Ogawa, T., Okazaki, T. 1980. Discontinuous DNA replication. *Ann. Rev. Biochem.* 49:421–57
46. Saito, H., Uchida, H. 1978. Organization and expression of the *dnaJ* and *dnaK* genes of *Escherichia coli* K-12. *Mol. Gen. Genet.* 16:1–8
47. Sakakibara, Y., Mizukami, T. 1980. A temperature-sensitive *Escherichia coli* mutant defective in DNA replication:

dnaN, a new gene adjacent to the *dnaA* gene. *Mol. Gen. Genet.* 178:541–553
48. Scherer, S., Davis, R. W. 1979. Replacement of chromosome segments with altered DNA sequences constructed *in vitro*. *Proc. Natl. Acad. Sci. USA* 76:4951–55
49. Shortle, D., DiMaio, D., Nathans, D. 1981. Directed mutagenesis. *Ann. Rev. Genet.* 15:265–94
50. Simchen, G. 1978. Cell cycle mutants. *Ann. Rev. Genet.* 12:161–91
51. Stewart, C. R. 1969. Physical heterogeneity among *Bacillus subtilis* deoxyribonucleic acid molecules carrying particular genetic markers. *J. Bacteriol.* 98:1239–47
52. St. John, T. P., Davis, R. W. 1979. Isolation of galactose-inducible DNA sequences from *Saccharomyces cerevisiae* by differential plaque hybridization. *Cell* 16:443–52
52a. Sussman, M., Godfrey, S. 1982. The genetics of development in *Dictyostelium discoideum*. *Ann. Rev. Genet.* 16
53. Timberlake, W. E. 1980. Developmental gene regulation in *Aspergillus nidulans*. *Dev. Biol.* 78:497–510
54. Timberlake, W. E., Barnard, E. C.. 1981. Organization of a gene cluster expressed specifically in the asexual spores of *A. nidulans*. *Cell* 26:29–37
55. Tomizawa, J., Selzer, G. 1979. Initiation of DNA synthesis in *Escherichia coli*. *Ann. Rev. Biochem.* 48:999–1034
56. Wechsler, J. A. 1978. The genetics of *E. coli* DNA replication. In *DNA Synthesis, Present and Future*, ed. I Molineux, M. Kohiyama, pp. 49–70. New York: Plenum. 1161 pp.
57. Wickner, S. H. 1978. DNA replication proteins of *Escherichia coli*. *Ann. Rev. Biochem.* 47:1163–91
58. Zimmermann, C. R., Orr, W. C., Leclerc, R. F., Barnard, E. C., Timberlake, W. E. 1980. Molecular cloning and selection of genes regulated in *Aspergillus* development. *Cell* 21:709–15

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CONTENTS