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(Ph. D. Thesis)

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Contents

Contents

Contents

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ABSTRACT

Super-suppressor genes in Saccharomyces cerevisiae are known to phenotypically reverse the effects of about 25% of ultraviolet-induced mutants chosen at random. The action of one of these suppressors on 32 mutants at a single locus is determined and related to other genetic and biochemical properties of the mutants.

The tryptophan synthetase gene-enzyme system in yeast was selected for these studies because it has been proved so useful for studies of gene action in Escherichia coli and Neurospora crassa. The yeast system is found to be more like Neurospora than like E. coli.

In order to construct an allelic map of the tryptophan synthetase locus (tr_5) a new method is developed. This method is based on the use of x rays to induce allelic recombination at mitosis in heteroallelic diploids. The map is constructed from the slopes of x-ray dose-frequency curves.

Of 32 tr_5 mutants, 13 are suppressed to prototrophy by a single super-suppressor, S_d. One other is changed to a different mutant phenotype by the same suppressor. These suppressible mutants map along the entire length of the x-ray allelic map, with four of them being repeat mutations at a site near one end of the map. There is a strong exclusion between complementation and suppressibility. Only 2 of the 13 suppressible alleles gave any evidence of allelic complementation, whereas all

but five of the nonsuppressible ones complemented with at least one other.

Possible mechanism of super-suppression are discussed. The results of the experiments are used to evaluate the plausibility of different models. Biochemical experiments to help further test the validity of the most plausible ones are suggested.

INTRODUCTION

The Problem

Suppressor mutations restore the function lost as the result of a mutation at another locus. Although most suppressors studied have been specific for a single allele, Hawthorne and Mortimer (1963) described a class of suppressors in Saccharomyces cerevisiae that suppressed the phenotypes of about 25% of all the nutritional mutants they tested. These suppressible mutants represent 11 different phenotypes, and yet at any given locus the super-suppressors act only on certain alleles and not on others. They compared three independently isolated suppressors of this type, finding no specificity differences among them. Two of the three were shown to be allelic.

Multiple suppressor action, usually on a smaller scale, has been reported in other organisms. Early, more restricted, cases were found in Drosophila (E. B. Lewis, unpublished, cited in Wagner and Mitchell 1955), and in Neurospora (Mitchell and Mitchell 1952; Giles 1951). More recently, Campbell (1961) reported suppressors in certain strains of Escherichia coli K that suppress a variety of mutations both in the bacterial genome and in the bacteriophage lambda, when it is grown in the suppressor-bearing bacterial strain. Similar suppressors in E. coli suppress mutants of bacteriophage T4. These suppressors act on numerous "ambivalent" rII mutants (Benzer and Champe 1962), and also suppress certain mutants of the structural gene for alkaline phosphatase in the bacterium (Garen and Siddiqi 1962). Of 220 independently isolated phosphatase-negative mutants examined by Garen and Siddiqi, 15 were suppressed by the same suppressor. Although this is a much lower frequency of suppressibility than found in yeast mutants, Witkin (1963) has presented evidence suggesting this type of suppressible mutant may be more common among randomly selected auxotrophic strains derived from E. coli B/r and K-12. She studied a class of auxotrophs with particular reversion characteristics—a class comprising about one-third of all auxotrophs. About half of all double-auxotrophic mutants (obtained by serial selection for

this special class of mutant) would revert to prototrophy by a single-step event. Witkin proposed that multiple-action suppressors account for these reversions, a hypothesis supported by her further experiments.

Although no conclusive evidence has been presented that all these suppressors in E. coli–bacteriophage systems involve the same mechanism, they appear to be quite similar and demonstrate that super-suppression is not unique to yeast.

How widespread are super-suppressors? The limited cases in Drosophila and Neurospora quoted above could conceivably be examples. Many additional mutants would have to be tested to decide. Indeed, the fact that large-scale multiple suppression has not been reported in these and other organisms may merely reflect the difficulty of studying simultaneous segregation of many genes, rather than absence of super-suppression. In yeast it is possible to analyze tetrads for simultaneous segregation of more than 20 markers. This possibility facilitated the discovery of these suppressors (Hawthorne and Mortimer 1963). In bacteriophage the situation is even more favorable since the suppressible mutants are conditional lethals; they can reproduce in one host (the one with the suppressor), but not in other, closely related strains (which lack the suppressor). This system permits rapid screening of many mutant phages and suppressor-bearing bacteria (Campbell 1961).

Brody and Yanofsky (1963) have carried out a remarkably detailed examination of suppression of mutations affecting the A protein of tryptophan synthetase in E. coli. Although many of these mutants are suppressible, each suppressor appears to be specific for a single mutant (Yanofsky, Helinski and Maling 1961; Allen and Yanofsky 1963). Three suppressors that Brody and Yanofsky studied in detail were each tested against 50 nonidentical A mutants, with no occurrence of multiple suppression. These suppressors, then, are clearly different, at least in specificity, from those described above. The general approach to the study of suppressor action, employed in this case, however, proved very powerful and rewarding. By studying the A protein produced by mutant and suppressed strains, they were able to demonstrate

that the suppressible mutants form an A protein with altered primary structure (by a single amino acid substitution) and that the suppressor restores the wild-type structure in a small percent of the A-protein molecules synthesized.

Characterization of super-suppressors and the mutants they suppress has, for the most part, been limited to their genetic properties; only in E. coli alkaline phosphatase mutants have the enzymes been examined. Garen and Siddiqi (1962) measured enzyme and CRM (immunological cross-reacting material) activities in 15 suppressible P^- mutants, with and without the suppressor. When unsuppressed, the mutants produced essentially no enzyme and no CRM, but enzyme and CRM were restored by the suppressor to levels ranging from 3 to 100% of wild-type levels.

It has been suggested that suppressor genes may be involved in the genetic control of protein synthesis (Yanofsky and St. Lawrence 1960; Yanofsky, Helinski and Maling 1961; Benzer and Champe 1962; Brody and Yanofsky 1963). If this is true, then the elucidation of their detailed mechanisms may also require chemical studies of the protein-synthesizing machinery, and indeed such suppressors may in turn be expected to provide a powerful tool for studying the biochemical genetics of protein biosynthesis.

On the one hand, yeast may offer several advantages for such studies. One genetic advantage—the facility for studying simultaneous segregation of many loci—has already been discussed. In addition, Saccharomyces cerevisiae can be maintained vegetatively in several stable ploidies (Lindegren and Lindegren 1951), facilitating studies of dominance and gene dosage. Yeast cells are easy to culture in large quantities and contain relatively high concentrations of RNA (ribonucleic acid). These are advantages for biochemical purposes. On the other hand, a suitable gene-protein system has not been previously developed in this organism, nor has there been a really satisfactory method for allelic mapping in Saccharomyces, an important adjunct to studies of gene action.

Since a well-developed gene-enzyme system and a convenient sensitive method for allelic mapping are essential for most studies of gene action, development of these tools was necessary. The striking successes obtained with tryptophan synthetase systems in Neurospora crassa and E. coli (Yanofsky 1960; Bonner, Suyama and DeMoss 1960; Yanofsky and St. Lawrence 1960; Yanofsky, Helinski and Maling 1961; and Yanofsky, Henning, Helinski and Carlton 1963), together with the availability of tryptophan synthetase-defective mutants (tr_5) in yeast (Mortimer, Lerner and Barr 1957; Hawthorne and Mortimer 1960), made this system attractive. The choice of a suitable method for allelic mapping was less obvious.

Tryptophan Synthetase System

Properties of the Enzyme

In Neurospora crassa (DeMoss and Bonner 1959) and in E. coli (Yanofsky and Rachmeler 1958) the enzyme tryptophan synthetase has been shown to catalyze three reactions:

$$\text{Indole} + \text{serine} \longrightarrow \text{tryptophan} \qquad (1)$$

$$\text{Indole-3-glycerol phosphate} \underset{(r)}{\overset{(f)}{\rightleftharpoons}} \text{indole} + \text{triose phosphate} \qquad (2)$$

$$\text{Indole-3-glycerol phosphate} + \text{serine} \longrightarrow \text{tryptophan} + \text{triose phosphate} \qquad (3)$$

Reaction (3) is the one of physiological importance, since it has been demonstrated that free indole does not occur as an intermediate product in this reaction in vitro (Yanofsky and Rachmeler 1958; Crawford and Yanofsky 1958; DeMoss 1962). Pyridoxal phosphate is required as a cofactor for reactions (2) and (3) but not for reaction (1)(Yanofsky 1955; Yanofsky and Rachmeler 1958; Umbreit, Wood and Gunsalus 1946). In Neurospora the enzyme behaves as a single protein during purification (Mohler and Suskind 1960), and attempts to separate it into two components have been unsuccessful (Yanofsky 1960). However, Carsiotis, Appella and Suskind (1963) have reported studies that indicate the enzyme is comprised of two or more subunits. On the basis of kinetics studies, DeMoss (1962) proposed that the molecule has two different active sites, each with a different affinity for indole, and that

these sites correspond to reactions (1) and (2r). In contrast to Neurospora, the tryptophan synthetase of E. coli is readily separated into two different protein subunits, designated A and B (Crawford and Yanofsky 1958, Yanofsky 1959). Either component can be assayed for any one of the three reactions in the presence of an excess of the other component. Further, component A, by itself, has trace activity in catalyzing reaction (2), whereas component B by itself will catalyze reaction (1) very slowly. Only a mixture of the two components will catalyze reaction (3), and such a mixture has more activity in any of the three reactions than either component by itself (Yanofsky, Helinski, and Maling 1961).

The genetics and biochemistry of these systems have been extensively investigated, with particular attention to the characterization of mutationally altered proteins. Since several excellent reviews are available (Yanofsky 1960; Yanofsky and St. Lawrence 1960; Bonner, Suyama, and DeMoss 1960; Yanofsky, Helinski and Maling 1961; Yanofsky, Henning, Helinsky and Carlton 1963), the discussion here will be limited to aspects of these systems that motivated the selection of the yeast tryptophan synthetase system for the study of super-suppressors.

Effects of Mutations

Tryptophan synthetase mutants are generally isolated as tryptophan auxotrophs (the td mutants in Neurospora) and accordingly lack activity in reaction (3). Such mutants can be further classified, on the basis of the other two reactions, into three categories: (a) having no activity in reactions (1) and (2); (b) having some activity in reaction (1), conferring the ability to utilize indole; and (c) having some activity in reaction (2), resulting in the accumulation of indole in the growth medium when growth is limited by tryptophan (Yanofsky and Crawford 1959; Bonner, Suyama and DeMoss 1960). In Neurospora, most td mutants lack all three activities; mutants that accumulate indole and mutants capable of utilizing it are relatively rare (Rachmeler and Yanofsky 1959). In E. coli, however, all mutants either accumulate indole or can utilize it; mutants lacking all three activities are not

found (Yanofsky 1960).

The indole-utilizing mutants of E. coli have mutations affecting the A protein; either they make no A protein at all or they make an altered form that is enzymatically inactive itself, but capable of activating the normal B component. Indole-accumulating mutants have mutations altering the B protein (Yanofsky, Helinski and Maling 1961). In Neurospora, with what is apparently a single protein possessing both functional sites, the more rare indole-accumulating or indole-utilizing mutants appear to represent somewhat special classes of mutants in which the effect of the mutational alteration is localized in one region of the enzyme, and leaves another region functional. Consistent with this interpretation is the finding that mutants of these two types are localized in different halves of the allelic map of the Neurospora td locus (Bonner, Suyama and DeMoss 1960).

The existence of these easily distinguishable types of mutants is one of the features of the tryptophan synthetase system that makes it particularly attractive for the study of super-suppressors. Comparison of suppressible and nonsuppressible mutants on the basis of all available criteria should help eliminate some of the many prospect models for suppressor action.

Mutationally Altered Proteins

A particularly powerful tool for studying mutations has resulted from the application of immunochemical techniques to the study of mutationally altered protein. By the use of antisera containing specific antibodies against the tryptophan synthetase protein, it has been possible to detect and characterize an immunologically related protein in some mutants, a consequence of mutational alteration of the active enzyme (Suskind, Yanofsky and Bonner 1955; Suskind 1957; Yanofsky, Helinski and Maling 1961). In E. coli, mutationally altered A or B protein can be detected an assayed by its ability to activate the normal form of the other component in reaction (1) or (2) (Yanofsky and Crawford 1959). In Neurospora, however, this type of complementary activation does not occur, and altered enzymes lacking activity are detected by their immunological properties. This technique has made it possible to purify such

CRM and compare its properties with those of normal enzyme (Yanofsky and Stadler 1958; Mohler and Suskind 1960).

Examination of mutationally altered enzymes has been most extensive and most successful with the E. coli A protein. This is a relatively small protein that is easily purified (Henning, Helinski, Chao and Yanofsky 1962), and consists of a single polypeptide chain (Carlton and Yanofsky 1962). Yanofsky and various collaborators (see Yanofsky, Henning, Helinski, and Carlton 1963) have examined the protein produced by the wild-type bacterium (Helinski and Yanofsky 1962a) and by many mutants, using the "fingerprinting" techniques developed by Ingram (1956). They have been able to detect amino acid replacements due to forward mutation (Helinski and Yanofsky 1962**b**; Henning and Yanofsky 1962a), reversion (Henning and Yanofsky 1962b; Helinski and Yanofsky 1963; Allen and Yanofsky 1963), recombination (Henning and Yanofsky 1962b), and suppression (Brody and Yanofsky 1963). They have also studied altered electrophoretic properties of mutationally altered A protein (Henning and Yanofsky 1963). These studies have produced elegant experimental demonstrations of the genetic determination of the primary structure of proteins. The possibility that yeast tryptophan synthetase could be as favorable for this type of study was another consideration in its selection.

The use of immunochemical tests permits further subdivision of mutants, according to whether they produce a cross-reacting material (CRM+) or not (CRM-). In addition, genetic tests for allelic complementation, suppressor mutations, frequency and types of reversions, and allelic mapping provide further criteria for characterizing mutants.

Allelic Complementation

There is an interesting formal similarity between complementation and suppression, in that both appear to involve some kind of cytoplasmic interaction, between gene products, which restores a function. When genomes with different mutations of the same gene are in a common cytoplasma, function is sometimes partially restored. This phenomenon is allelic complementation, and perhaps more than any other single genetic test it reveals the complexity of the relationship between genotype and phenotype.

In vivo complementation. The test for this is achieved in diverse ways in different organisms: with heterokaryons in Neurospora, diploids in yeast and higher organisms, and abortive transductants in bacteria. It has been studied most extensively in Neurospora, notably at the ad-4 locus (Woodward, Partridge and Giles 1958; Woodward 1959; Partridge 1960), the pan-2 locus (Case and Giles 1960), the am locus (Fincham 1959), the ad-3B locus (de Serres 1963), the leu-2 locus (Gross 1962), among isoleucine-valine mutants (Wagner, Somers, and Bergquist 1960; Bernstein and Miller 1961), as well as among td mutants (Lacy and Bonner 1961; Yanofsky 1960). In the yeast Schizosaccharomyces pombe interallelic complementation has been demonstrated at both the ad_1 and ad_6 loci (Leupold 1961) and, in Saccharomyces cerevisiae at several ad loci (Dorfman 1963) and at the ar_4 locus and others (Mortimer, private communication). Examples in bacteria are the his B locus in Salmonella (Loper 1961) and the alkaline phosphatase mutants of E. coli (Garen and Garen 1963).

In most cases it has been possible to represent the results in the form of a one-dimensional array (complementation map) in which each mutant or group of mutants (with only a few exceptions--for example, see Gross 1962) is represented by a single uninterrupted line, so that overlapping groups do not complement and complementing groups do not overlap. Where comparisons have been possible, there has been general colinearity between the complementation and recombination maps (for example, Case and Giles 1960).

In vitro complementation. From the initial observations of complementation between genomes residing in separate nuclei that share a common cytoplasm in Neurospora heterokaryons, it became evident that it involved a cytoplasmic interaction, rather than a genetic one. This motivated a hypothesis of cytoplasmic recombination between gene products--RNA or polypeptide--"perhaps analogous to crossing over..." (Woodward, Partridge, and Giles 1958). However, when Woodward (1959) demonstrated in vitro complementation by recovering adenylosuccinase activity from extracts of mixed mycelia of ad-4 mutants, which had been cultured separately, an interaction between mutationally

altered proteins became most tenable. A more specific model was provided by the results of Singer and Itano (1959). They found that mixture of suitably labeled human hemoglobin molecules could be dissociated at acid pH and reassociated into hybrids, which resulted from exchange of asymmetrical subunits. Several additional cases of in vitro complementation have been reported, including Neurospora tryptophan synthetase (Suyama 1963), and glutamic dehydrogenase (am locus) (Fincham and Coddington 1963), Salmonella imidazoleglycerol phosphate dehydrase (his B mutants) (Loper 1961), and E. coli alkaline phosphatase (Schlesinger and Levinthal 1963). In the last case the investigators were able to demonstrate dissociation of the two differently altered enzymes and reassociation of subunits into an active hybrid. They further demonstrated that the hybrid formed in vitro had properties similar to those found by Garen and Garen (1963) for the enzyme formed in vivo by the same pair of mutants. Studies on other systems have demonstrated that enzymes formed by allelic complementation generally have properties quite different from the wild-type enzyme, supporting the model of their being hybrids of proteins that are normally composed of identical subunits (Partridge 1960; Fincham 1962; Fincham and Coddington 1963).

A case for altered proteins. Taken as a whole, the evidence from these diverse enzyme systems in bacteria, fungi, and humans would seem to justify the conclusion that allelic complementation has a universal mechanism. Therefore,

1. Any mutant that complements at least one other allele may be assumed to make an altered form of the corresponding enzyme, and
2. Enzymes for which complementation occurs are composed of two or more identical subunits.

This makes allelic complementation of particular interest in the present study, for it would appear to be an especially convenient detector of altered enzyme. Lacy and Bonner (1961) found that, among Neurospora td mutants, all that complemented in vivo also made CRM.

The concept of a cistron. The very useful concept of a cistron as a genetically defined unit of function proposed by Benzer (1957) on the basis of the cis-trans test of Lewis (1951) has been subjected to some confusion by the existence of allelic complementation. The use of the term "cistron" in reference to the subdivisions of allelic complementation maps (Woodward, Partridge, and Giles 1958; Catcheside and Overton 1958) has been subsequently criticized (Case and Giles 1960; de Serres 1963). Demerec and Hartman (1959) proposed the term complementation unit, which de Serres (1963) proposed shortening to complon, consistent with current genetical jargon. This still leaves open the question of the ambiguity of the cis-trans test as a definition of the unit of function. Fincham (1960) proposed a distinction between interlocus and intralocus complementation based on the nature of the enzyme produced. With interlocus complementation normal enzymes are produced and account for the activity. With intralocus complementation abnormal enzymes result from formation of hybrids of normally identical subunits. This, however, is not a genetic test but instead is a biochemical one and thereby lacks the general utility inherent in a purely genetic criterion. Distinction can be made, perhaps, on the basis of whether or not overlapping complementation groups exist, with the requirement that "true cistrons" cannot overlap. This can result in erroneous conclusions based on an insufficient number of mutants (as in the case of the ad_5-ad_7 locus in Saccharomyces, which now appears to be a single cistron; see Dorfman 1963).

Allelic Mapping in Yeast

Genetic Basis

Roman (1956b) analyzed a number of independently isolated adenine auxotrophs of Saccharomyces, which, by allelism tests, he could associate with seven genetic loci. When he combined two independently isolated mutants at the same locus (heteroalleles), in repulsion in the same cell (e.g., ad_{3-1}/ad_{3-3}), he found that this diploid had a much higher frequency of reversion to wild type during mitotic division than either of the homoallelic combinations (ad_{3-1}/ad_{3-1} or ad_{3-3}/ad_{3-3}).

He also found that heteroallelic diploids revert with greater frequency at meiosis (during sporulation) than do the homoallelic strains. Roman (1956b) investigated 119 individual cases of heteroallelic reversion by tetrad analysis of the prototrophic strains. He did not find any doubly defective genes. Had the prototrophs resulted from reciprocal crossing over between the alleles, followed by random segregation of parental and recombinant chromatids, half of the prototrophs should have carried the reciprocal, doubly defective gene. He proposed a mechanism of non-reciprocal recombination to explain his results. The phenomenon appeared quite similar to some observed in Neurospora (Mitchell 1955; St. Lawrence 1956).

Roman (1956b) noted that the reversion frequency depended in an orderly way on the particular pair of alleles, and he suggested the possibility of using the effect to study genetic fine structure. Leupold (1957, 1958) used both mitotic and meiotic reversion frequencies in heteroallelic strains of Schizosaccharomyces pombe to construct allelic maps. Measurement of spontaneous reversion frequencies during mitosis is complicated by the clonal distribution of revertants (Luria and Delbrück 1943). Also, in certain strains of Saccharomyces that do not sporulate readily, it is difficult to make accurate measurements of meiotic reversion frequencies. That these problems can be solved has been admirably demonstrated by Magni and von Borstel (1962) for homoallelic strains.

A New Approach

Roman and Jacob (1957) found that ultraviolet light stimulates heteroallelic reversion, and Mortimer (1959) observed a similar effect with x rays. Although for ultraviolet light a nonlinear dose-effect relation is observed, with sublethal doses of x rays the number of induced revertants is proportional to the dose.

A simple hypothesis that would predict such a linear response is that x rays produce lesions, distributed at random in the genetic material, and a lesion of the appropriate type anywhere in the region between the two mutations leads to a recombination. This hypothesis is suggested in part by the results of Jacob and Wollman (1955), who

found that ultraviolet light increases the frequency of recombination between different mutants in crosses of the temperate bacteriophage lambda. Their studies of the kinetics of recombination following irradiation of the parental phages support the conclusion that recombination occurs with high probability at the sites of radiation-induced lesions.

A further prediction is that the slope of the x-ray dose-effect curve is proportional to the distance between the two alleles, since the slope is the probability of producing a lesion in that region and that probability is assumed to be proportional to the length of the region. Therefore, it should be possible to construct allelic maps from the slopes of such x-ray curves for reversion of diploids bearing different combinations of heteroalleles.

Approach to the Problem

For the purpose of further defining the nature of super-suppressors and the types of mutants they suppress, and with the hope of being able to narrow the field of possible mechanisms for such suppression, the following experiments were undertaken and the results described in this dissertation:

1. A series of independent mutants of Saccharomyces affected in the ability to produce tryptophan synthetase were isolated.
2. These mutants were characterized with respect to
    a. Their position on an allelic map, constructed by the x-ray method,
    b. Allelic complementation, and
    c. Accumulation and utilization of indole.
3. Their suppressibility by a super-suppressor was determined and correlated with their other properties.

METHODS AND MATERIALS

Yeast Strains

Heterothallic strains of Saccharomyces cerevisiae were studied. All were obtained from Robert K. Mortimer of the University of California. Nomenclature and symbols for genetic markers in this yeast were standardized at the Carbondale Yeast Genetics Conference in 1961 (von Borstel 1963). These conventions, except the symbols for suppressors, will be followed.

The genetic markers, their symbols, and the phenotypes of the strains used in these experiments are listed in Tables A-1 through A-IX. The first subscript number following a symbol refers to the locus, the second to the allele. When only one allele is used the second number is omitted.

Media

The culture media used for these strains, their compositions, and their uses are as follows:

YEPD Complex medium for stock maintenance, routine culturing, and assaying viability: Difco yeast extract, 1%; Difco Bacto-Peptone, 2%; dextrose, 2%; and agar, 2% (agar was omitted for liquid medium).

YEPAD YEPD supplemented with adenine (20 mg/liter), used for culturing adenine auxotrophs to prevent selection of spontaneous revertants.

2× YEPAD YEPAD with all concentrations doubled.

GNA High-glucose medium, used for presporulation growth (Miller, Colvin, and Tremaine 1955): dextrose, 5%; Difco yeast extract, 1%; Difco Bacto-nutrient agar, 2.3%; and additional agar, 0.5%.

RA Sporulation medium (Fowell 1952): raffinose, 0.022%; sodium acetate, 0.3%; agar, 2% (agar omitted for liquid medium).

SC Synthetic complete medium, used as control medium when scoring nutritional phenotypes: MV supplemented with adenine, 20 mg/liter; arginine, 20 mg/liter; leucine, 30 mg/liter; lysine, 20 mg/liter; histidine, 10 mg/liter; methionine, 20 mg/liter; tryptophan, 20 mg/liter; threonine, 350 mg/liter; phenylalanine, 20 mg/liter; serine, 375 mg/liter; tyrosine, 20 mg/liter; and uracil, 20 mg/liter.

-X Omission media, used to score phenotypes of auxotrophs. SC with one of the supplements omitted. These media are designated by the omitted nutrient, e.g., -Tr means SC without tryptophan.

PET A medium containing glycerol as the carbon source, used to score respiratory deficiency (petites): glycerol, 3%; dextrose, 0.025%; Difco yeast extract, 1%; Difco Bacto-Peptone, 2%; and agar, 2%.

SM Starvation medium: dextrose, 5%; and KH_2PO_4, 0.05 M.

Genetic Methods

Isolation of Auxotropic Mutants

Tryptophan auxotrophs were induced by ultraviolet light and isolated by a procedure based on that by Mortimer, Lerner and Barr (1957). Mortimer (private communication) refined his original procedure by using the replica-plating method of Lederberg and Lederberg (1952). The strains from which new tryptophan auxotrophs were derived will be described in the next section; their genotypes are given in Table A-III.

These haploid strains were cultured for 48 hours at 30°C in 30 ml of liquid YEPD in 30-ml Erlenmeyer flasks. Vigorous swirling on a rotary shaker aerated the cultures. To minimize the number of spontaneous revertants, single-colony isolates were used for inocula. Cells were harvested by centrifugation, washed, and resuspended in distilled water. About 10 ml of a suspension containing 10^5 cells/ml was irradiated (in an open Petri dish) with ultraviolet light for 30 sec.

Survival was between 20 and 30% in different experiments. The irradiated suspension was diluted with cold distilled water, plated on YEPD, and incubated at 30°C for 2 days. Each plate, which then contained 150 to 200 visible colonies, was replica plated [by the velvetten method of Lederberg and Lederberg (1952)] onto -Tr. The two plates--master and replica--were incubated overnight. Each replica plate was screened for traces of colonies that had failed to grow, and the corresponding colonies on the master plates were sampled and streaked on YEPD for rescreening; 32 suspected tryptophan auxotrophs were streaked on each of these secondary masters. After overnight incubation, these were replica plated onto SC, -Tr, and -Ty (SC without tyrosine). The SC detected auxotrophs that required some nutrient, other than tryptophan, not included in the synthetic media. The -Ty detected mutants blocked before the branching point in the aromatic biosynthetic pathway; these mutants require tyrosine, phenylalanine, and para-aminobenzoic acid, in addition to tryptophan (Mortimer, Lerner and Barr 1957). Only those mutants that grew on SC and -Ty but not on -Tr were tested further.

Tests for Allelism and Allelic Complementation

The criterion for allelism was the trans part of the cis-trans test (Roman 1956a; Mortimer, Lerner, and Barr 1957), which is potentially ambiguous because of allelic complementation. If a non-complementing mutant happens to be chosen as the tester strain for a locus, no difficulty ensues. If a complementing mutant is selected, however, straightforward application of the criterion leads to the erroneous conclusion that two or more cistrons exist, where there is only one. Eventually, single-site mutants that fail to complement either group will be found and reveal the ambiguity. This appears to be the history of the ad_5-ad_7 locus in Saccharomyces (Roman 1956a; Dorfman 1963). The strains selected as testers for the tr loci appear to be noncomplementing. These strains and their genotypes are given in Table A-I.

The complementation test employed replica plating. Overnight streak cultures of the mutants to be tested and tester strains of opposite mating type were mixed on YEPD, with the aid of sterile toothpicks,

in an array of five columns (one for each tr locus) and as many as seven rows per plate (one for each mutant). These mass-mated cultures were incubated overnight and replica plated on -Tr and SC. Results were recorded on the second or third day.

The same procedure was used to test for allelic complementation. Doubtful cases, in which cross-feeding from other cultures on the plate appeared to contribute, were retested individually. To score a complementation test negative it is necessary to ascertain that mating has occurred. The mixed cultures on YEPD were examined under the microscope for zygotes after 3 to 6 hours. Yeast zygotes have a distinguishing morphology that makes them easy to recognize.

Isolation of Diploid Hybrid Clones

To establish diploid hybrid strains, haploids of opposite mating types were mass mated on YEPD. Three hours later individual zygotes were isolated by micromanipulation, and allowed to form colonies (Hawthorne and Mortimer 1960).

Tetrad Analysis

Hybrid strains, precultured 2 days on GNA slants, sporulated in liquid RA. At first, RA slants in capped vials were used, but sporulation was low. Later, liquid RA in cotton-plugged tubes was found to give better results, so this method was adopted. Asci were dissected by the method of Johnston and Mortimer (1959). After 3 days in liquid RA the cells and asci were resuspended in a solution of snail digestive juice. This digested the ascus wall, and left the spores free from the wall but still together. The digested sporulation mixture was spread on the edge of a thin YEPD slab with a sterile loop, and the slab, supported on a coverslip 40 by 22 mm, was inverted over a moist chamber. Spores from four-spored asci were then distributed at 2-mm intervals on the agar with a microneedle. The slab was lifted from the coverslip with a sterile spatula and placed, spores up, on a YEPD plate. After 2 or 3 days the spore colonies were picked with sterile toothpicks and streaked on YEPD for replica plating.

Nutritional phenotypes were assayed by replica plating on appropriate media. To score mating type, a pair of tester strains with

nutritional markers complementary to those segregating was selected. A YEPD replica of the master plate of spore cultures was prepared, and the cultures were cross streaked with each of the two testers--mating type a at the top and mating type α at the bottom. This operation was most conveniently accomplished with aluminum "combs" having blunt teeth spaced at the same intervals as the streaks of yeast on the plate. The tester strain was picked up with a "comb" from a confluent growth on a YEPD plate and cross streaked to eight spore cultures simultaneously. The cross-streaked plate was incubated overnight and replica plated onto MV. Only diploids, formed by matings between a and α haploids, grew.

Measurement of X-Ray-Induced Reversion Frequencies

The method for allelic mapping used in this work is based on x-ray-induced reversion in heteroallelic diploids (Manney and Mortimer 1954). Hybrids were cultured in 2X YEPAD, either from small inocula (100 to 500 cells) or from inocula that were essentially whole clones from single-cell isolates. Three different conditions were used: (a) 30 ml of suspension contained in a cotton-plugged 250-ml Erlenmeyer flask and incubated on a rotary shaker; (b) 10 ml of suspension contained in a cotton-plugged culture tube (22 mm by 175 mm) and incubated on an inclined rotating wheel; and (c) 10 ml of suspension contained in a metal-covered 50-ml Du Long flask and incubated on a vibrating shaker.

After 48 hours, or more, an aliquot of the suspension was diluted, centrifuged, and washed and resuspended in 10 ml of cold distilled water at a concentration of approximately 10^8 cells/ml. This suspension was serially diluted by factors of 10 or 100. Between 10^6 and 10^8 cells (the number depending on the alleles and the dose) were spread on -Tr. About 200 cells were spread on YEPD to assay viability. X rays from a beryllium-window tube (Machlett OEG 60) were delivered to the cells on the agar surface. The tube was operated at 50 kVp and either 5 or 20 mA. The dose rate at the position of the cells was about 52 r/sec at 5 mA and 200 r/sec at 20 mA. The dosimetry of these x rays was discussed by Mortimer (1953) and by Sayeg, Birge, Beam and Tobias (1959). The latter authors calculated

that the absorbed dose, in rads, is numerically equal to the air dose, in r, the standard error on the conversion factor being ± 10%.

YEPD plates were counted after 2 days and at intervals thereafter until there was no further increase in the number of colonies. Because additional colonies, which proved upon retesting to be auxotrophs, eventually appeared on some -Tr plates, the final count was recorded on the fifth day after plating. If there was any increase of prototrophic colonies after that time it was less than 1% and therefore negligible. Plates were incubated at 30°C.

Measurement of Growth Rates

Cell concentrations were routinely determined by counting appropriately diluted suspensions in a hemacytometer. Growth rates in liquid media were determined by measuring turbidity changes with a colorimeter (Bausch and Lomb Spectronic 20). Cultures were grown in tubes that could be placed in the colorimeter for direct reading of optical density. A calibration curve was constructed by plotting hemacytometer counts vs optical density (O. D.) readings (650 mμ) from measurements on a growing culture at various times (Fig. 1). This relationship is essentially linear between 3×10^6 and 1.5×10^7 cells/ml, corresponding to optical densities of 0.1 and 0.5.

Biochemical Methods

Tests for Accumulation of Precursors

Indoleglycerol phosphate. Mutant strains were grown in -Tr medium supplemented with tryptophan (4 mg/liter). Under these conditions tryptophan becomes growth limiting. Tubes were incubated for 2 days at 30°C and refrigerated overnight to allow the cells to settle. The medium was drawn off and 1 ml was mixed with 2 ml of ferric chloride reagent (1 ml of 0.5 M $FeCl_3$ plus 50 ml of water plus 30 ml of concentrated H_2SO_4; Yanofsky 1956). The development of either a pink or a red color was evidence of indoleglycerol or indoleglycerol phosphate (Yanofsky 1956; Gots and Ross 1957).

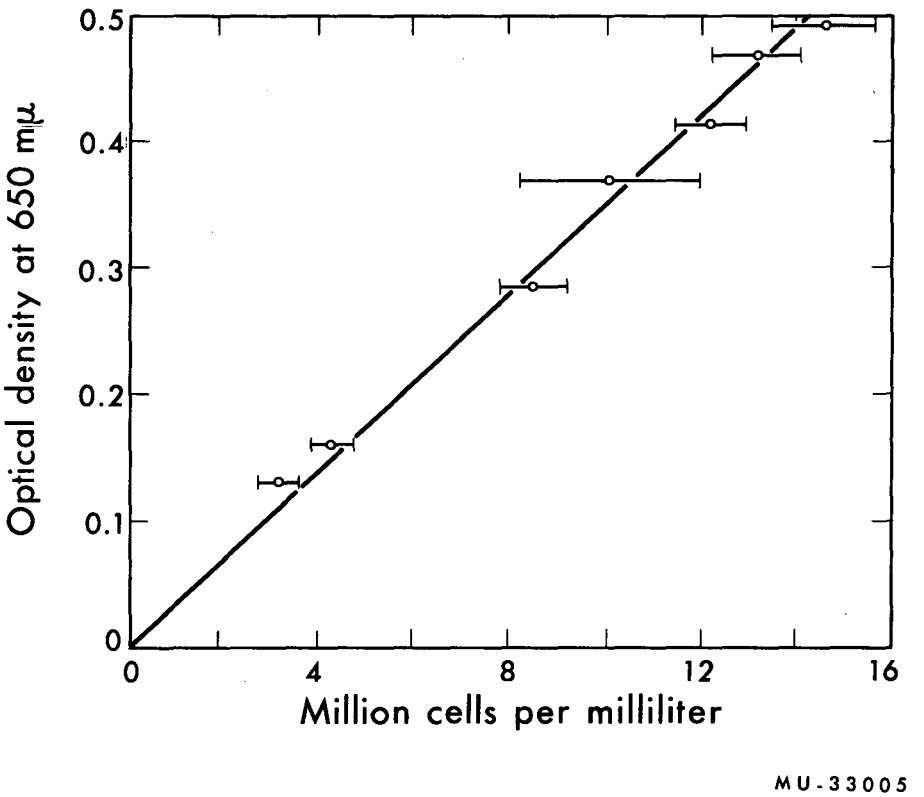

Fig. 1. Calibration curve for colorimeter (Bausch and Lomb Spectronic 20). Optical density at 650 mμ is plotted as a function of concentration of log-phase, diploid yeast in synthetic medium (SC).

Indole. One-milliliter samples of the tryptophan-limited growth media described above were tested for indole with 2 ml of color reagent (see method for tryptophan synthetase assay described below). Appearance of a red color was evidence of indole.

Test for Ability to Utilize Indole

Streak cultures, grown overnight on YEPD plates, were replica plated onto -Tr, and -Tr supplemented with indole (10 mg/liter. If growth was visibly greater on the supplemented medium the mutant was classified as indole utilizing.

Measurement of Tryptophan Synthetase Specific Activity

Preparation of crude extracts. Cultures were grown in liquid YEPD. One liter of YEPD in a 2 liter bottle was heavily inoculated from a fresh streak culture (YEPD). Gentle bubbling with filtered air kept the cells aerated and in suspension. After 2 days at 30°C, the cells were allowed to settle at 4°C. Most of the medium was decanted and the cells were resuspended and transferred to a polyethylene bottle for centrifugation. Throughout the rest of the procedure the material was kept between 0° and 5°C. After they were centrifuged and washed with distilled water, the cells were frozen to the walls of the bottle and lyophilized overnight. The lyophilized cells were then ground (dry) in a mortar in the cold room. Enough ground yeast was stirred into cold buffer [0.1 M PO_4 (potassium), pH 7.8; 0.01 M EDTA; 0.25 mg/liter pyridoxal phosphate; and 0.005 M reduced glutathione] to make a thin paste. This mixture was kept in an ice bath for 30 min and then centrifuged at 10,000 × g for 30 min at 3°C. The supernatant was used in the following assays.

Assay of tryptophan synthetase activity. The activity of crude extracts in converting indole (present in excess) to tryptophan in the presence of excess serine and pyridoxal phosphate was measured by a slightly modified version of the method by Yanofsky (1955) (Maling, private communication).

The reaction mixture [0.4 μM indole; 6.3 mg serine; 40 μg pyridoxal phosphate; 0.1 M PO_4 (potassium), pH 7.8; crude extract and water to give total volume of 1.0 ml] was mixed in an ice bath and then

incubated at 37°C for various times. To stop the reaction, 0.1 ml of 1% NaOH was added. Unconverted indole was extracted with 4.0 ml of toluene. The tube was shaken vigorously and allowed to stand until the mixture separated into two layers. The toluene (top) layer contained the indole. One milliliter of the toluene layer was transferred to a tube containing 4.0 ml of 95% ethanol and 2.0 ml of color reagent (36 g p-dimethylaminobenzaldehyde in 500 ml ethanol, 180 ml concentrated HCl, and water to make a total volume of 1 liter). The color was allowed to develop for 30 min, and the optical density measured at 565 mμ in a Bausch and Lomb Spectronic 20 colorimeter. The colorimeter was calibrated with indole solutions of known concentration. One unit of tryptophan synthetase activity was defined by the disappearance of 0.1 μM of indole in 20 min under the above conditions.

Assay of total protein. To compare extracts on the basis of specific enzyme activity (units of tryptophan synthetase activity per milligram of protein), it was necessary to measure the total-protein concentration of each extract. The method of Lowry, Rosebrough, Farr, and Randall (1951) was used. Protein solution (crude extract or bovine plasma albumin), diluted with distilled water, was mixed with fresh $CuSO_4$ reagent (2% Na_2CO_3 in 1 N NaOH and 0.5% $CuSO_4$ in 1% sodium tartrate, mixed 50:1 just before using) and allowed to stand at room temperature for 10 min. To this, 0.5 ml of Folin-Ciocalteu phenol reagent (Van Waters and Rogers, Inc., two parts reagent: three parts water) was added with a blowout pipet. After 30 min, the color was read at 750 mμ with a recording spectrophotometer (Beckman model DK-1). Protein standard solutions were prepared from crystalline bovine plasma albumin (Armour Pharmaceutical Co.). Calibration curves of optical density vs relative protein concentration (Fig. 2) were nonlinear and somewhat different for bovine plasma albumin and a crude yeast extract. This difference introduced a relative error of about 3.5% over the range of concentrations of bovine plasma albumin between 50 and 200 μg per ml.

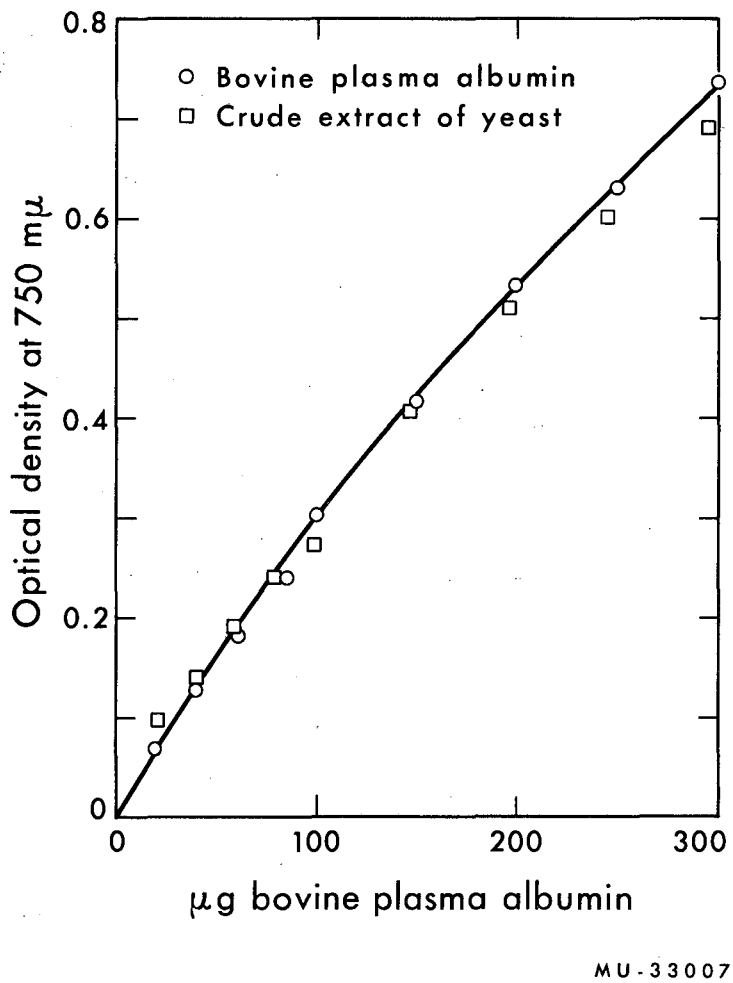

Fig. 2. Protein standard curve for determining protein by method of Lowry, Rosebrough, Farr and Randall (1951). Results for a dilution series of crude extract of yeast, diluted parallel to the bovine plasma albumin are shown for comparison.

TRYPTOPHAN SYNTHETASE MUTANTS OF SACCHAROMYCES

Genetics

Genealogy

Thirty-six ultraviolet-induced tr_5 mutants were studied, 14 from Mortimer's stocks and 22 original isolates. Mortimer's early mutants were isolated from a haploid strain, S288C (Mortimer, Lerner and Barr 1957), which has no amino acid, purine, or pyrimidine requirements, and grows satisfactorily on MV medium. Later, he derived mutants from genetically marked strains, themselves mutants of S288C (see Fig. 3). Following this practice, I isolated seven mutants from the doubly marked S288C derivative, JB97 (ad_{2-2} le_1). All these mutants, like S288C, have the α mating-type allele.

It was desirable (for the allelic-mapping procedure to be described in a following section) for some of the mutants to have the a-mating-type allele. Two of Mortimer's tr_5 mutants, JC2 and JC20, were derived from S1285C, mating-type a, but they grow in clumps and are difficult to use. Additional a-mating-type mutants were therefore derived from a spore isolate, XT300-4A, which is closely related to S288C. The 36 tr_5 mutant strains, their allele designations, parents, and genotypes are listed in Tables A-III and A-IV. Their genealogy is represented in Fig. 3.

Linkage and Allelism

Mutant DT29 (tr_{5-1}) is the tester allele for this locus and has been used extensively in linkage studies (Hawthorne and Mortimer 1960). Every mutant in Table A-IV fails to complement with DT29. Hawthorne and Mortimer (1960) mapped the tr_5 locus on the left arm of chromosome VII, about 15 centimorgan units from the centromere, distal to le_1, which is 4 units from the centromere. Among tetrad data for 10 different tr_5 alleles, I found 39 parental-ditype (PD), 17 tetra-type (T) and 0 nonparental-ditype (NPD) asci, for segregation with le_1, in satisfactory agreement with their results for DT29.

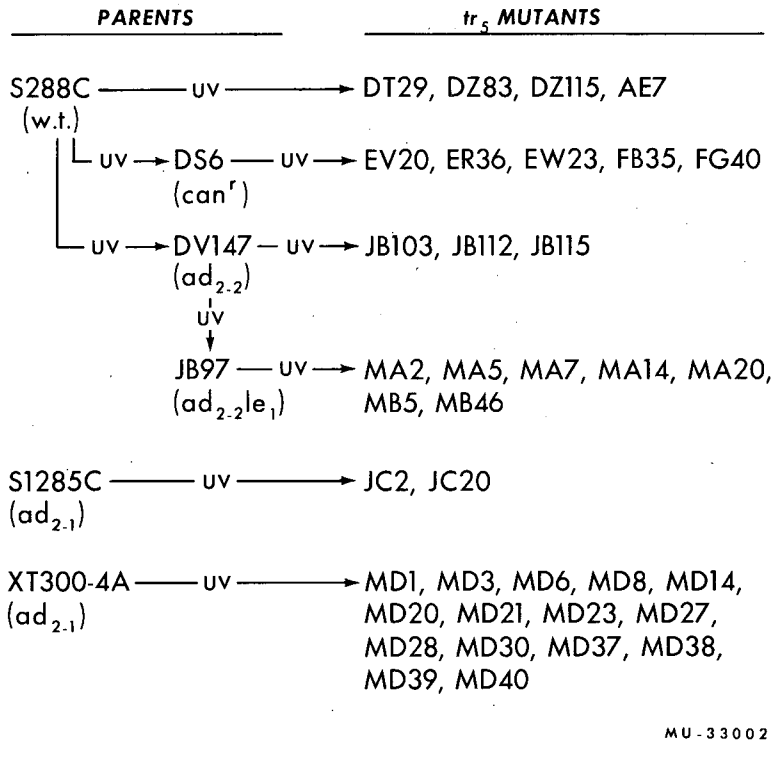

Fig. 3. Genealogy of tr_5 mutants.

Allelic-Complementation

These alleles were tested for complementation in diploids representing all possible trans combinations, by use of the mutants and strains derived from them by dissection of hybrid asci. Most combinations were tested at least twice, and all positive tests were confirmed by retesting, in many cases by isolating zygotes and testing diploid clones. In all cases—positive and negative—formation of zygotes was confirmed by microscopic observation. The results of these tests are summarized in Fig. 4. Of the 35 alleles tested, 19 complement with at least one other and 17 do not. It is of interest to notice, however, that if merely three alleles—tr_{5-7}, tr_{5-11}, and tr_{5-18} or (tr_{5-29})—had been omitted, no complementation would have been found. Some of the 17 that gave negative results may yet give positive results if tested with a sufficiently large number of alleles. When the number of alleles tested is small, negative results are inconclusive.

A complementation map representing these results is shown in Fig. 5. Each allele is represented by a line. If two alleles did not complement, their lines overlap. If they did complement, their lines do not overlap. The bottom line represents the alleles that did not complement any others. Since every allele can be represented by a continuous line, the map is linear. It will be compared with a recombination map in the next chapter.

Allelic Mapping and Suppressors

In subsequent sections, the sequence of these alleles, their linkages to each other, and the action of suppressors on them will be described.

Biochemistry

Properties of Mutants

All the mutants accumulated indoleglycerol phosphate (InGP) in the growth medium, as evidenced by the $FeCl_3$ test. Three of them—JC2 (tr_{5-6}), FB35 (tr_{5-18}), and MD14 (tr_{5-26})—also accumulated indole (In). One mutant—MA14 (tr_{5-11})—grew slowly on -Tr medium supplemented with indole (10 mg/liter). Several grew slowly on -Tr without supplementations.

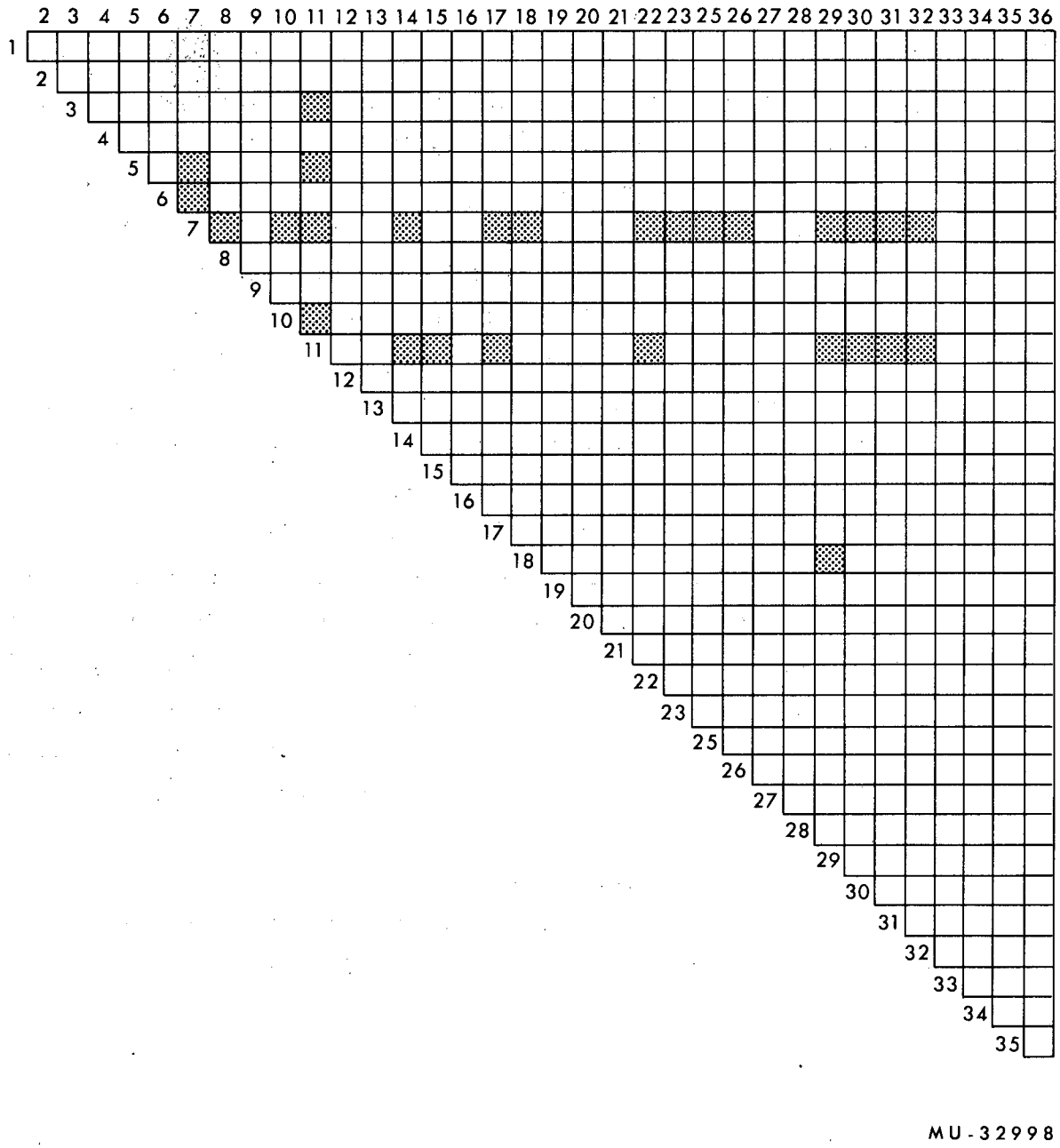

Fig. 4. Results of all possible allelic complementation tests with 35 tr_5 mutants in heteroallelic diploids. Squares corresponding to complementing pairs are shaded. Open squares correspond to pairs that did not complement each other.

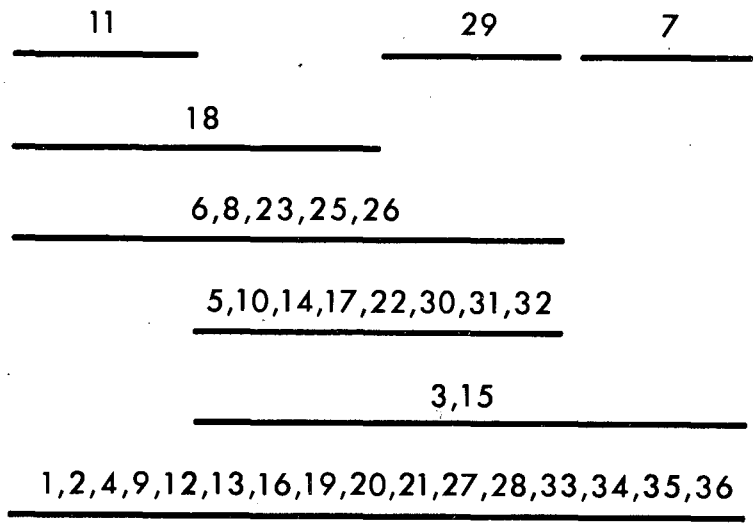

Fig. 5. Complementation map constructed from results in Fig. 4. Overlapping lines represent combinations that gave only negative results. Nonoverlapping lines represent combinations that gave positive results.

Properties of Crude Extracts

Crude extracts from the parent strain, S288C, and from two mutants, DT29 (tr_{5-1}) and EV20 (tr_{5-2}), were tested for ability to convert indole in the presence of serine and pyridoxal phosphate. Figure 6 illustrates the activity of the S288C extract. On the ordinate is plotted the amount of indole removed per ml of crude extract; on the abcissa, time of incubation at 37°C.

Results of tryptophan synthetase assays on three crude extracts are summarized in Table I. The trace of activity measured for EV20 is probably not significant, as it corresponds to the difference between optical densities of 0.475 and 0.485 in the colorimetric measurement of indole. Both mutants are clearly affected in their ability to make active tryptophan synthetase.

Summary and Conclusions

The results of these genetic and biochemical tests are summarized in Table II. On the basis of allelic complementation alone, 35 mutants are divided into eight different groups (Fig. 5). Some of these are divided into subgroups on the basis of indole accumulation and utilization. It will be seen in following sections that even further subdivision is possible.

The finding of indole-accumulating and indole-utilizing mutants indicates that in yeast, as in Neurospora and E. coli, tryptophan synthetase possesses three catalytic activities. The pattern of allelic complementation indicates that these three activities are embodied in a single protein molecule, whose structure is specified by a single gene. If separate genes specified separable proteins with these functions one would expect the indole-accumulating mutants to complement with the indole-utilizing mutant, but they do not. That most mutants lack all three activities is consistent with this view. In these respects, yeast appears to be more like Neurospora than like E. coli. This is perhaps not surprising, as yeast and Neurospora belong to the same phylogenetic class-ascomycetes.

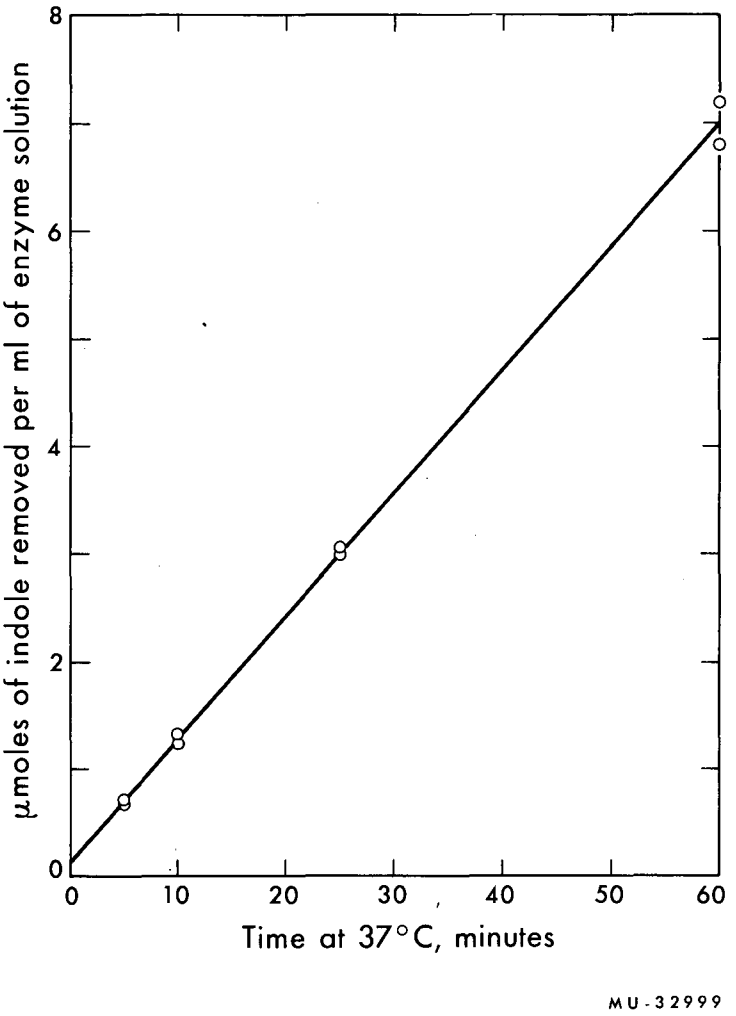

Fig. 6. Assay curve for tryptophan synthetase activity in crude extract from wild-type Saccharomyces cerevisiae (S288C). Assay conditions are detailed in text.

Table I. Tryptophan synthetase specific activities in wild-type and tr_5 mutant strains of Saccharomyces.

Extract from	Volume assayed (ml)	Indole removed in 20 min (μM)	Protein (mg/ml)	Specific activity (units per mg protein)[a]
S288C	0.06	0.22	38	0.97
EV20	0.3	0.01	66	--
DT29	0.3	0.00	44	--

a. One unit corresponds to the disappearance of 0.1 μM of indole in 20 min.

Table II. Genetic and biochemical properties of tr_5 mutants.

Properties	Number of mutants	Alleles
I. Noncomplementing	16	1, 2, 4, 9, 12, 13, 16, 19, 20, 21, 27, 28, 33, 34, 35, 36
II. Complementing		
A. Indole utilizing	1	11
B. Indole accumulating	3	6, 18, 26
C. Others	15	3, 5, 7, 8, 10, 14, 15, 17, 23, 25, 26, 29, 30, 31, 32
Total	35	

ALLELIC MAPPING

Preliminary Studies

Mortimer's studies (1959, 1961) of x-ray-induced reversion in heteroallelic diploid yeast were primarily restricted to a single pair of alleles: ar_{4-1} and ar_{4-2}. Although he consistently obtained linear dose-frequency curves for these heteroalleles (private communication), it remained to be demonstrated that they were not unique in this respect. Further, for the slopes of such dose-frequency curves to be used to determine the sequence of mutational sites required that they be additive, i.e., given any three alleles (a_1, a_2, and a_3), the curves for two of the three possible heteroallelic combinations (say, a_1/a_2 and a_2/a_3) should have slopes whose sum equals the slope for the third combination (a_1/a_3). This remained to be tested. Finally, before the method could be used generally, the influence of some genetic and physiological variables had to be investigated.

Dose-Frequency Curves: Linearity and Additivity of Slopes

For these preliminary experiments, heteroallelic diploids were obtained from Mortimer. Three heteroallelic combinations—ar_{4-1}/ar_{4-2}, ar_{4-2}/ar_{4-3}, and ar_{4-1}/ar_{4-3}—and two homoallelic combinations for comparison—ar_{4-1}/ar_{4-1} and ar_{4-2}/ar_{4-2}—were used. Complete genotypes for these diploids are in Table A-5. Dose-frequency curves for x-ray-induced reversion to arginine-independence for these strains are plotted in Fig. 7 from data given in Table III. Survival of colony-forming ability on complete medium (YEPD) was greater than 95% at all doses shown.

The curves for heteroallelic combinations are clearly linear over the range of doses used. The curve for ar_{4-2}/ar_{4-2} was significantly nonlinear, but, as may be seen in Fig. 7, this has a negligible effect on the heteroallelic curves. These curves can be represented by the following equation

$$F = F_0 + \beta D$$

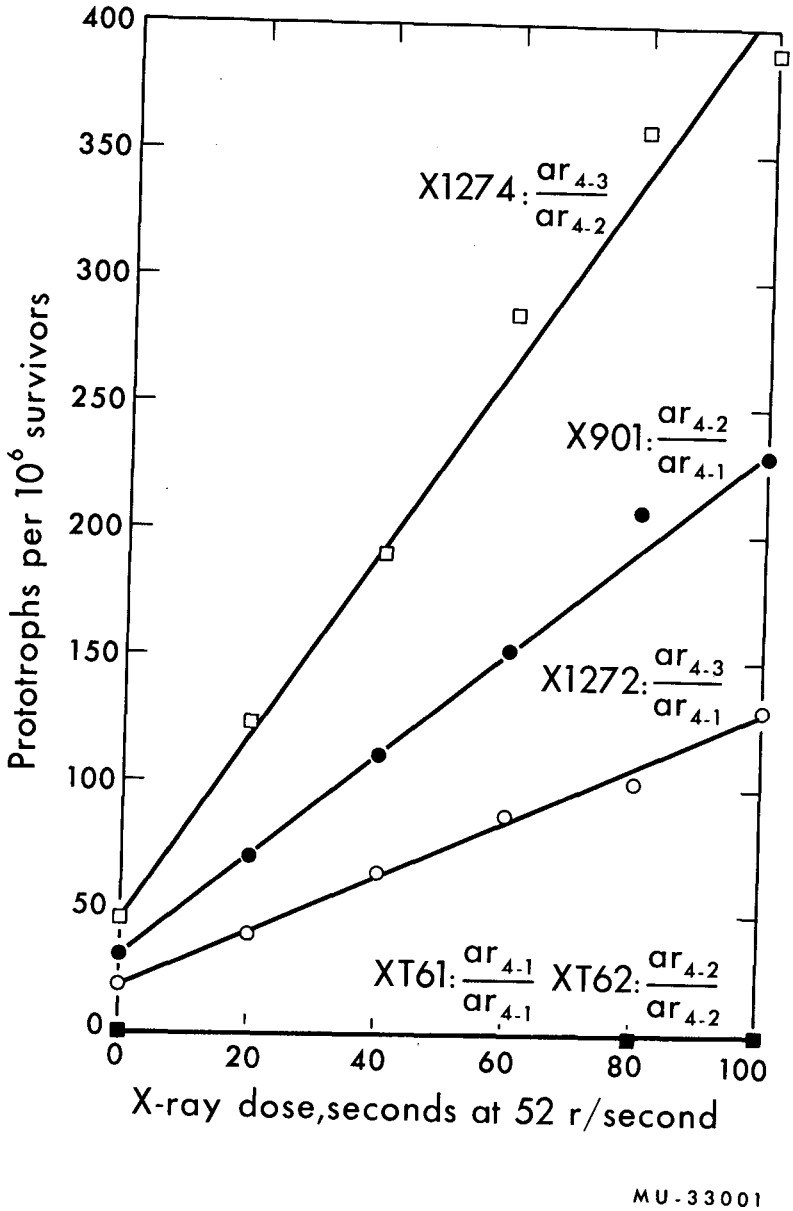

Fig. 7. Dose-frequency curves for x-ray-induction of mitotic reversion in ar_4 heteroallelic and related homoallelic diploid strains of Saccharomyces.

Table III. Frequencies of x-ray-induced reversion in heteroallelic ar_4 hybrids.

X-ray dose (sec)[a]	Prototrophs per 10^6 survivors				
	X901 $\frac{ar_{4-1}}{ar_{4-2}}$	X1272 $\frac{ar_{4-1}}{ar_{4-3}}$	X1274 $\frac{ar_{4-2}}{ar_{4-3}}$	XT61 $\frac{ar_{4-1}}{ar_{4-1}}$	XT62 $\frac{ar_{4-2}}{ar_{4-2}}$
0	30	19	21	<0.1	0.02
20	71	40	123	-	0.1
40	112	65	192	-	0.4
60	154	87	286	-	0.8
80	209	102	358	0.48	1.2
100	231	131	390	1.4	2.6

a. Dose rate = 52 r/sec.

where F is the frequency of prototrophs per 10^6 survivors after a dose, D, of x rays, and F_0 is the frequency of prototrophs in the same culture before irradiation. The slope, β, is the radiosensitivity for reversion and has units of prototrophs per 10^6 survivors per unit of dose. Since x-ray exposures were administered in units of time (sec) at a constant dose rate (either 52 or 200 r/sec), it is convenient to express the dose in sec.

The conditions for additivity are also satisfied by the results in Fig. 7

$$\beta\,(ar_{4\text{-}1}/ar_{4\text{-}3}) + \beta\,(ar_{4\text{-}1}/ar_{4\text{-}2}) = (1.10 \pm 0.04) + (2.03 \pm 0.08)$$
$$= 3.13 \pm 0.09$$

and $$\beta\,(ar_{4\text{-}2}/ar_{4\text{-}3}) = 3.7 \pm 0.23.$$

The discrepancy only slightly exceeds the standard deviations. These results predict the order $ar_{4\text{-}3}\ ar_{4\text{-}1}\ ar_{4\text{-}2}$, and this has been confirmed by three-point tests (Manney and Mortimer 1964) with the cis double mutant $ar_{4\text{-}1}\ ar_{4\text{-}2}$.

Genetic and Physiological Factors

Diploids, heteroallelic for the same pair of alleles but with differing nutritional phenotypes and genotypes at other loci, were derived. The complete genotypes for two strains, XT294 and XT297, with the same tr_5 heteroalleles ($tr_{5\text{-}8}/tr_{5\text{-}10}$) are given in Table A-V. Although the slope, when measured in separate experiments, was reproducible for either of these strains, XT297 was consistently more sensitive than XT294. Dose-frequency curves from two experiments are plotted in Fig. 8. XT297 is more than three times as sensitive as XT294. Although this was the most extreme example found, other strains, with these and other alleles, revealed a similar influence of the genetic background.

The differences in the nutritional phenotypes of these two strains suggested that the physiological state of the culture may influence its sensitivity. To examine this, the sensitivity of XT294 was measured first when the culture was just reaching the plateau of the growth curve

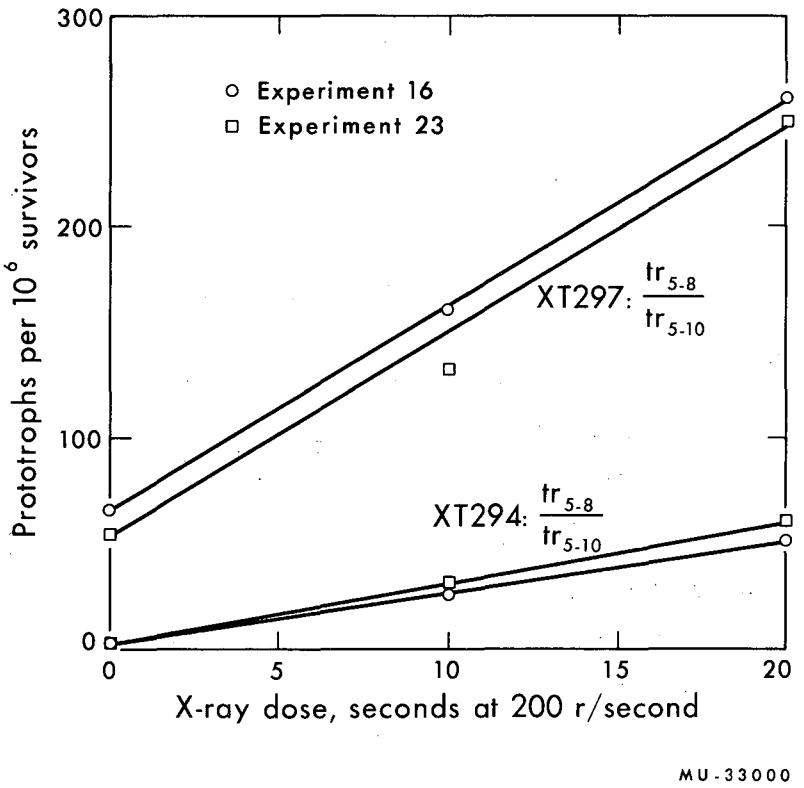

Fig. 8. Dose-frequency curves for x-ray-induction of mitotic reversion in two strains of Saccharomyces that are heteroallelic tr_{5-8}/tr_{5-10}. These illustrate possible influence of genotype at other loci.

(36 hours in YEPD), again 12 hours later, and subsequently after 24 and 48 hours of nitrogen starvation in dextrose-phosphate buffer (SM). After nitrogen starvation in this buffer, haploid yeasts are nearly three times as resistant to killing by x rays as when freshly harvested late in the logarithmic phase of growth (Elkind and Beam 1955). The influence of these conditions on the x-ray reversion sensitivity of XT294 is shown in Table IV. The reversion sensitivity was not appreciably influenced by this starvation.

In summary, then, it appears that the sensitivity of a particular heteroallelic combination can be profoundly influenced by the genotype (at other loci), but not by physiological conditions known to be determinants of radiosensitivity for killing.

Mapping tr_5 Mutation Sites

Toward a Uniform Genetic Background

The preliminary studies demonstrated that the genetic background of heteroallelic hybrids influences their reversion sensitivity. Diploids for mapping experiments were therefore constructed by mating mutant strains of mating type a with mutant strains of mating type α. Since all a mutants, except JC2 and JC20, were derived from XT300-4A and all α mutants were derived (by one or more rounds of selection) from S288C, this procedure minimized genotypic and phenotypic differences. The only phenotypic difference was that some hybrids, being hemozygous ad_2/ad_2, required adenine, whereas others, being heterozygous ad_2/AD_2, did not. The culture medium was supplemented with adenine (YEPAD) to minimize possible effects of this difference.

Two-Point Mapping

Limiting the hybrids to those that could be made directly from two mutant strains precluded, in principle, direct verification of additivity. Therefore, the following scheme was adopted to give indirect verification. In each of three series of experiments a pair of reference mutants of one mating type was chosen and several mutants of the other mating type were mapped against each of them. The distance between the two reference mutants was thereby determined, indirectly, as

Table IV. Influence of culture age and of starvation on the reversion sensitivity of XT294.

Culture conditions	Prototrophs per 10^6 survivor's per second
YEPD, 36 h	3.1 ± 0.06
YEPD, 48 h	3.0 ± 0.06
YEPD, 48 h + dextrose-PO_4, 24 h	3.8 ± 0.2
YEPD, 48 h + dextrose-PO_4, 48 h	2.9 ± 0.3

either the sum or the difference of their distances from a third mutant, the choice of sum or difference depending on whether that mutant was between the reference mutants or not. In this way, the distance was independently measured with each mutant mapped. In each series of experiments a different pair of reference mutants was selected, but the pairs overlapped and were mapped against each other so that a single map could be constructed.

In the first series, the reference mutants were JC2 (tr_{5-6}) and JC20 (tr_{5-7}), both mating-type a. Five mutants of mating-type α were mapped against this pair. The results are summarized in Table V. The data from which this table was derived are tabulated in Table A-X. Slopes and standard deviations were calculated by the method of least squares. The errors of the individual sums and differences were calculated by applying a propagation-of-error formula* to the standard deviation of the two slopes.

In the second series MB5 (tr_{5-20}) and MB46 (tr_{5-21}) were selected as reference points and 15 mutants of mating-type a were mapped against them. These results are summarized in Table VI, and complete data are tabulated in Table A-XI. In some cases, the measured slopes were based on a single dose point, so the least-squares analysis was not applicable. These values were excluded when the mean of the sums or differences was calculated. Owing to the small distance between 20 and 21 relative to many of the other distances in this series, these data do not unambiguously determine the sequence of all the alleles.

The results of the third series of experiments are similarly summarized in Table VII and tabulated in Table A-XII. In this series the reference mutants were MD39 (tr_{5-35}) and MD40 (tr_{5-36}), mating-type a; and eight mating-type α mutants were mapped. Of the three series, this one had the most favorable choice of reference points, and, consequently, these data provide the best confirmation of the additivity of the method.

* $\sigma_p^2 = \sum_i \sigma_i^2$ where σ_p is the propagated error and σ_i is the least-squares standard deviation.

Table V. X-ray mapping data with JC2 and JC20 used as reference mutants.

Mutant	tr_5 Genotype	×JC2 (tr_{5-6})		×JC20 (tr_{5-7})		Sum or difference
		Hybrid	Prototrophs per 10^6 survivors per sec	Hybrid	Prototrophs per 10^6 survivors per sec	
MA5	5-9	XT393	3.4 ± 0.08		10.2 ± 0.8	6.8 ± 0.8
MA20	5-12	XT395	2.5 ± 0.6	XT396	1.1 ± 0.1	3.6 ± 0.6
MB5	5-20	XT397	7.0 ± 0.1	XT398	12.7 ± 0.6	5.7 ± 0.6
MB46	5-21	XT399	9.8 ± 1.7	XT400	15.1 ± 0.5	5.3 ± 1.8
FB35	5-18	XT403	0.6 ± 0.05			
					Mean	5.4 ± 1.3

Table VI. X-ray mapping data with MB5 and MB46 used as reference mutants (experiment 46).

Mutant	tr_5 Genotype	× MB (tr_{5-20})		× MB46 (tr_{5-21})		Sum or difference
		Hybrid	Prototrophs per 10^6 survivors per sec	Hybrid	Prototrophs per 10^6 survivors per sec	
MD1	5-22	XT418	2.0 ± 0.4	XT419	7.1 ± 0.3	5.1 ± 0.5
MD3	5-23	XT412	8.8 ± 0.2	XT413	11.0 ± 0.6	2.2 ± 0.6
MD6	5-24	XT414	0.68± 0.12	XT415	5.0 ± 0.7	4.3 ± 0.7
MD8	5-25	XT416	11.0 ± 1.1	XT417	12.5 ± 0.1	1.5 ± 1.1
MD14	5-26			XT421	12.2 ± 2.5	
MD20	5-27	XT422	3.5 ± 0.32	XT423	0.8 ± 0.3	2.7 ± 0.4
MD21	5-28	XT424	12.6 ± 0.2	XT425	10.6 ± 0.4	2.0 ± 0.5
MD23	5-29	XT426	13.1 ± 0.7	XT427	16.5 ± 0.2	3.4 ± 0.7
MD27	5-30	XT428	9.4 ± 0.3	XT429	11.5	2.1[a]
MD28	5-31	XT430	10.7 ± 0.4	XT431	13.7	3.0[a]
MD30	5-32	XT432	11.6 ± 0.05	XT433	13.9 ± 0.2	2.3 ± 0.2
MD37	5-33	XT434	9.5 ± 0.8	XT435	10.4 ± 1.1	0.9 ± 1.3
MD38	5-34			XT437	7.6 ± 1.2	
MD39	5-35	XT438	2.4 ± 0.14	XT439	5.3 ± 0.1	2.9 ± 0.17
MD40	5-36	XT440	5.5		0.0[b]	5.5[a]
					Mean	2.7 ± 1.3

a. Not included in average.

b. See Table IX for values for this group.

Table VII. X-ray mapping data with MD39 and MD40 used as reference mutants (experiment 57).

Mutant	tr_5 Genotype	× MD39 (tr_{5-35}) Hybrid	× MD39 (tr_{5-35}) Prototrophs per 10^6 survivors per sec	× MD40 (tr_{5-36}) Hybrid	× MD40 (tr_{5-36}) Prototrophs per 10^6 survivors per sec	Sum or difference
MA5	5-9	XT469	0.4 ± 0.01	XT470	7.0 ± 0.12	7.4 ± 0.1
MA14	5-11	XT459	4.0 ± 0.06	XT460	3.0 ± 0.17	7.0 ± 0.2
ER36	5-16	XT471	3.0 ± 0.3	XT472	10.3 ± 0.8	7.3 ± 0.9
FG40	5-19	XT463	1.7 ± 0.12	XT464	4.9 ± 1.2	6.6 ± 1.2
MB5	5-20	XT438	2.4 ± 0.2	XT440	5.0 ± 0.12	7.4 ± 0.2
MB46	5-21	XT439	7.2 ± 0.5	a	0.0[a]	7.2 ± 0.5
EV20	5-2	XT465	10.8 ± 0.8	a	0.0[a]	10.8 ± 0.8
JB112	5-4	XT467	7.0 ± 0.9	a	0.0[a]	7.0 ± 0.9
					Mean	7.6 ± 1.3

a. See Table IX for this group.

To verify some of the results obtained in these three series of experiments, and to map additional alleles, another series was conducted. This time the induced-reversion frequency was measured at one dose point only. Consequently, the least-squares analysis could not be applied. These results are summarized in Table VIII and the data tabulated in Table A-XIII.

Hot Spot: Contribution of Homoallelic Reversion

Homoallelic-reversion sensitivities, as observed with the ar_4 alleles, are usually lower by orders of magnitude than most heteroallelic sensitivities. A few combinations of mutants, however, had very low heteroallelic sensitivities. In these cases the homoallelic sensitivity had to be determined, and the heteroallelic value corrected appropriately. Homoallelic reversions presumably result from back-mutations or from dominant suppressors. These would be expected to be the same for a given allele whether homoallelic or heteroallelic.

In the above results there are two cases in which the homoallelic correction is of special interest. The alleles 2, 4, and 21 all appeared to be extremely close to 36. These combinations has sensitivities an order of magnitude smaller than any others and nearly three orders of magnitude smaller than some. The alleles 6 and 18 also appeared close together, although not as close as the others just mentioned. These two, however, accumulate indole and are of interest for that reason.

Table IX summarizes the results obtained with homoallelic and heteroallelic strains of these alleles. The data are tabulated in Table A-XIV. There are no significant differences between homoallelic and heteroallelic combinations of the alleles 2, 4, 21, and 36. Either they are repeat mutations at the same site or they are so close together that they cannot be resolved by this method. The homoallelic reversion sensitivity of 6 is about four times that of 18 and is still small relative to the heteroallelic sensitivity of this pair (0.6 ± 0.05).

Additivity of Intervals

In each series of experiments, the distance between the reference alleles has been estimated from the sum or difference of the distances between each reference allele and another allele. This assumes

Table VIII. X-ray mapping data for miscellaneous hybrids.

a Mutant	a Mutant	Hybrid	Genotype	Prototrophs per 10^6 survivors per sec	Expt.
JC2	FB35	XT518	$\frac{5-6}{5-18}$	0.52	63
JC2	DZ83	XT526	$\frac{5-6}{5-13}$	2.2	69
JC2	JB103	XT528	$\frac{5-3}{5-6}$	4.5	69
MD39	JB103	XT531	$\frac{5-3}{5-35}$	1.0	70
MD39	FB35	XT540	$\frac{5-18}{5-35}$	5.4	70
MD40	FB35	XT541	$\frac{5-18}{5-36}$	11.5	70
MD21	FB35	XT538	$\frac{5-18}{5-28}$	2.7	70
JC20	FB35	XT536	$\frac{5-18}{5-7}$	8.9	70
JC20	MA20	XT532	$\frac{5-7}{5-12}$	3.2	70
MD23	MA20	XT534	$\frac{5-29}{5-12}$	5.1	70
MD39	MA20	XT535	$\frac{5-12}{5-35}$	7.5	70
MD38	FB35	XT527	$\frac{5-34}{5-18}$	2.4	69

Table IX. X-ray-induced reversion sensitivities for some homoallelic and very closely linked heteroallelic hybrids.

Hybrid	tr_5 Genotype	Prototrophs per 10^6 survivors per sec	Expt.
XT506	$\frac{5-2}{5-2}$	0.015	62
		0.024	63
XT507	$\frac{5-4}{5-4}$	0.017	62
XT505	$\frac{5-21}{5-21}$	0.018	63
XT504	$\frac{5-36}{5-36}$	0.015	63
XT466	$\frac{5-2}{5-36}$	0.016	63
XT468	$\frac{5-4}{5-36}$	0.014	62
		0.012	63
XT441	$\frac{5-21}{5-36}$	0.017	62
XT520	$\frac{5-6}{5-6}$	0.016	68
XT519	$\frac{5-18}{5-18}$	0.0037	68

additivity. If the assumption is valid, the length of a particular interval, determined by this indirect method, should be independent of the position of the third allele employed in the measurement, within the limits of statistical fluctuations. From the set of n independent determinations of an interval, x, and the standard deviation of each measurement, σ, it is possible to calculate two statistical measures: (a) the standard deviation of the mean, σ_m, where

$$\sigma_m^2 = \sum_i \frac{|x_i - \bar{x}|^2}{n - 1},$$

which is a measure of the actual distribution of these measurements, and (b) the propagated error, σ_p, where

$$\sigma_p^2 = \sum_i \sigma_i^2,$$

which is an estimate of what the standard deviation should be if there were no systematic differences among the measurements. If the method is not additive, the standard deviation of the mean value of the independent measurements should exceed the propagated errors of those measurements. Further, the difference between the two estimates of error is a rough quantitive measure of the deviation from additivity. In Table X the results of such calculations are given. The differences are expressed as percent of the mean value of the interval. In the most favorable case, tr_{5-36} to tr_{5-35}, the difference appears insignificant, and even in the others the discrepancies are not serious. Therefore this method can be used to determine the sequence of mutational sites from two-point tests.

Construction of Maps

The data summarized in Tables V, VI, VII, and VIII can be used to determine the sequence of many of the alleles. For an illustration of the procedure used, consider the data in Table V. There are four indirect measurements of the interval between 6 and 7. The most consistent set of values for the length of the interval is obtained by assumming that 12 lies within and that 9, 20, and 21 lie outside.

Table X. Standard deviations and propagated errors of indirect measurements of reference intervals used for x-ray mapping.

Reference interval	Mean	Standard deviation	Propagated error	Difference	(%)
tr_{5-6} to tr_{5-7}	5.4	1.3	0.5	0.8	15
tr_{5-21} to tr_{5-20}	2.7	1.3	0.7	0.6	22
tr_{5-36} to tr_{5-35}	7.6	1.3	1.1	0.2	2.6

Further, these three are all farther from 7 than from 6, and 21 is farther than 20, which in turn is farther than 9. Hence, the order is 21, 20, 9, 6, 12, 7.

The results of applying this procedure to all of the data are summarized in Fig. 9. These are sequence maps. The alleles are grouped in clusters; the sequence of the clusters has been established, but the sequence of the alleles within each cluster has not. Map a) was derived from the data in Tables V and VI. Maps b) and c) were constructed from Tables VII and VIII, respectively, and Map d) combines the results from a) through c). In certain cases it has been established that pairs of mutants in the same cluster are actually at different sites. These cases are summarized in Table XI.

Relative distances between the mutation sites may also be derived from these data. Appreciably greater variations occurred from one series of experiments to the next than were found within each series. The reasons for these variations are not clear. In a continuing effort to improve the accuracy and sensitivity of the method, minor changes in the culture procedure were made in some of the experiments. The medium, however, was the same throughout (2× YEPAD). In the first two series (Tables V and VI), however, 30-ml cultures were grown in 250-ml Erlenmeyer flasks and were incubated on a rotary shaker. In the third series (Table VII) 10-ml cultures were incubated in tubes on an inclined, rotating wheel. The smaller volume leads to a lower frequency of spontaneous revertants. In the last experiments (Table VIII) 10-ml cultures in 50-ml Du Long flasks were incubated on a vibrating shaker. These differences seem small and hardly sufficient to account for the observed discrepancies. These discrepancies are illustrated in Table XII for the interval between tr_{5-36} and tr_{5-35}, measured under each of the three culture conditions. Reasonable agreement for all intervals that were measured under more than one of these conditions can be obtained by multiplying the intervals in each series by an appropriate factor chosen to make the interval between tr_{5-36} and tr_{5-35} the same (7.6 prototrophs per 10^6 survivors per sec). The results of this normalizing operation are summarized in

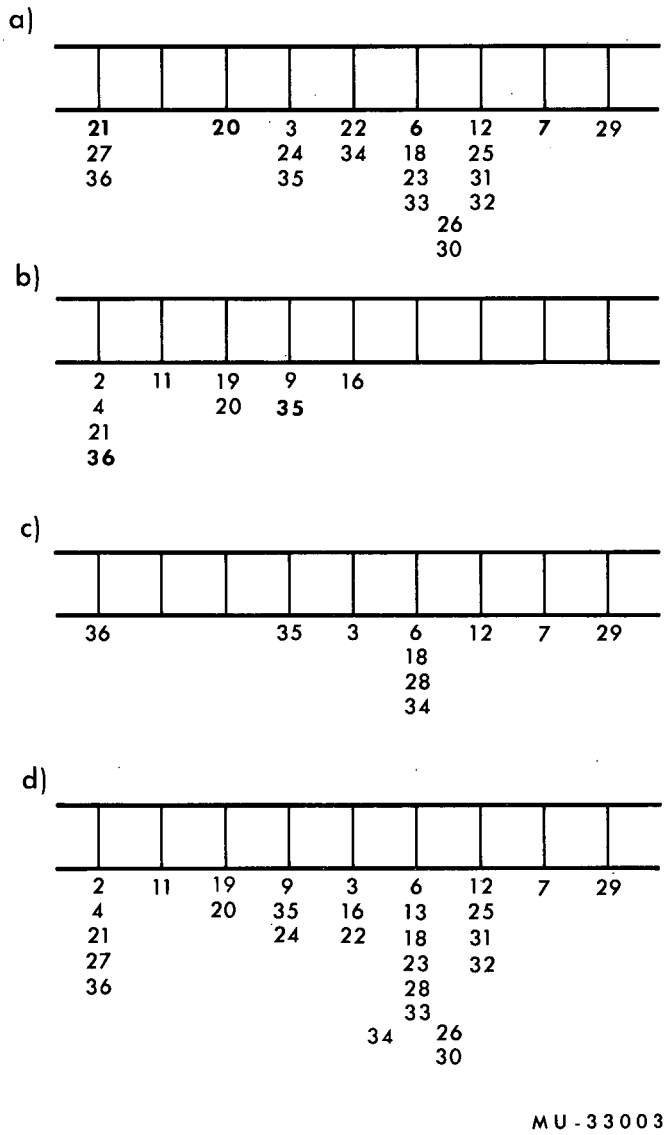

Fig. 9. Sequence maps of tr_5 mutants determined by x-ray method. The sequence of clusters was established by two-point tests, but the sequence within each cluster is not established. (a). Sequence determined from data in Tables V and VI; (b). Sequence determined from data in Table VII; (c). Sequence determined from data in Table VIII; (d). Complete sequence determined by combining (a), (b), and (c). Mutants shown in boldface were used as reference points in two-point tests.

Table XI. X-ray mapping data demonstrating that certain mutations are at different sites.

Alleles	Hybrid	Slope	Expt.
$\frac{5\text{-}27}{5\text{-}21}$	XT423	0.8 ± 0.3	46
$\frac{5\text{-}9}{5\text{-}35}$	XT469	0.4 ± 0.01	57
$\frac{5\text{-}6}{5\text{-}18}$	XT403	0.6 ± 0.05	41
$\frac{5\text{-}6}{5\text{-}13}$	XT526	2.2	69
$\frac{5\text{-}18}{5\text{-}28}$	XT538	2.7	70
$\frac{5\text{-}18}{5\text{-}34}$	XT527	2.4	70

Table XII. Interval between tr_{5-36} and tr_{5-35} measured under slightly different culture conditions.

Source	Hybrids	Interval between tr_{5-36} and tr_{5-35}
Table VI	XT439	5.3 ± 0.1
Table VII	XT439	7.2 ± 0.5
Table VIII	XT541-XT540	6.1

Table XIII for intervals measured under more than one condition. Figure 10 is a map, constructed on this basis, illustrating the relative distances between the mutation sites, in units of prototrophs per 10^6 survivors per sec.

Comparison of X-Ray Map with Complementation Map

In both the x-ray map in Fig. 9d) and the complementation map in Fig. 5, mutants are assigned sequences. These maps were derived from observations of quite unrelated properties of the mutants. The x-ray map reflects genetic relationships among the alleles. The simplest interpretation is that it represents the sequence of mutational sites. The complementation map, however, reflects functional relationships among mutant-gene products. There is no reason to expect this to be a simple relationship, because the structure of the functional enzyme is undoubtedly quite complicated.

Not all of the complementing alleles have been mapped by the x-ray method too. For those that have, the two kinds of maps are compared in Fig. 11. For the most part the sequences are colinear. There is one exception, however. The order of alleles 7 and 29 relative to the rest of the alleles is different. On the complementation map 7 is on the end, whereas on the x-ray map 29 is beyond 7. This discrepancy will be seen to be of considerable theoretical importance with respect to a model for the action of super-suppressors; this is discussed in the next chapter.

Discussion

Interpretation of Allelic Maps

Ideally a mapping method should permit one to measure the distance between mutational sites in physical units and with sufficient accuracy to ascertain the length of a mutational alteration. This ideal is not realized by any method yet available. It is therefore necessary to consider realistic criteria by which available methods can be judged, in order to understand what information is actually contained in allelic maps such as those in Figs. 9 and 10.

Table XIII. Comparison of normalized map intervals from different experiments.

tr_5 Allele	Distance from tr_{5-36} after normalizing data in			
	Tables V and VI	Table VII	Table VIII	Mean
5-6	14.3 ± 3.1		13.8	14.0
5-7	22.2 ± 2.2		21.0	21.6
5-18	15.2 ± 3.1		14.4	14.8
5-20	3.9 ± 1.9	5.1 ± 1.3		4.5
5-29	23.7 ± 1.9		23.4	23.6
5-34	15.0 ± 2.6		11.4	13.1
5-35	7.6 ± 1.9	7.6 ± 1.3	7.6	7.6
5-9	8.3 ± 3.9	7.1 ± 1.3		7.7
5-12	19.3 ± 3.9		17.0	18.2

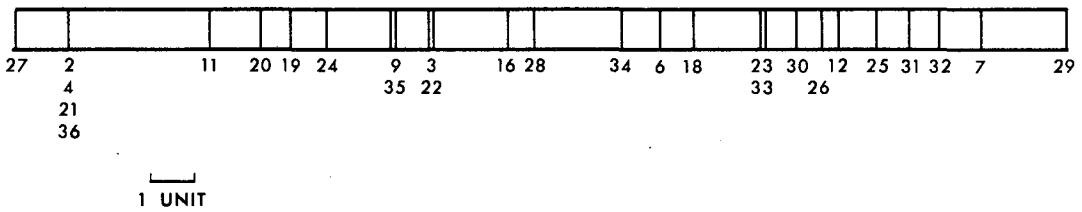

Fig. 10. Map of tr_5 mutants determined by x-ray method. One unit corresponds to one prototroph per 10^6 survisors per second of x rays at a dose rate of 200 r/sec. Values are normalized according to procedure discussed in text .

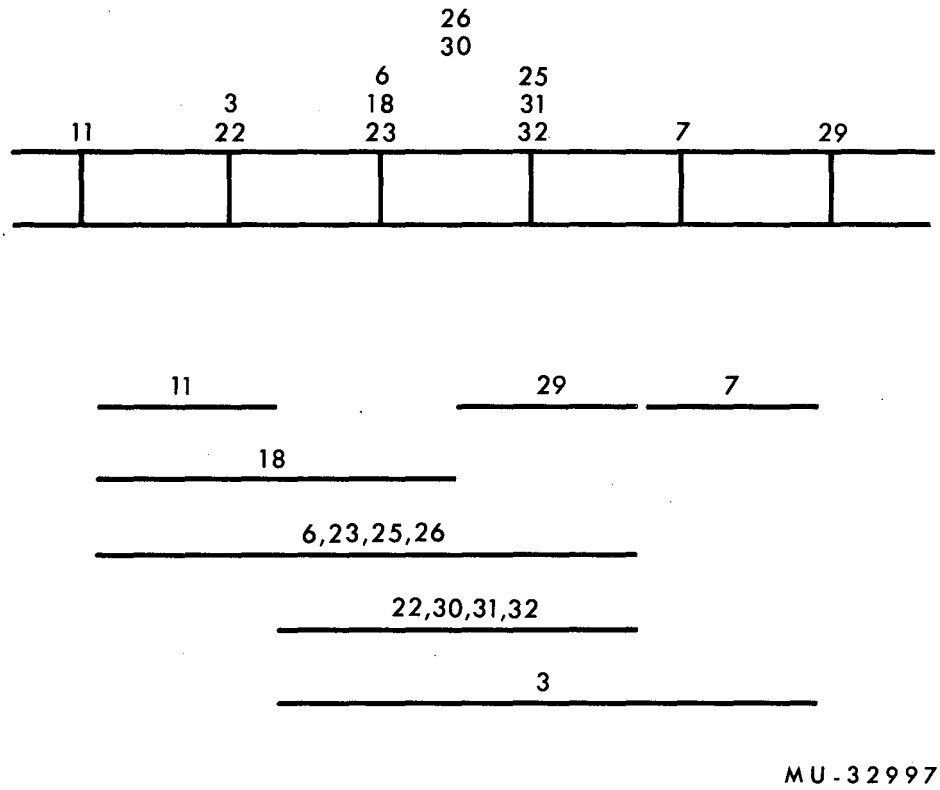

Fig. 11. X-ray sequence map of complementing tr_5 mutants compared with allelic complementation map.

To understand what any genetic map means requires an understanding of the molecular basis of the process used to determine it. The term recombination is generally (but not universally) used for these processes. But with the impressive variety of biological events employed to observe recombination--meiosis, mitosis, bacterial conjugation, transduction, bacterial transformation, bacteriophage replication--the term certainly cannot be construed to imply a single mechanism. Recombination is, however, strikingly similar in all these cases. Genetic elements, which have been demonstrated to be associated with more or less linear structures, become arranged in new combinations. It is not surprising that similar mechanisms have been postulated for all these systems, although in different cases the postulates derive support from quite different kinds of experimental observations.

Nearly all postulated mechanisms of recombination invoke one (or both) of two types or processes: breakage and rejoining of existing linear structures, and "copy-choice," copying from two different existing elements during replication of the genetic material. Assume that heteroallelic reversion results from one or both of these types of mechanisms.

Additivity. If the parameter measured by any mapping method is strictly proportional to the physical distance between the mutational sites, then the measured values of the intervals will be consistently additive. Additivity, then, is a necessary condition for any method. It does not follow, however, that if the measured intervals are additive, they are therefore proportional to the physical distances between sites. It means only that the probability of exchange in any given region is constant, independent of the presence or absence of markers.

If additivity is realized with a particular method, then the method can be used to determine the sequence of mutational sites. The sequence determined will not depend on the measured intervals' being proportional to physical distances. If one accepts this statement, then the sequence of mutational sites, as represented in Fig. 9, is the most unequivocal information one can derive from the data.

Relative sensitivity. The results of experiments described above demonstrate that the reversion sensitivity of any particular heteroallelic combination is subject to environmental and genetic modification. In this sense the sensitivity is clearly not constant. However, if all relevant environmental and genetic determinants are held constant, what determines the sensitivity? Is the probability (per unit of dose) of inducing a recombination proportional to the physical length of the region, or are certain portions of the genetic material more sensitive than others? This question must be answered before biological information can be derived from the relative distances between the alleles shown in Fig. 10. Not knowing the answer, I can only speculate.

Speculations

Assume the following:

(a) The genetic material in Saccharomyces is nucleic acid, each locus being a monotene sequence of coding nucleotides (or nucleotide pairs); each successive sequence of three corresponds to an amino acid residue, in loci that specify the primary structure of proteins.

(b) There is a constant proportionality between the map intervals measured by this method and the number of nucleotides between the mutational sites involved.

(c) Yeast tryptophan synthetase consists of two identical protein subunits, each containing about 500 amino acid residues.

(d) The alterations in the alleles tr_{5-27} and tr_{5-29} are near opposite ends of the tr_5 locus.

Resolving power. The length of the locus in units of prototrophs per 10^6 survivors per sec is about 25 units. One unit, then, corresponds to 20 amino acid residues, or one amino acid residue corresponds to 0.05 units.

The reversion sensitivity of homoallelic and heteroallelic combinations of alleles 2, 4, 21, and 36 were equal, within experimental uncertainties. The mean value from nine measurements (Table IX) was 0.016 ± 0.003 in the above units. This corresponds to about one-third of an amino acid residue, or to one nucleotide (or nucleotide pair). The standard deviation corresponds to less than 0.1 nucleotide. Therefore,

if the stated assumptions are true, these four alleles arose from repeat mutations at the same site.

On these same assumption, the distance between alleles 9 and 36 (0.4 ± 0.01) corresponds to about eight amino acid residues. This is the smallest nonzero interval measured in these experiments.

Randomness. Another question is: Are the mutations distributed at random along the length of the map? Only assumptions 1 and 2 are needed to answer this. The map in Fig. 10 was divided arbitrarily into 26 equal segments and the distribution of alleles among these segments determined. A Poisson distribution was calculated to give the observed number of intervals containing no mutants. These two distributions are compared in Table XIV. The discrepancies are smaller than the uncertainties in the map. There is no indication, therefore, of a significant deviation from randomness in the distribution of mutants. In this analysis the four mutants that are apparently repeats were considered to be distributed only within a one-unit interval. Their occurrence at the same site is not consistent with randomness.

Table XIV. Observed and calculated distributions of mutants on x-ray map.

n	Number of intervals having n mutations	Fraction	$m^n/n!$	$P_m(n)$[a]	$26 \times P_m(n)$
0	8	0.31	1	0.31	8.0
1	11	0.42	1.17	0.36	9.4
2	5	0.19	0.68	0.21	5.5
3	1	0.04	0.27	0.08	2.1
4	1	0.04	0.08	0.03	0.8
Totals	26	1.00		0.99	25.8

a. $P_m(n) = \frac{m^n}{n!} e^{-m}$ $\qquad P_m(0) = e^{-m} = 0.31$

$m = 1.17$

ACTION OF A SUPER-SUPPRESSOR ON tr_5 MUTANTS

Origin of Super-Suppressor S_d

In their initial studies Hawthorne and Mortimer (1963) investigated three super-suppressors, which they called S_a, S_b, and S_c. Although they found no differences in the spectra of mutants on which these acted, the three suppressors represent at least two different loci. S_a and S_c are either very closely linked or are allelic, but show no linkage to S_b. A fourth super-suppressor, S_d, which will be described, evidently represents an additional locus, because, unlike the first three, it is closely centromere-linked. This suppressor has been used to study the suppressibility of 32 tryptophan synthetase mutants.

S_d was isolated as a dominant suppressor of the allele hi_{5-2} in a homoallelic diploid, X841 (See Table A-V for complete genotype). Histidine-independent colonies were isolated following irradiation (10 krad) with a beam of 120-MeV carbon nuclei from the Berkeley heavy-ion linear accelerator (Mortimer, Brustad and Cormack 1960). Revertants were sporulated and asci dissected. A histidine-independent spore culture from each of five revertants was backcrossed to wild type and the resulting hybrid was analyzed. In each case the recessive hi_{5-2} allele was found, with segregation ratios characteristic of an unlinked suppressor. All five of the reversions had resulted from dominant-suppressor mutations, unlinked to the hi_5 locus. Several other markers in the cross also had irregular segregation ratios. The segregation of four markers is shown in Table XV. These are completely consistent with the behavior expected of a super-suppressor. If a suppressor and the locus it suppresses segregate independently, and at least one of them is not centromere-linked, 4+:0-, 3+:1- and 2+:2- asci occur in the ratios 1:4:1, respectively. The significant deviation from these ratios for ar_{4-2} and ly_{1-1}, which are both centromere-linked (Hawthorne and Mortimer 1960), indicates that the suppressor, S_d, is also centromere-linked. Recently Mortimer (private communication) has mapped S_d on chromosome VI, proximal to hi_2 and about two units from the centromere.

Table XV. Segregation of super-suppressible markers in X841 histidine revertants.

Genotype	Numbers of asci having positive to negative phenotype ratios of:			Percent SDS[a]
	2:2	3:1	4:0	
hi_{5-2}	5	21	1	
ad_{2-1}	1	6	4	76.9
ly_{1-1}	4	4	4	54.5
ar_{4-2}	5	2	6	15.9

a. Second-division segregations. Data from Hawthorne and Mortimer 1960.

It suppresses many of the same alleles as the previous super-suppressors.

Action of S_d on tr_5 Alleles

Test for Suppressibility

It is relatively simple to test whether a particular super-suppressor suppresses a given recessive allele. A hybrid is derived that is heterozygous for the allele to be tested and for the suppressor, and homozygous for a suppressible allele at another locus (preferably concerned with a different phenotype). The suppressor is thereby "marked" by the positive phenotype of this other locus, and its presence or absence in any spore can be ascertained with certainty. If the tr_5 allele being tested is suppressible, one expects to find an excess of tryptophan-independent phenotypes, and these should be consistent with the segregation of the suppressor; no suppressor-bearing spore will have the mutant phenotype of a suppressible allele. Accordingly, the occurrence of the mutant phenotype, coincident with the suppressor in a spore culture, is conclusive evidence that the allele responsible for that phentotype is not suppressed. The test does not actually require tetrad analysis. Among a random selection of spores there will be a significant excess of positive phenotypes for any allele that is suppressed, and there will be no spores with the negative phenotype and the suppressor.

Results with Tr_5 Alleles

In the tests with the tr_5 alleles, ad_2 was employed to "mark" the suppressor. Unfortunately, however, two different alleles at the ad_2 locus were inadvertently used and one of them is not suppressible. Consequently, in some crosses the suppressor could not be scored completely. The above criteria for suppressibility are still valid in these cases, but the test is less certain when only a small number of asci were examined. The negative results are still conclusive. Complete genotypes of the hybrids used are given in Tables A-VI through A-IX.

Thirty-two tr_5 alleles were tested for suppression by S_d. Thirteen were suppressed and 19 were not. The data for the 19 non-suppressed ones are summarized in Table XVI. Three types of evidence are tabulated: (a) segregation ratios of $Tr^+:tr^-$ phenotypes among four-spored asci, (b) segregation ratios of $Tr^+:tr^-$ phenotypes among suppressor-bearing spores, and (c) segregation ratios of $Tr^+:tr^-$ phenotypes among all spores examined. The tetrad ratios are based on data from complete asci. Those with inviable spores and those with irregular segregation for markers not suppressed by S_d were excluded. In each cross several unsuppressed markers were included to detect "false tetrads," which occasionally arise from chance association of nonsister spores (Johnston and Mortimer 1959). Spores from incomplete and "false" asci were included in the spore ratios shown in the last two columns. Therefore, these data do not merely duplicate the tetrad ratios. In all cases for which the suppressor could be scored, the strongest evidence that these alleles are not suppressed is the occurrence of suppressor-bearing spores that require tryptophan. In every case at least two of the three criteria are satisfied for these 19 alleles. In the two hybrids for which the suppressor could not be scored directly (XT317 and XT451), there is a significant absence of excess positive phenotypes.

Data for the 13 suppressible alleles are shown in Table XVII. These results were confirmed by backcrossing a tryptophan-independent spore presumed to have the genotype $tr_{5\text{-}x}S_d$ with a strain bearing several suppressible alleles at other loci. Tetrad ratios observed in these backcrosses are shown in Table XVIII and the spore ratios are included in Table XVII. Segregation of tryptophan-requiring spores from these hybrids confirms the presumed genotype of the spores that were backcrossed, and proves that they are suppressible. The backcross was not performed with $tr_{5\text{-}16}$, but the conclusion of suppressibility for this allele was confirmed by another method. A hybrid was made from a presumed $tr_{5\text{-}16}\ S_d$ spore and an unsuppressed $tr_{5\text{-}16}$ strain. The diploid required tryptophan. Therefore, it must have been homozygous for $tr_{5\text{-}16}$, and S_d must be a recessive suppressor of this allele.

Table XVI. Segregation data establishing nonsuppressibility by S_d for 19 tr_5 mutants.

Hybrid	tr_5 Genotype	+:-Tetrad Ratios			Spores	
		4:0	3:1	2:2	$S_d^+Tr^+ : S_d^+tr^-$	$Tr^+ : tr^-$
XT301	5-1	0	0	4	6:4	14:11
XT305	5-5	0	0	4	8:6	15:11
XT442	5-6	0	0	5	3:4	13:11
XT443	5-7	0	0	4	6:6	13:11
XT308	5-8	0	0	5	3:8	11:10
XT310	5-10	0	0	7	4:5	14:14
XT311	5-11	0	0	5	7:8	13:13
XT317	5-17	0	0	4		13:13
XT318	5-18	0	0	6	4:8	13:14
XT409	5-19	0	0	5	2:0	11:13
XT320	5-20	0	0	4	4:3	13:10
XT445	5-23	0	0	2	3:3	8:6
XT446	5-25	0	0	6	8:13	14:13
XT448	5-27	0	0	5	6:6	13:12
XT449	5-28	0	1	1	2:6	9:8
XT451	5-30	0	0	5		12:11
XT452	5-31	0	0	5	7:4	14:12
XT453	5-32	0	0	5	6:8	14:15
XT456	5-35	0	0	5	4:7	11:12
	Totals	0	1	87	83:99	238:220

Table XVII. Segregation data establishing suppressibility by S_d for 13 tr_5 mutants.

Hybrid	tr_5 Genotype	+: -Tetrad Ratios 4:0	3:1	2:2	Spores $S_d^+Tr^+ : S_d^+tr^-$	$Tr^+ : tr^-$	Backcross Hybrid	Backcross Spores $Tr^+ : tr^-$
XT302	5-2	2	2	2	10:1	18:6	XT473	18:5
XT405	5-3	1	4	2	7:0	21:4	XT474	20:4
XT304	5-4	2	2	2	13:0	21:6	XT475	17:4
XT309	5-9	2	3	2	9:0	21:7	XT476	21:7
XT312	5-12	3	1	1	13:0	20:5	XT495	18:6
XT313	5-13	1	2	1	11:0	19:5	XT478	20:3
XT408	5-16	3	1	2	4:0	21:5		
XT321	5-21	2	0	1	6:0	18:6	XT481	21:6
XT458	5-24	2	0	2	13:0	21:5	XT482	19:4
XT450	5-29	3	2	1	14:0	23:5	XT483	24:3
XT454	5-33	3	3	0	12:0	25:7	XT497	17:7
XT455	5-34	3	0	1	13:0	24:2	XT485	16:7
XT457	5-36	2	2	1	15:0	24:5	XT486	20:5
	Totals	29	22	18		276:68		231:61

Table XVIII. Segregation ratios from backcrosses of suppressed tr_5 mutants.

Hybrid	tr_5 Genotype	+: - Tetrad Ratios 4:0	3:1	2:2
XT473	5-2	2	1	2
XT474	5-3	3	2	1
XT475	5-4	1	0	2
XT476	5-9	2	3	2
XT495	5-12	1	3	1
XT478	5-13	3	0	1
XT481	5-21	2	3	2
XT482	5-24	2	1	1
XT483	5-29	3	3	0
XT497	5-33	2	1	3
XT485	5-34	1	1	3
XT486	5-36	3	1	1
	Totals	25	19	19

The evidence clearly supports the conclusion that S_d suppresses these 13 alleles. The single-suppressor-bearing tryptophan-requiring spore in XT302 must be considered an error, in light of the consistency of the other data for that hybrid.

Effect of S_d on tr_{5-19}

Although tr_{5-19} is not suppressible as judged by the tests described, it is affected by the super-suppressor. It appears to be "suppressed" to a different type of mutant, one that can utilize indole slowly. The spore cultures in many of the tests described above were scored on -Tr medium supplemented with indole (10 mg/liter) and on unsupplemented -Tr. Some of the segregations observed for XT409 (tr_{5-19}) are shown in Table XIX. This hybrid is not homozygous for a suppressible marker, so unfortunately the suppressor cannot be scored in every ascus. The 3:1 segregations on -Tr + In, however, are consistent with the suppressor restoring the enzyme's ability to convert indole to tryptophan. Spore XT409-4C was backcrossed to a strain with the genotype $a\ tr_5^+\ ad_{2-1}\ hi_{5-2}\ ly_{1-1}$. Segregation in the two asci from this hybrid shown in Table XX confirms the presence of the suppressor in XT409-4C and its action on tr_{5-19}. The phenotypes of the four spore strains from ascus 4 of XT409 are demonstrated quantitatively in Fig. 12. Growth rates at 30°C were measured in liquid -Tr medium with and without supplemental indole. Spore culture 4C was clearly able to utilize indole. The allele tr_{5-19} is classed as suppressible, since S_d clearly has an effect on it.

After a suggestion by B. D. Maling (private communication), an attempt was made to isolate a suppressor that would suppress tr_{5-19} to prototrophy. Not only was the attempt unsuccessful, but this allele could not be made to revert at all. Even after irradiation with ultraviolet light (to survival level of about 20%) no prototrophs were recovered from more than 10^9 cells. However, when the cells were plated on -Tr supplemented with indole (10 mg/liter) and given the same dose of ultraviolet light, about five colonies were formed for every 10^6 cells plated. When these indole-utilizing mutants, derived from tr_{5-19}, were cultured and plated on -Tr, prototrophs were obtained.

Table XIX. Segregation of tryptophan-dependence and indole-utilization phenotypes in spores from XT409.

Ascus	Spore	Phenotype on			
		-Tr	-Tr+In	-Ad	Pet
2	A	-	+	+	-
	B	+	+	+	+
	C	+	+	+	+
	D	-	-	-	-
3	A	+	+	+	-
	B	-	-	-	-
	C	+	+	+	+
	D	-	-	-	+
4	A	+	+	+	-
	B	+	+	-	+
	C	-	+	+	-
	D	-	-	+	+
5	A	+	+	+	+
	B	+	+	-	-
	C	-	-	+	-
	D	-	+	+	+

Table XX. Segregation tryptophan-dependence and indole-utilization phenotypes in spores from backcross of suppressor-bearing tryptophan-independent spore from XT409.

Ascus	Spore	Phenotype on				
		-Tr	-Tr+In	-Ad	-Hi	-Ly
3	A	-	+	+	+	+
	B	+	+	+	-	+
	C	+	+	-	-	+
	D	-	+	+	+	+
4	A	+	+	-	-	-
	B	-	+	+	+	+
	C	-	+	+	+	+
	D	+	+	+	+	+

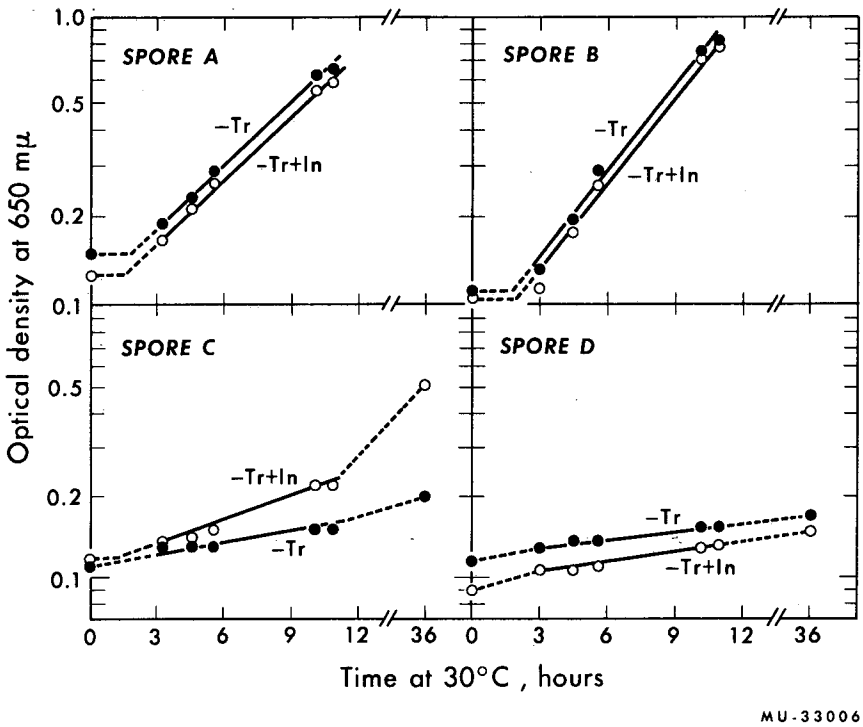

Fig. 12. Growth curves for spore cultures from ascus XT409-4.

It appears, then, that tr_{5-19} reverts only by a two-step event. There are two tenable explanations for the behavior of this mutant. It could actually be a double mutant, one alteration being suppressible and the other capable of utilizing indole. It could also be a small rearrangement, such as an inversion, that requires two base changes for reversion. Experiments employing S_d and designed to distinguish between these two possibilities are in progress.

Properties of Suppressible Mutants

We are now in a position to compare the properties of mutants that are suppressible by S_d with those that are not. Several questions about these suppressible mutants can be answered:

(a) How are they distributed along the genetic map? Are they clustered, or do they occur at random along the entire region?

(b) How are suppressible mutants distributed on the complementation map? Is there a correlation between suppression and complementation?

(c) Is suppressibility correlated with the biochemical properties of the mutants?

Distribution of Suppressible Alleles on the Genetic Map

In Fig. 13, the allelic map from Fig. 10 has been redrawn to show the distribution of suppressible alleles, which are the ones in the circles. They are distributed along the full length of the map, ruling out the possibility that the suppressor acts only on mutants at a specific site. There is, in fact, no discernible pattern. Application of the test for randomness supports this conclusion, if the four suppressible mutants at the left end are considered as one. We may conclude, then, that suppressible mutation sites are randomly distributed along the map, but some are significantly more sensitive to mutation-induction by ultraviolet light than others. If we assume that there are no repeat mutations at other sites, then the 32 mutants tested for suppressibility are distributed among 29 sites. Of these, 10 (or 35%) are suppressible. Hawthorne and Mortimer (1963) tested 50 alleles at different loci and found 14 (or 28%) suppressible. Since their mutants were at many different loci, it is appropriate to compare numbers of mutation sites, rather than mutants. On

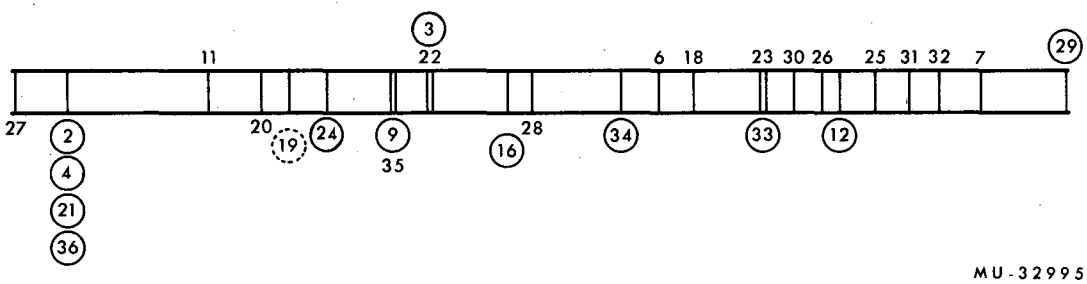

Fig. 13. Map of tr_5 mutants determined by x-ray method, redrawn from Fig. 10 to show distribution of complementing and suppressible alleles. Mutants plotted above the line are complementing, whereas those below are noncomplementing. The symbols circled represent mutants suppressible by S_d.

this basis the frequency of suppressible alleles among tr_5 mutants appears to be about the same as among nutritional mutants in general. This is also consistent with the hypothesis that super-suppressible mutations can occur at every locus.

Distribution of Suppressible Alleles on the Complementation Map

In contrast to their nearly uniform distribution along the genetic map, suppressible mutants are almost completely restricted to one group of the complementation map, the group of noncomplementing alleles. There are only two exceptions: tr_{5-3} and tr_{5-29}. The distribution of 31 alleles with respect to suppressibility and complementation is summarized in Table XXI. (One suppressible allele, tr_{5-24}, could not be scored for complementation, owing to high frequency of spontaneous reversion.) Of the noncomplementing alleles 11 of 16 are suppressible, whereas only 2 of 15 complementing ones are. Only five of these 31 mutants failed to function either by complementation or by suppression.

The lower frequency of suppressibility among complementing mutants than among noncomplementing mutants is highly significant. The two exceptional alleles, tr_{5-3} and tr_{5-29}, however, cannot be ignored. An acceptable model must explain these mutants as well as the almost complete mutual exclusion between complementation and suppressibility.

Biochemical Properties

The number of mutants that accumulate or utilize indole is too small for a significant comparison of these properties with suppressibility. These four alleles are not suppressible, but they are all complementing alleles. One would not be likely to find suppressible alleles among four complementing mutants chosen at random.

Summary

The characteristic properties of the class of mutants that is suppressed by S_d may be summarized as follows:

(a) They are distributed along the entire length of the mapped portion of the tr_5 locus; they apparently are distributed throughout the entire genome of Saccharomyces as well, perhaps at every locus.

Table XXI. Summary of results of complementation and supersuppressibility tests for 31 tr_5 mutants.

Suppressible	Complementing	Number of alleles	Alleles
+	-	11	2, 4, 9, 12, 13, 16, 19, 21, 33, 34, 36
+	+	2	3, 29
-	+	13	5, 6, 7, 8, 10, 11, 17, 18, 23, 25, 30, 31, 32
-	-	5	1, 20, 27, 28, 35
	Total	31	

(b) Some of the sites at which these mutations occur have differing sensitivities to mutation induction (prototrophy to auxotrophy) by ultraviolet light, as evidenced by the occurrence of four suppressible mutations at the same site.

(c) Most of these suppressible mutants are noncomplementing, but there are exceptions.

Comparison with Suppressible Mutants in Other Organisms

These characteristics of suppressible tr_5 mutants appear to be similar to those typical of mutants in E. coli and bacteriophage that are suppressed by multiple-action suppressors. The ambivalent rII mutants of bacteriophage T4 are distributed at several sites in the rII A region (Benzer and Champe 1962). The 15 suppressible alkaline phosphatase mutants studied by Garen and Siddiqi (1962) are distributed among at least seven sites. Further, they appear to be noncomplementing. The suppressor-sensitive mutants of bacteriophage lambda studied by Campbell (1961) are similarly found at many sites, which he was able to group into nonoverlapping complementation groups (cistrons). Complementation was not found within each group.

These similarities are striking. They motivate the working hypothesis that the mechanism of suppression in all these systems, including Saccharomyces, is basically the same. The demonstration of these similarities is one of the most significant results of these studies. On the one hand, it demonstrates that this mechanism of gene action, whatever it is, is not merely a curiosity, unique to a single genetic system, for genetically, yeast and the E. coli-bacteriophage systems are conspicuously dissimilar. On the other hand, if this working hypothesis is valid, the various systems--because of their differences--together provide a unique assemblage of genetic and biochemical approaches to the study of a mechanism of suppressor-gene action.

Models for Super-Suppressors

There are many plausible models for the action of super-suppressors. In fact, there are too many plausible models. Our understanding of mechanisms of genetic control of protein synthesis and enzyme activity

is sufficiently advanced to permit us to conceive several quite specific mechanisms for super-suppressors, yet we have not advanced far enough to easily determine the validity of a particular model. Most of what is known about these suppressors has been inferred from genetic experiments. The purpose now is to use this information to construct models that can be tested biochemically.

An adequate model must answer two questions: (a) what is the nature of suppressible mutations, and (b) what is the normal function of suppressor loci, and how do mutations at these loci restore the function of suppressible mutants? The possibilities may be explored by listing the types of mutations that have been demonstrated or proposed and then considering how the effects of these mutations could be reversed at the phenotypic level.

Classification of Mutations

For a locus that is responsible for the production of an enzyme, we may formally distinguish between mutations that affect the enzyme activity by altering its structure and those that affect the activity by altering the number of enzyme molecules produced by the cell. These may be denoted as structural mutations and regulatory mutations, respectively. We may presume that it is the region of the genetic material in which a mutation occurs that determines which type it is, and that the same kinds of alterations of the genetic material can result in either.

Assuming that the genetic information is carried and transmitted as a coded sequence of bases in a nucleic acid polymer, there are apparently only a few possible types of heritable alterations: (a) replacement of one base by another, (b) deletion of one or more bases, (c) addition of one or more bases, and (d) rearrangement of the existing bases. The primary mutational event may involve some other kind of alteration, but after replication mut assume one of these forms to be heritable. We consider the consequences of these individually.

Base replacements. There are two types of possible base replacements. If a purine is replaced by another purine, or a pyrimidine replaced by another pyrimidine, the change is called a transition. If a purine is replaced by a pyrimidine, or a pyrimidine by a purine, it is

called a transversion (Freese 1959a). According to current understanding of the genetic code (Crick 1963), replacement of one base by another may lead to a "missense" mutation (replacement of one amino acid by another in the protein), to a "nonsense" mutation (a noncoding sequence), or to no mutation at all (owing to degeneracy of the code). A missense mutation may go undetected if the amino acid substitution does not alter the enzyme activity appreciably, or it may result in an enzymatically altered or inactive protein. A nonsense mutation would be expected to prevent completion of the protein.

Deletions and additions of bases. The effect of a deletion or addition of genetic material most likely depends on the extent of the change. Large deletions undoubtedly result in complete absence of the protein. The remarkable experiments of Crick, Barnett, Brenner, and Watts-Tobin (1961), however, suggest that very small deletions or additions of bases can in some cases be accommodated by the system. Such alterations are believed to shift the reading frame that governs the translation of an amino acid sequence from a nucleotide sequence. If the reading-frame shift does not introduce a nonsense sequence a protein may still be formed. If the deletion or addition consists of a small integral number of coding units, the reading frame will not be shifted and the rest of the protein will presumably be unaltered. It is conceivable that in some regions of a protein, deletion or addition of a few amino acid residues would not significantly alter the function of the protein and would go undetected as a mutation.

Rearrangements of existing sequences. For completeness, and by analogy with gross chromosome aberrations, we may suppose that inversions of nucleotide sequences within a gene may occur. Assuming no material is added or lost in the process, such inversions would have no effect on the reading frame. They would be equivalent to several adjacent base-replacement mutations, leading to either missense or nonsense. It might be expected that this type of mutation would revert only at an extremely low frequency.

Mechanisms of Forward and Reverse Mutation

In many cases the strongest evidence for a particular type of mutation is derived from knowledge of the agent that induced it or agents that will induce it to revert (Freese 1959a; Crick, Barnett, Brenner and Watts-Tobin 1961). Since the effects of suppressors mimic reverse mutations, we may consider what is known of the mechanisms of reversion for clues to the mechanisms of supersupressors.

Watson and Crick (1953) proposed a mechanism for spontaneous base replacement at the time of DNA replication based on tautomeric shifts in the structure and consequent changes of the hydrogen-bonding characteristics of the bases. Subsequently Freese (1959b) proposed a similar mechanism to explain the mutagenic action of certain base analogs. Similarly, chemical and physical agents (such as nitrous acid and ultraviolet light) that can transform normal bases to analogs can induce transitions (Freese 1959b; Drake 1963).

Transition mutations revert by transition, and transversions by transversion. Restoration of the original base sequence, however, may not be necessary for restoration of function. Because of the apparent degeneracy of the code (Crick 1963), and because in some cases an enzyme will function with any of several amino acids at a particular position (Henning and Yanofsky 1962b), any of several base replacements may suffice to restore function. In addition, some mutations result in enzymes that are inactive because they are sensitive to certain factors such as temperature, pH, or metal-ion concentration (Suskind and Kurek 1959). In these cases function may be restored by altering the environment rather than the enzyme itself. Such sensitive mutations could conceivably arise from other types of mutational alterations as well as simple base replacements.

Mutations resulting from small deletions and additions could, of course, be reverted by adding back the deleted material or by removing the added material, but this would be unlikely. The experiments of Crick, Barnett, Brenner and Watts-Tobin (1961) on rII mutants of T4, however, suggest that the correction need not necessarily be at exactly

the same site as the original mutation. If a deletion and an addition of equal length are sufficiently close together in certain regions of the rII B cistron, function is restored. In these cases it appears necessary only to have the reading frame in proper register.

It is believed that mutations that result from shifting the reading frame cannot be made to revert by simple base replacements, and that a reading-frame shift cannot cause reversion of a base-replacement mutation (Crick, Barnett, Brenner, and Watts-Tobin 1961; Benzer and Champe 1962).

Reversion of small inversions and other similar rearrangements would probably be rather unlikely occurrences. In some special cases involving only two or three bases a functional sequence could possibly be restored by a single base replacement. In general, however, it would seem that little less than precise reversal of the original rearrangement would do. This would be an extremely rare event. If such mutations occur they probably are not found to revert.

Possible Super-Suppressor Loci

Initially, we may consider every locus involved in each of the steps by which the genotype is translated into phenotype as a possible candidate for a super-suppressor locus. It is immediately evident that current knowledge of the mechanism of protein synthesis is much too limited to allow us to even guess all the loci involved. A generalized scheme, however, will illustrate some of the obvious possibilities. Let us consider some possible steps involved in translating from genotype to phenotype the ability to convert indoleglycerol phosphate to tryptophan. At this point it becomes necessary to distinguish between structural mutations and regulatory mutations.

The genotype presumably consists of a sequence of nucleotide pairs carrying the information necessary to determine the amino-acid sequence of the tryptophan synthetase enzyme. There may be other genetic elements involved in the regulation of the amount of tryptophan synthetase formed. These elements have not been identified in yeast, but let us postulate another sequence of nucleotide pairs endowed with this function. This regulatory locus either could be specific for

tryptophan synthetase, or it could be involved in the regulation of the entire tryptophan biosynthetic pathway or even several biosynthetic pathways.

The general nature of the reactions by which the sequence of base pairs in the structural gene is translated into a functional enzyme is known at least in part, and many of the enzymatic steps have been isolated and studied in vitro:

(a) The sequence of deoxyribonucleotides in the genome is first transcribed into a sequence of ribonucleotides, under the influence of a polymerase (Hurwitz and August 1963).

(b) This messenger RNA must then travel to sites of protein synthesis in the cytoplasm. The stability of this RNA is probably determined by several enzymes, including at least one RNA-specific nuclease. The messenger presumably acts as a template for protein synthesis.

(c) Other enzymes activate amino acids and catalyze their attachment to adapter molecules of sRNA. The adaptors bear the specificity for translating the base sequence into an amino acid sequence.

(d) When the amino acids are polymerized in the sequence determined by the messenger, the peptide chain must then fold, forming secondary and tertiary bonds. These bonds will be sensitive to the chemical environment in the cytoplasm--pH, ionic strength, etc.

(e) In some cases polypeptide chains associate in polymers to become proteins with quaternary structure.

(f) When the final enzymatic structure is formed, it will still depend on the proper chemical environment for its activity. This cytoplasmic environment itself may be considered to be under the control of enzymes that govern the biosynthesis of various small molecules.

The necessity for eliminating some of these loci as possible candidates for super-suppressor loci should be clear.

Structural or Regulatory Mutants?

The properties of the tr_5 mutants may be used to argue that the suppressible mutations affect the gene controlling the structure of the tryptophan synthetase enzyme rather than a gene with only

regulatory function. The suppressible mutations are distributed along the entire mapped length of the tr_5 locus (see Fig. 13). Four mutants at this locus (three indole-accumulating and one indole-utilizing have properties that cannot be readily explained on the basis that they merely produce less enzyme (fewer molecules). Therefore, we must conclude that the tr_5 locus determines the structure of yeast tryptophan synthetase, and accordingly that the suppressible mutations studied at this locus also affect the structure of the enzyme. This argument does not exclude the possibility that in yeast regulation and structure are controlled by the same loci.

What Types of Mutations are Super-Suppressible?

It is difficult to think of mechanisms by which reading-frame mutants at different sites in different genes could be suppressed by a single suppressor locus. There is, however, no definitive evidence in yeast on this question. In fact, the observed partial exclusion between complementation and suppressibility is quite consistent with suppressible mutants being of this type. Benzer and Champe (1962) have found that ambivalent rII mutants can be induced to revert by 2-aminopurine. This is taken as evidence that they are transition mutants and therefore not of the reading-frame type. Similar reversion tests will have to be performed on yeast mutants before final conclusions can be drawn.

It is equally difficult to think of mechanisms by which mutants arising from inversions could be suppressed by a super-suppressor. For base-replacement mutations, however, there are many conceivable mechanisms. Most of the steps in enzyme formation outlined above could be modified to compensate for some mutations of this type. It is useful at this point, then, to try to narrow the field of possible suppressible mutations on the basis of the observed properties of super-suppressible mutants.

Properties of Suppressible Mutants

The most apparent property of the super-suppressible tr_5 mutants is that they are, for the most part, noncomplementing. This is not a likely characteristic of base-replacement mutants in general, and

therefore the suppressible ones must be a special class. This would argue strongly against any mechanism that merely reverses the effect of one particular base replacement by a change in the specificity of an activating enzyme or an sRNA [step (c)]. This type of model has been developed to explain specific suppressors (Yanofsky and St. Lawrence 1960; Yanofsky, Helinski, and Maling 1961; Brody and Yanofsky 1963). There are other objections to this as a model for these super-suppressors. In its general formulation the model predicts that a given suppressor should suppress all mutations that arise from a particular amino acid replacement (which could result from any of several base replacements). It is unreasonable, however, to consider that any single-amino-acid replacement could account for 28% of the ultraviolet-induced nutritional mutants in yeast, let alone 40% of the tr_5 mutants.

There remain several categories of base-replacement mutants that are consistent with the observed exclusion between complementation and suppressibility. These are of at least two general types: nonsense mutations and mutation to sensitivity. Nonsense mutation should be mostly noncomplementing because the nonsense sequence presumably terminates the translation process before a complete protein is made. Mutants that are sensitive to specific compounds on to such conditions as high as low pH or high or low ionic strength could, however, also be mostely noncomplementing. It could be that the ability of the protein subunits to polymerize can become sensitive to cellular conditions [step (e)]. Such mutants would necessarily to noncomplementing. Inhibitors that act specifically on the active centers of the enzyme could also preclude complementation [step (f)].

To summarize, then, these considerations allow us to narrow the field of plausible suppressible mutations. We are now in position to consider specific mechanisms for the action of the super-suppressor on these mutations and to consider biochemical experiments to test these models.

The Nonsense Model

Derivation from phage experiments. Benzer and Champe (1962) devised a genetic test for nonsense mutations. In standard type T4

the rII region consists of two cistrons, A and B. The mutant r1589 has a deletion that spans the two cistrons, joining them together as a single cistron, but without inactivating the B function; it complements any mutant having a functional A cistron. When the two cistrons are not joined as a result of this deletion, a mutation limited to the A portion will have no influence on the B function. However, some mutations of the A cistron, when placed in series with r1589, abolish the B function too. Some of these have been interpreted as very small deletions or additions of nucleotides that shift the reading frame (Crick, Barnett, Brenner, and Watts-Tobin 1961). A requisite characteristic of such reading-frame mutants is that they cannot revert by a simple base substitution. There is however, a class of rII mutants that map as point mutations in the A cistron, abolish the B activity when in series with r1589, and are base-analog revertible. Benzer and Champe (1962) interpret these as nonsense mutations; the B protein cannot be formed, they postulate, because translation stops at the nonsense. They found that five ambivalent mutants suppressed by a common suppressor (in the host KB-3) all satisfy the above criteria for nonsense. They also report that four additional mutants satisfied these criteria but were not suppressed in KB-3.

The suppressor, according to Benzer and Champe, could change nonsense to "sense" by changing the coding properties or amino acid specificity of one sRNA adaptor. The suppressor mutation could be either in a gene specifying an sRNA or in one specifying an activating enzyme [step (c)].

This model is quite consistent with properties of the suppressible mutants at the tr_5 locus in Saccharomyces. It also leads to some predictions that may provide a critical test of the hypothesis.

Application to tr_5 mutants. Nonsense mutants should be noncomplementing. With two significant exceptions, the suppressible tr_5 mutants are noncomplementing. Nonsense mutants, in theory, could arise through a number of different base changes: a particular sequence of three different bases can result from any of nine different single base

changes in other three-base sequences. Ultraviolet light would not be expected to induce all of these with equal efficiency. This is consistent with the occurrence of repeat mutations that are suppressible.

Perhaps the strongest support for this hypothesis is applied to yeast comes from the behavior of tr_{5-19}, which is "suppressed" in only one of the three tryptophan synthetase reactions. This mutant is apparently altered near one of the catalytic sites of the enzyme. It is very likely that the activity of that site requires the presence of one specific amino acid in this position. Some other amino acid in this position, however, might not interfere with the activity of the other catalytic site. Thus tr_{5-19} could be a case of the suppressor changing nonsense to missense. If tr_{5-19} is actually a double mutant, however, it can be explained by nearly any model.

How can the two complementing suppressible mutants (tr_{5-3} and tr_{5-29}) be reconciled with this model? Can there be any exceptions to the "rule" that nonsense mutants do not complement? Necessary conditions for complementation (according to evidence discussed in the INTRODUCTION) is that the two mutants have nonoverlapping alterations in the protein, and that they are able to combine to form the quaternary structure necessary for activity. It is highly plausible that tr_{5-29} could be a nonsense mutant and satisfy these requirements. The alteration in this mutant is possibly very near one end of the locus. If transcription proceeds from the other end, a nearly complete protein would be made before the nonsense terminated the process. The protein would be completed beyond the sites of the alterations in the mutants that it complements with. But what about tr_{5-3}? This mutant would transcribe no more than half of the protein before the nonsense site was reached. Could half of a protein complement? Since the only mutant that complements with tr_{5-3} is the indole-utilizing one, tr_{5-11}, it is not impossible. Most of the effect of the alteration in tr_{5-11} is localized to the catalytic activity responsible for cleaving indoleglycerol phosphate, and tr_{5-3} would be expected, if translation is from the left, to be unaltered in this region. This possibility raises another question. Does the protein fragment produced in the nonsense mutant

remain on the ribosome, or is it released? If it remains attached, then the active hybrid formed by complementation would also be attached to a ribosome. This should be testable. In either event, if the active hybrid formed by this pair could be purified enough that its molecular weight could be measured, one would expect to find a significantly lower value than for the normal enzyme. If it consists of only one subunit from each mutant, the molecular weight of the hybrid should be only about 75% of the molecular weight of normal tryptophan synthetase.

These two "exceptional" cases, then, appear to be reconcilable with the nonsense hypothesis. They are also exceptional in other ways. Of all the complementing mutants, only two do not complement with tr_{5-7}. One is tr_{5-3} and the other one is tr_{5-15}, which has not been tested for suppressibility, unfortunately. The single exception to colinearity between the complementation and genetic maps results from the situation of tr_{5-29} to the "right" of tr_{5-7}, a situation necessary for their complementation to be reconcilable with the interpretation that tr_{5-29} is a nonsense mutant.

To generalize, the nonsense model imposed restrictions on the location of alleles that can complement with a super-suppressible allele. At any locus all alleles that complement super-suppressible alleles must map in the same direction from the suppressible one they complement. That is, since a nonsense mutant translates only the part of the protein from the "beginning" to the nonsense sequence, the resulting protein fragment can only be expected to complement proteins from other mutants altered in that region. Therefore it one super-suppressible mutant at a locus complements with a mutant to its left on the allelic map, then the nonsense hypothesis requires that no super-suppressible mutant at the locus complement with one to its right.

This makes the validity of the model vulnerable to relatively simple genetic experiments. It would be a routine task, for instance, to screen a large number of mutants for exceptions to the above conditions.

The nonsense model is very appealing because it explains so many observations and is readily testable. Therefore, its logical limitations, which Benzer and Champe (1962) acknowledge, should be examined. In their interpretation of the behavior of r1589 double mutants they assume that the product of the A-cistron fragment does not affect the B activity. They argue: "It seems most unlikely, however, that the present data could be due to such an effect, since it is implausible that the effects would be the same for the interaction of the A with the B fragment in rII and for the interaction of the phosphatase molecule with itself." There is, however, one type of interaction that, to me at least, seems plausible-allelic complementation.

Sensitive Enzyme Models

Many mutations affecting enzymes result in mutants that are abnormally sensitive to various agents or conditions such as pH and concentration of certain metal ions (Suskind and Kurek 1959; Campbell 1961). In principle either the formation of the enzyme [steps (d) and (e)] or its activity could be sensitive [step (f)]. In cases for which the inhibiting condition is the result of a particular concentration (or the presence or absence) of some small molecule in the cytoplasm, this condition would presumably be under genetic control. If the rest of the cell's metabolic apparatus can tolerate sufficient variation of these conditions, then the function of the sensitive mutants could be restored by action of the genes controlling the formation of the compounds involved. Those genes would then behave as super-suppressors.

General properties of such models. The detailed predictions of this kind of model depend on the specific nature of the inhibiting conditions, but some common characteristics are predicted. According to these models a complete protein should be formed. If the activity of the protein as an enzyme is the aspect that is sensitive, then the protein formed may be quite similar to wild-type protein and would be expected to show some activity in vitro if the proper conditions could be achieved. If, on the other hand, the folding of the protein or the association of protein monomers into active polymers is the sensitive step, then the

inactive protein may be quite different from the wild type. So, although a complete protein is predicted by this kind of model, that protein may be so drastically altered in its tertiary and quaternary structure as to be undetectable by available methods.

A specific inhibitor model can be formulated to explain many of the observed properties of the suppressible tr_5 mutants and the super-suppressors that suppress them.

The super-inhibitor model. Assume that wild-type yeast produces some compound that can act as an inhibitor to a variety of enzymes. In that same wild-type organism all active enzyme would of necessity be resistant to inhibition by this agent. Many of these enzymes, however, could become sensitive to it by mutation. If this super-inhibitor itself is a compound synthesized through a several-step pathway, each locus controlling a step of that pathway could be a super-suppressor locus. This would account for several super-suppressor loci with effectively the same spectrum of action. Further, if tr_{5-19} is a double tr_5 mutant bearing a suppressible alteration and an indole-utilizing one, removal of the inhibitor would result in an indole-utilizing phenotype.

Predictions Based on Super-Suppressor Models

The above models are highly speculative. They contribute to our understanding of biology mainly to the extent that they suggest useful experiments. Each of the models discussed leads to predictions of specific biochemical properties of suppressible and suppressed mutants. These will provide the basis for further studies on the mechanisms of super-suppression.

Formation of altered proteins. Knowledge of whether a complete protein chain is produced by suppressible mutants would help distinguish between some of the possible alternative models. The nonsense model specifically predicts that only incomplete proteins are synthesized. Mutationally altered protein can be recognized in favorable cases by immunochemical tests and by compementation tests (Suskind, Yanofsky and Bonner 1955; Suyama 1963). When such tests are negative, however, they are not conclusive. Only from positive evidence

of altered protein formation can conclusions be drawn. Accordingly, experiments of this nature may provide a critical test of the nonsense hypothesis, but not directly of the sensitive enzyme hypothesis. If an altered protein is identified, however, the latter model predicts properties that can provide such a test.

Sensitive enzymes. If super-suppressible mutants produce enzymes that are reversibly inhibited by something normally present in yeast cytoplasm, then sufficient purification of the enzyme preparation should restore some activity. Here again negative results from partial purification would not be conclusive, although a positive result would be. The specific hypothesis of a super-inhibitor, however, can be more critically tested. This hypothesis states that the suppressible mutant contains a sensitive enzyme plus an inhibitor. The suppressed strain contains the sensitive enzyme but not the inhibitor. A mixture of extracts from the two strains should contain the sensitive enzyme plus the inhibitor, and since several suppressible mutations can exist in the same strain the inhibitor would have to be in excess. Therefore the hypothesis predicts that the mixture of extracts should also be inactive.

Properties of pure enzymes. If the enzyme produced by suppressible and suppressed mutants could be purified sufficiently to permit amino-acid-composition analysis and fingerprinting and sequence analysis, then one might hope to determine how suppressible mutations and super-suppressors affect the primary structure of the protein, as Brody and Yanofsky (1963) have shown for a specific suppressor. The feasibility of this type of experiment with yeast tryptophan synthetase, however, cannot be judged until the properties of the enzyme are further investigated.

SUMMARY

The tryptophan synthetase system of Saccharomyces cerevisiae has been investigated and used to study the action of a super-suppressor. A new method for allelic mapping was developed and employed in these studies.

(a) Thirty-six allelic tr_5 mutants were isolated and characterized. Enzyme assays of crude extracts demonstrated that this locus controls the formation of tryptophan synthetase activity. Three mutants accumulate indole when grown on tryptophan-limited medium, and one mutant is able to utilize indole.

(b) Of 35 mutants tested in all possible combinations, two at a time, 19 complemented with at least one other. A linear complementation map with four complons was constructed from these results.

(c) An allelic map for 29 mutants was constructed by the x-ray method. The mutants are randomly distributed along the length of this map with the exception of four that are repeat mutants at one site. This map is colinear with the complementation map with one exception.

(d) Thirteen of 32 mutants tested are suppressible by the super-suppressor S_d. Of these, only two are of the complementing type. One other mutant is altered by S_d to an indole-utilizing phenotype. This mutant has unusual reversion properties.

(e) The two mutants that are both suppressible and complementing complement only with alleles that are to their left on the x-ray allelic map. They are unique in this respect

(f) Two types of models to explain these results are considered, and biochemical experiments to test them are proposed.

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APPENDIX

Table A-I. Tryptophan tester strains.

Locus	Strain	Genotype
tr_1	S732C	a ga_1 tr_1 ad_1 hi_2
tr_1	S733A	α ga_1 tr_1 ad_1 hi_2 le_1 ur_1
tr_2	D160-4D	a ur_3 thr_3 hi_1 ar_6 is_1 tr_2 ad_1
tr_2	D160-2C	α ur_3 thr_3 hi_1 ar_6 is_1 tr_2 ad_1
tr_3	S1783D	a ga_1 hi_6 thr_1 ar_4 ur_2 ad_1 ur_1 ly_1 tr_3
tr_3	S1788B	α ur_2 ur_1 ly_1 tr_3
tr_4	X1011-1B	a tr_4 ad_1 ad_2
tr_4	X1011-6D	α hi_6 tr_4 ur_2 ad_1 thr_5
tr_5	S1795A	a hi_4 tr_{5-1} ad_6 ur_1
tr_5	S1799D	α hi_4 tr_{5-1} ad_6

Table A-II. Genetic markers and symbols.[a]

Amino acids	Symbol
Arginine	ar
Histidine	hi
Leucine	le
Lysine	ly
Methionine	met
Phenylalanine	pha
Serine	ser
Threonine	thr
Tryptophan	tr
Tyrosine	ty
Bases	
Adenine	ad
Uracil	ur
Others	
Actidione (resistance)	ac^r
Canavanine (resistance)	can^r
Mating type	a, α
Heterozygote	a/α
Petite	p

a. From Carbondale Yeast Genetics Conference, Microbial Genetics Bulletin, Supplement to No. 19, January 1963.

Table A-III. Parents of tr_5 mutants.

Strain	Genotype
S288C	α ga_2^a
DS6	α ga_2 can^r
DV147	α ga_2 ad_{2-2}
JB97	α ga_2 ad_{2-2} le_1
S1285C	a ad_{2-1}
XT300-4A	a ad_{2-1}

a. Fermentation markers not scored in present experiments.

Table A-IV. tr_5 mutants.

tr_5 Allele	Mutant	Parent	tr_5 Allele	Mutant	Parent
1	DT29[a]	S288C	19	FG40[a]	DS6
2	EV20[a]	DS6	20	MB5	JB97
3	JB103[a]	DV147	21	MB46	JB97
4	JB112[a]	DV147	22	MD1	XT300-4A
5	JB115[a]	DV147	23	MD3	XT300-4A
6	JC2[a]	S1285C	24	MD6	XT300-4A
7	JC20[a]	S1285C	25	MD8	XT300-4A
8	MA2	JB97	26	MD14	XT300-4A
9	MA5	JB97	27	MD20	XT300-4A
10	MA7	JB97	28	MD21	XT300-4A
11	MA14	JB97	29	MD23	XT300-4A
12	MA20	JB97	30	MD27	XT300-4A
13	DZ83[a]	S288C	31	MD28	XT300-4A
14	DZ115[a]	S288C	32	MD30	XT300-4A
15	AE7[a]	S288C	33	MD37	XT300-4A
16	ER36[a]	DS6	34	MD38	XT300-4A
17	EW23[a]	DS6	35	MD39	XT300-4A
18	FB35[a]	DS6	36	MD40	XT300-4A

a. Mutants obtained from Robert K. Mortimer, University of California.

Table A-V. Miscellaneous genotypes.

Hybrid	Genotype
X901	$\frac{a}{a}\ \frac{thr_2\ ty_4}{+\ ty_2}\ \frac{me_1}{me_1}\ \frac{+\ +\ +}{tr_5\ le_1\ ad_6}\ \frac{ly_1\ +}{+\ hi_6}\ \frac{hi_2}{+}\ \frac{tr_1}{+}\ \frac{ar_{4-1}\ thr_1\ CU_1}{ar_{4-2}\ +\ cu_1}\ \frac{+}{ur_1}$
X1272	$\frac{a}{a}\ \frac{+}{ad_{2-2}}\ \frac{ar_{4-1}\ thr_1}{ar_{4-3}\ +}\ \frac{ty_4}{+}\ \frac{tr_5\ \ le_1\ \ ad_6}{+\ \ +\ \ +}\ \frac{hi_6}{+}\ \frac{hi_2}{+}\ \frac{tr_1}{+}\ \frac{ur_1}{+}\ \frac{thr_2}{+}$
X1274	$\frac{a}{a}\ \frac{+}{ad_{2-2}}\ \frac{ar_{4-2}\ thr_1}{ar_{4-3}\ +}\ \frac{ty_4}{+}\ \frac{tr_5}{+}\ \frac{hi_6}{+}\ \frac{hi_2}{+}\ \frac{ur_1}{+}\ \frac{thr_2}{+}\ \frac{CU_1}{cu_1}$
XT61	$\frac{a}{a}\ \frac{ar_{4-1}\ thr_1}{ar_{4-1}\ thr_1}\ \frac{ty_4}{ty_4}\ \frac{tr_5\ \ le_1\ \ ad_6}{tr_5\ \ le_1\ \ ad_6}\ \frac{hi_6}{hi_6}\ \frac{hi_2}{+}\ \frac{tr_1}{+}\ \frac{ur_1}{+}\ \frac{thr_2}{thr_2}\ \frac{+}{ly_1}$
XT62	$\frac{a}{a}\ \frac{ar_{4-2}\ thr_1}{ar_{4-2}\ thr_1}\ \frac{ty_4}{+}\ \frac{+}{ty_2}\ \frac{tr_5}{+}\ \frac{hi_6}{+}\ \frac{hi_2}{+}\ \frac{ur_1}{ur_1}\ \frac{thr_2}{+}\ \frac{CU_1}{CU_1}$
XT294	$\frac{a}{a}\ \frac{thr_4}{+}\ \frac{tr_{5-8}\ le_1}{tr_{5-10}\ le_1}\ \frac{ur_3}{+}\ \frac{hi_8\ \ ser_1\ \ ad_2}{hi_8\ \ ser_1\ \ ad_2}$
XT297	$\frac{a}{a}\ \frac{hi_4\ \ le_2\ \ thr_4}{+\ \ +\ \ +}\ \frac{tr_{5-10}\ le_1}{tr_{5-8}\ \ le_1}\ \frac{ly_7}{ly_7}\ \frac{ad_2\ \ +}{ad_2\ \ hi_8}$
X841	$\frac{a}{a}\ \frac{me_1}{+}\ \frac{ur_1}{+}\ \frac{ad_2}{+}\ \frac{hi_{5-2}}{hi_{5-2}}\ \frac{tr_1}{tr_1}\ \frac{+\ \ ar_{4-1}\ thr_1}{p_1\ \ ar_{4-1}\ +}\ \frac{ly_1}{+}\ \frac{le_1}{+}$

Table A-VI. Haploid parent strains of hybrids used to study action of S_d.

Hybrid	a Parent	α Parent	tr_5 Allele	Hybrid	a Parent	α Parent	tr_5 Allele
XT301	XT39-6D	XT101-10D	1	XT449	MD21	XT304-6B	28
XT302	XT39-6D	XT102-1B	2	XT450	MD23	XT304-6B	29
XT405	XT39-6D	JB103	3	XT451	MD27	XT304-6B	30
XT304	XT39-6D	XT104-13C	4	XT452	MD28	XT304-6B	31
XT305	XT30-6D	XT105-9D	5	XT453	MD30	XT304-6B	32
XT442	JC2	XT304-6B	6	XT454	MD37	XT304-6B	33
XT443	JC20	XT304-6B	7	XT455	MD38	XT304-6B	34
XT308	XT39-6D	XT108-1A	8	XT456	MD39	XT304-6B	35
XT309	XT39-6D	XT109-1C	9	XT457	MD40	XT304-6B	36
XT310	XT39-6D	XT110-8A	10	XT473	XT302-4D	XT39-2A	2
XT311	XT39-6D	XT111-6A	11	XT474	XT405-3A	XT39-2A	3
XT312	XT39-6D	XT112-5B	12	XT475	XT304-5C	XT39-2A	4
XT313	XT39-6D	XT113-2B	13	XT476	XT309-7A	XT39-2A	9
XT408	XT39-6D	ER36	16	XT495	XT312-7A	XT39-2A	12
XT317	XT39-6D	XT117-1A	17	XT478	XT313-1A	XT39-2A	13
XT318	XT39-6D	XT118-9B	18	XT494	XT409-4C	XT39-2A	19
XT409	XT39-6D	FG40	19	XT481	XT321-6C	XT39-2A	21
XT320	XT39-6D	MB5	20	XT482	XT458-5C	XT39-2A	24
XT321	XT39-6D	MB47	21	XT483	XT450-5B	XT39-2A	29
XT445	MD3	XT304-6B	23	XT497	XT454-4D	XT39-2A	33
XT458	MD6	XT304-6B	24	XT485	XT455-3A	XT39-2A	34
XT446	MD8	XT304-6B	25	XT486	XT457-5A	XT39-2A	36
XT448	MD20	XT304-6B	27				

Table A-VII. Genotypes of hybrids XT301 to XT321.

Hybrid	tr_5 Allele	tr_5	hi_8	ser_1	ad_2	ly_7	p_1	thr_4	ur_3	le_1	le_2	hi_4	S_d [a]
XT301	1	+/-	+/-	+/-	-/-	+/-	-/+	+/+	+/+	+/+	+/+	+/+	+/-
XT302	2	+/-	+/-	+/-	-/-	+/-	-/+	+/-	+/-	+/+	+/+	+/+	+/-
XT405	3	+/-	+/+	+/+	-/-	+/-	+/+	+/+	+/+	+/+	+/+	+/+	+/-
XT304	4	+/-	+/-	+/+	-/-	+/+	-/+	+/+	+/-	+/+	+/+	+/+	+/-
XT305	5	+/-	+/-	+/+	-/-	+/-	-/+	+/-	+/+	+/+	+/+	+/+	+/-
XT442	6	+/-	+/-	+/+	-/- [b]	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/+
XT443	7	+/-	+/-	+/+	-/-	+/+	+/+	+/+	+/+	+/+	+/+	+/+	-/+
XT308	8	+/-	+/-	+/+	-/-	+/-	-/+	+/+	+/+	+/-	+/+	+/+	+/-
XT309	9	+/-	+/+	+/+	-/- [b]	+/+	-/+	+/+	+/+	+/-	+/-	+/-	+/-
XT310	10	+/-	+/-	+/-	-/- [b]	+/+	-/+	+/+	+/+	+/+	+/-	+/+	+/-
XT311	11	+/-	+/-	+/-	-/-	+/+	-/+	+/-	+/+	+/+	+/+	+/+	+/-
XT312	12	+/-	+/-	+/-	-/-	+/-	-/+	+/-	+/+	+/+	+/+	+/+	+/-
XT313	13	+/-	+/-	+/+	-/-	+/+	-/+	+/-	+/-	+/+	+/+	+/+	+/-
XT408	16	+/-	+/+	+/+	-/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-
XT317	17	+/-	+/+	+/+	-/+	+/+	-/+	+/+	+/-	+/+	+/+	+/+	+/-
XT318	18	+/-	+/-	+/-	-/-	+/+	-/+	+/+	+/-	+/+	+/+	+/+	+/-
XT409	19	+/-	+/+	+/+	-/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-
XT320	20	+/-	+/+	+/+	-/- [b]	+/+	-/+	+/+	+/+	+/-	+/+	+/+	+/-
XT321	21	+/-	+/+	+/+	-/- [b]	+/+	-/+	+/+	+/+	+/-	+/+	+/+	+/-

a. Active form of S_d = +.

b. Nonsuppressible allele of ad_2.

Table A-VIII. Genotypes of hybrids XT445 to XT457.

Hybrid	tr_5 Allele	tr_5	hi_8	ad_2	S_d [a]
XT445	23	-/+	+/-	-/-	-/+
XT458	24	-/+	+/-	-/-	-/+
XT446	25	-/+	+/-	-/-	-/+
XT448	27	-/+	+/-	-/-	-/+
XT449	28	-/+	+/-	-/-	-/+
XT450	29	-/+	+/-	-/-	-/+
XT451	30	-/+	+/-	-/-	-/+
XT452	31	-/+	+/-	-/-	-/+
XT453	32	-/+	+/-	-/-	-/+
XT454	33	-/+	+/-	-/-	-/+
XT455	34	-/+	+/-	-/-	-/+
XT456	35	-/+	+/-	-/-	-/+
XT457	36	-/+	+/-	-/-	-/+

a. Active form of S_d = + .

Table A-IX. Genotypes of hybrids XT473 to XT486.

Hybrid	tr_5 Allele	tr_5	ad_2	p_1	ur_3	ly_1	hi_{5-2}	S_d [a]
XT473	2	-/+	-/-	+/+	-/+	+/-	+/-	+/-
XT474	3	-/+	-/-	+/+	+/+	+/-	+/-	+/-
XT475	4	-/+	-/-	+/+	-/+	+/-	+/-	+/-
XT476	9	-/+	-/- [b]	+/+	+/+	+/-	+/-	+/-
XT495	12	-/+	-/-	+/+	+/+	+/-	+/-	+/-
XT478	13	-/+	-/-	+/-	+/+	+/-	+/-	+/-
XT494	19	-/+	+/-	+/+	+/+	+/-	+/-	+/-
XT481	21	-/+	-/-	+/-	+/+	+/-	+/-	+/-
XT482	24	-/+	-/-	+/+	+/+	+/-	+/-	+/-
XT483	29	-/+	-/-	+/+	+/+	+/-	+/-	+/-
XT497	33	-/+	-/-	+/+	+/+	+/-	+/-	+/-
XT485	34	-/+	-/-	+/+	+/+	+/-	+/-	+/-
XT486	36	-/+	-/-	+/+	+/+	+/-	+/-	+/-

a. Active form of S_d = +.

b. Nonsuppressible allele of ad_2.

Table A-X. Data from which Table V was derived.

Hybrid	$\frac{tr_5}{\text{Genotype}}$	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec	Expt.
XT393	$\frac{5\text{-}9}{5\text{-}6}$	0	27 (2)[b]	138(4)[b]	1.0	4.4 ± 0.6	37
		10	74 (2)	137(3)	1.0		
		20	73 (3)	134(3)	0.5		
XT394	$\frac{5\text{-}9}{5\text{-}7}$	0	106 (4)	134(4)	1.0	12.3 ± 1.0	37
		10	252 (4)	137(3)	1.0		
		20	212 (4)	130(3)	0.5		
XT395	$\frac{5\text{-}12}{5\text{-}6}$	0	13 (4)	146(4)	1.0	2.5 ± 0.6	37
		10	60 (4)	145(3)	1.0		
		20	42 (3)	140(3)	0.5		
XT396	$\frac{5\text{-}12}{5\text{-}7}$	0	8 (4)	128(4)	1.0	1.1 ± 0.1	37
		10	22 (4)	131(3)	1.0		
		20	18 (4)	123(3)	0.5		
XT393	$\frac{5\text{-}9}{5\text{-}6}$	0	91 (4)	165(4)	1.0	3.4 ± 0.08	41
		10	140 (4)	163(3)	1.0		
		20	98 (4)	156(3)	0.5		
XT394	$\frac{5\text{-}9}{5\text{-}7}$	0	199 (4)	159(4)	1.0	10.2 ± 0.8	41
		10	334 (4)	154(3)	1.0		
		20	255 (5)	144(3)	0.5		
XT397	$\frac{5\text{-}20}{5\text{-}6}$	0	37 (4)	156(4)	1.0	7.0 ± 0.1	41
		10	148 (4)	173(3)	1.0		
		20	134 (4)	164(3)	0.5		

a. Dose rate = 200 r/sec.
b. Number of plates for each point shown in parentheses.

(Continued) Table A-X. Data from which Table V was derived.

Hybrid	$\frac{tr_5}{\text{Genotype}}$	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec	Expt.
XT398	$\frac{5\text{-}20}{5\text{-}7}$	0	146 (4)	154 (3)	1.0	12.7 ± 0.6	41
		10	355 (4)	155 (3)	1.0		
		20	272 (4)	163 (3)	0.5		
XT399	$\frac{5\text{-}21}{5\text{-}6}$	0	53 (4)	136 (4)	1.0	9.8 ± 1.7	41
		10	204 (4)	116 (3)	1.0		
		20	152 (4)	130 (3)	0.5		
XT400	$\frac{5\text{-}21}{5\text{-}7}$	0	146 (4)	131 (4)	1.0	15.1 ± 0.5	41
		10	333 (4)	133 (3)	1.0		
		20	272 (4)	130 (2)	0.5		
XT401	$\frac{5\text{-}6}{5\text{-}1}$	0	16 (3)	110 (4)	1.0	11.4 ± 0.5	41
		10	152 (4)	113 (3)	1.0		
		20	133 (4)	111 (3)	0.5		
XT402	$\frac{5\text{-}7}{5\text{-}1}$	0	365 (2)	128 (4)	1.0	29.6 ± 0.4	41
		10	730 (2)	123 (3)	1.0		
		20	558 (2)	131 (3)	0.5		
XT403	$\frac{5\text{-}18}{5\text{-}6}$	0	2 (4)	143 (3)	1.0	0.6 ± 0.05	41
		10	10 (4)	145 (3)	1.0		
		20	10 (4)	158 (3)	0.5		

a. Dose rate = 200 r/sec.

Table A-XI. Data from which Table VI was derived (experiment 46).

Hybrid	$\frac{tr_5}{\text{Genotype}}$	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec
XT412	$\frac{5-23}{5-20}$	0	28 (4)[b]	166 (4)[b]	1.0	8.8 ± 0.2
		10	173 (4)	178 (3)	1.0	
		20	163 (4)	164 (3)	0.5	
XT413	$\frac{5-23}{5-21}$	0	94 (4)	160 (4)	1.0	11.0 ± 0.8
		10	296 (4)	179 (3)	1.0	
		20	232 (4)	165 (3)	0.5	
XT414	$\frac{5-24}{5-20}$	0	3 (4)	172 (4)	1.0	0.68± 0.12
		10	18 (4)	168 (3)	1.0	
		20	12 (4)	155 (3)	0.5	
XT415	$\frac{5-24}{5-21}$	0	54 (4)	156 (4)	1.0	5.0 ± 0.7
		10	112 (4)	151 (3)	1.0	
		20	101 (3)	148 (3)	0.5	
XT416	$\frac{5-25}{5-20}$	0	77 (4)	145 (4)	1.0	11.0 ± 1.1
		10	266 (4)	149 (3)	1.0	
		20	200 (4)	146 (3)	0.5	
XT417	$\frac{5-25}{5-20}$	0	177 (4)	151 (4)	1.0	12.5 ± 0.1
		10	369 (4)	162 (3)	1.0	
		20	284 (4)	155 (3)	0.5	
XT418	$\frac{5-22}{5-20}$	0	126 (4)	207 (4)	1.0	2.0 ± 0.4
		10	180 (4)	191 (3)	1.0	
		20	103 (4)	214 (3)	0.5	

a. Dose rate = 200 r/sec.
b. Number of plates for each point shown in parentheses.

Table A-XI (continued). Data from which Table VI was derived (experiment 46).

Hybrid	$\frac{tr_5}{\text{Genotype}}$	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec
XT419	$\frac{5-22}{5-7}$	0	26 (4)	180 (4)	1.0	7.1 ± 0.3
		10	145 (4)	176 (3)	1.0	
		20	139 (4)	177 (3)	0.5	
XT421	$\frac{5-26}{5-21}$	0	345 (4)	132 (4)	1.0	12.2 ± 2.5
		10	564 (4)	132 (3)	1.0	
		20	334 (4)	129 (1)	0.5	
XT422	$\frac{5-27}{5-20}$	0	14 (4)	154 (4)	1.0	3.5 ± 0.32
		10	76 (4)	156 (3)	1.0	
		20	61 (4)	142 (2)	0.5	
XT423	$\frac{5-27}{5-21}$	0	42 (4)	164 (3)	1.0	0.8 ± 0.3
		10	60 (4)	159 (2)	1.0	
		20	29 (4)	143 (3)	0.5	
XT424	$\frac{5-28}{5-20}$	0	86 (4)	171 (3)	1.0	12.6 ± 0.2
		10	310 (4)	171 (3)	1.0	
		20	261 (4)	174 (3)	0.5	
XT425	$\frac{5-28}{5-21}$	0	158 (4)	163 (4)	1.0	10.6 ± 0.4
		10	347 (4)	172 (2)	1.0	
		20	254 (4)	163 (3)	0.5	
XT426	$\frac{5-29}{5-20}$	0	128 (4)	157 (4)	1.0	13.1 ± 0.7
		10	355 (4)	159 (3)	1.0	
		20	272 (4)	160 (2)	0.5	

a. Dose rate = 200 r/sec.

Table A-XI (continued). Data from which Table VI was derived (experiment 46).

Hybrid	tr_5 Genotype	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec
XT427	$\frac{5-29}{5-21}$	0	132 (4)	151 (4)	1.0	16.5 ± 0.2
		10	386 (4)	165 (3)	1.0	
		20	325 (4)	155 (2)	0.5	
XT428	$\frac{5-30}{5-20}$	0	33 (4)	158 (4)	1.0	9.4 ± 0.3
		10	179 (4)	143 (3)	1.0	
		20	154 (4)	137 (3)	0.5	
XT429	$\frac{5-30}{5-21}$	0	705 (1)	181 (4)	1.0	11.5
		20	554 (1)	182 (3)	0.5	
XT430	$\frac{5-31}{5-20}$	0	38 (4)	174 (4)	1.0	10.7 ± 0.4
		10	229 (4)	150 (3)	1.0	
		20	198 (4)	170 (3)	0.5	
XT431	$\frac{5-31}{5-21}$	0	601 (1)	153 (4)	1.0	13.7
		20	521 (1)	175 (3)	0.5	
XT432	$\frac{5-32}{5-20}$	0	28 (4)	168 (4)	1.0	11.6 ± 0.05
		10	226 (4)	169 (3)	1.0	
		20	212 (4)	177 (3)	0.5	
XT433	$\frac{5-32}{5-21}$	0	76 (4)	178 (4)	1.0	13.9 ± 0.2
		10	327 (4)	187 (3)	1.0	
		20	295 (4)	193 (3)	0.5	

a. Dose rate = 200 r/sec.

Table A-XI (continued). Data from which Table VI was derived (experiment 46).

Hybrid	$\frac{tr_5}{\text{Genotype}}$	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec
XT434	$\frac{5-33}{5-20}$	0	38 (4)	178 (4)	1.0	9.5 ± 0.8
		10	223 (4)	162 (3)	1.0	
		20	181 (4)	167 (3)	0.5	
XT435	$\frac{5-33}{5-21}$	0	62 (4)	162 (4)	1.0	10.4 ± 1.1
		10	269 (3)	164 (3)	1.0	
		20	204 (4)	170 (3)	0.5	
XT437	$\frac{5-34}{5-21}$	0	80 (4)	166 (4)	1.0	7.6 ± 1.2
		10	220 (4)	174 (3)	1.0	
		20	170 (4)	171 (3)	0.5	
XT438	$\frac{5-35}{5-20}$	0	37 (4)	137 (4)	1.0	2.4 ± 0.14
		10	66 (2)	128 (3)	1.0	
		20	50 (4)	130 (2)	0.5	
XT439	$\frac{5-35}{5-21}$	0	226 (4)	182 (4)	1.0	5.3 ± 0.1
		10	325 (4)	190 (3)	1.0	
		20	210 (3)	167 (2)	0.5	
XT440	$\frac{5-36}{5-20}$	0	707 (1)	204 (4)	1.0	5.5
		20	406 (2)	181 (3)	0.5	

a. Dose rate = 200 r/sec.

Table A-XII. Data from which Table VII was derived (experiment 57).

Hybrid	tr_5 Genotype	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec
XT438	$\frac{5-35}{5-20}$	0	15 (8)[b]	213 (3)[b]	1.0	2.4 ± 0.2
		10	74 (4)	197 (3)	1.0	
		20	58 (4)	218 (3)	0.5	
XT439	$\frac{5-35}{5-21}$	0	19 (7)	221 (3)	1.0	7.2 ± 0.5
		10	192 (4)	216 (3)	1.0	
		20	168 (4)	204 (3)	0.5	
XT440	$\frac{5-36}{5-20}$	0	19 (6)	204 (3)	1.0	5.0 ± 0.12
		10	122 (4)	210 (2)	1.0	
		20	111 (4)	192 (3)	0.5	
XT459	$\frac{5-11}{5-35}$	0	20 (8)	226 (4)	1.0	4.0 ± 0.06
		10	107 (4)	221 (3)	1.0	
		20	98 (4)	213 (3)	0.5	
XT460	$\frac{5-11}{5-36}$	0	5 (8)	209 (3)	1.0	3.0 ± 0.17
		10	74 (4)	210 (3)	1.0	
		20	65 (4)	216 (3)	0.5	
XT461	$\frac{5-18}{5-35}$	0	8 (8)	200 (4)	1.0	7.4 ± 0.12
		10	160 (4)	221 (3)	1.0	
		20	161 (4)	214 (3)	0.5	
XT462	$\frac{5-18}{5-36}$	0	11 (8)	172 (4)	1.0	14.0 ± 1.5
		10	308 (3)	187 (3)	1.0	
		20	256 (4)	179 (3)	0.5	
XT463	$\frac{5-19}{5-35}$	0	2 (7)	197 (4)	1.0	1.7 ± 0.12
		10	32 (4)	214 (3)	1.0	
		20	36 (4)	205 (3)	0.5	

a. Dose rate = 200 r/sec.
b. Number of plates for each point shown in parentheses.

Table A-XII (continued). Data from which Table VII was derived (experiment 57).

Hybrid	tr_5 Genotype	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec
XT464	$\frac{5-19}{5-36}$	0	8 (9)	219 (3)	1.0	4.9 ± 1.2
		10	134 (2)	213 (3)	1.0	
		20	108 (4)	204 (3)	0.5	
XT465	$\frac{5-2}{5-35}$	0	13 (4)	214 (4)	1.0	10.8 ± 0.8
		10	273 (4)	214 (3)	1.0	
		20	238 (4)	213 (3)	0.5	
XT467	$\frac{5-4}{5-35}$	0	581 (7)	195 (4)	1.0	7.0 ± 0.9
		10	742 (1)	183 (3)	1.0	
		20	419 (3)	177 (3)	0.5	
XT469	$\frac{5-4}{5-35}$	0	3 (8)	196 (4)	1.0	0.4 ± 0.0
		10	11 (4)	192 (3)	1.0	
		20	10 (4)	190 (3)	0.5	
XT470	$\frac{5-9}{5-36}$	0	15 (8)	199 (4)	1.0	7.0 ± 0.12
		10	158 (4)	219 (3)	1.0	
		20	143 (3)	219 (3)	0.5	
XT471	$\frac{5-16}{5-35}$	0	10 (8)	179 (3)	1.0	3.0 ± 0.3
		10	78 (4)	186 (2)	1.0	
		20	62 (4)	197 (3)	0.5	
XT472	$\frac{5-16}{5-36}$	0	46 (8)	191 (4)	1.0	10.3 ± 0.8
		10	264 (4)	187 (3)	1.0	
		20	216 (4)	180 (3)	0.5	

a. Dose rate = 200 r/sec.

Table A-XIII. Data from which Table VIII was derived.

Hybrid	$\frac{tr_5}{\text{Genotype}}$	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec	Expt.
XT518	$\frac{5\text{-}6}{5\text{-}18}$	0	11 (4)[b]	258 (4)[b]	10	0.52	63
		20	136 (4)	238 (3)	5		
XT519	$\frac{5\text{-}18}{5\text{-}18}$	0	0 (4)	86 (3)	100	0.0037	68
		20	6.2 (4)	80 (3)	100		
XT520	$\frac{5\text{-}6}{5\text{-}6}$	0	3 (4)	137 (3)	100	0.016	68
		20	45 (4)	125 (3)	100		
XT525	$\frac{5\text{-}28}{5\text{-}29}$	0	30 (4)	125 (4)	0.2	12.3	69
		20	46 (4)	128 (4)	0.1		
XT526	$\frac{5\text{-}6}{5\text{-}13}$	0	5 (4)	114 (4)	1.0	2.2	69
		20	29 (4)	122 (4)	0.5		
XT527	$\frac{5\text{-}34}{5\text{-}18}$	0	5 (4)	125 (4)	1.0	2.4	69
		20	32 (4)	125 (4)	0.5		
XT528	$\frac{5\text{-}6}{5\text{-}3}$	0	10 (4)	140 (4)	1.0	4.5	69
		20	70 (4)	155 (3)	0.5		
XT531	$\frac{5\text{-}3}{5\text{-}35}$	0	124 (4)	82 (4)	1.0	1.0	70
		20	70 (4)	76 (4)	0.5		
XT532	$\frac{5\text{-}7}{5\text{-}12}$	0	10 (4)	126 (4)	1.0	3.2	70
		20	48 (4)	136 (4)	0.5		
XT534	$\frac{5\text{-}29}{5\text{-}12}$	0	146 (4)	76 (4)	1.0	5.1	70
		20	114 (4)	84 (4)	0.5		

a. Dose rate = 200 r/sec.
b. Number of plates for each point shown in parentheses.

Table A-XIII (continued). Data from which Table VIII was derived.

Hybrid	Genotype	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec	Expt.
XT535	$\frac{5-12}{5-35}$	0	150 (4)	67 (4)	1.0	7.5	70
		20	126 (4)	70 (4)	0.5		
XT536	$\frac{5-7}{5-18}$	0	20 (4)	142 (4)	1.0	8.9	70
		20	133 (4)	134 (4)	0.5		
XT538	$\frac{5-18}{5-28}$	0	257 (4)	86 (3)	1.0	2.7	70
		20	151 (4)	82 (3)	0.5		
XT540	$\frac{5-18}{5-35}$	0	200 (4)	128 (4)	1.0	5.4	70
		20	170 (4)	131 (4)	0.5		
XT541	$\frac{5-18}{5-36}$	0	352 (2)	108 (4)	0.5	11.5	70
		20	472 (4)	102 (4)	0.5		

a. Dose rate = 200 r/sec.

Table A-XIV. Data from which Table IX was derived.

Hybrid	tr_5 Genotype	Dose (sec)[a]	Mean colonies per plate -Tr	Mean colonies per plate YEPD	Diln. factor ($\times 10^{-4}$)	Prototrophs per 10^6 survivors per sec	Expt.
XT441	$\frac{5\text{-}36}{5\text{-}21}$	0	10 (4)[b]	160 (4)[b]	50	0.017	62
		20	37 (5)	162 (3)	50		
XT466	$\frac{5\text{-}36}{5\text{-}2}$	0	0.4 (5)	195 (4)	50	0.016	62
		20	35 (5)	202 (3)	50		
XT468	$\frac{5\text{-}36}{5\text{-}4}$	0	8 (5)	176 (4)	50	0.014	62
		20	31 (5)	169 (3)	50		
XT506	$\frac{5\text{-}2}{5\text{-}2}$	0	0.4 (5)	90 (4)	100	0.015	62
		20	31 (5)	92 (4)	100		
XT507	$\frac{5\text{-}4}{5\text{-}4}$	0	0.2 (5)	109 (4)	50	0.017	62
		20	19 (5)	124 (3)	50		
XT468	$\frac{5\text{-}36}{5\text{-}4}$	0	4 (5)	256 (4)	50	0.012	63
		20	33 (5)	250 (4)	50		
XT504	$\frac{5\text{-}36}{5\text{-}36}$	0	0.6 (5)	138 (4)	50	0.015	63
		20	21 (5)	138 (4)	50		
XT505	$\frac{5\text{-}21}{5\text{-}21}$	0	6 (5)	227 (4)	50	0.018	63
		20	45 (5)	220 (4)	50		
XT506	$\frac{5\text{-}2}{5\text{-}2}$	0	1.4 (5)	273 (4)	50	0.024	63
		20	67 (5)	282 (4)	50		

a. Dose rate = 200 r/sec.
b. Number of plates for each point shown in parentheses.

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