much higher doses of ultraviolet, Kelner (*7*), and Kanazir and Errera (*8*) found DNA synthesis to be inhibited.

Even though the cells do not divide within at least 3 hours after irradiation when grown at 37°C in the C-1 medium, most of them apparently recover their ability to divide at a later time and give rise to a normal macroscopic colony.

Further results relating to division inhibition mechanism follow. (i) The action spectrum for division inhibition, for which some data are shown in the lower part of Fig. 1, has a maximum at 2652 A and a minimum at 2300 A. (ii) As is shown by the work of Errera (*2*), the division inhibition can be reversed by visible light if the light is given within 15 minutes after the ultraviolet. (iii) The procedure of Robinow (*9*) for staining cytoplasmic cell boundaries discloses dark-staining bands at intervals along the filament of about the same distance as the length of normal cells. In old filament cultures, fission was occasionally seen at the dark-staining regions. Microscopic observation of the manner of lysis of the filaments by T-1 phage also indicates some sort of transverse cell boundaries.

Some preliminary work has been done on division inhibition in synchronized cultures. Synchronization was done by a slight modification of the technique of McNair Scott (*10*). There appears to be some variation in ultraviolet sensitivity as the cells go through the different stages of division. However, the change in sensitivity is probably not over a factor of 2. No definite correlation of this change with steps of division can be made yet, except that division seems to be more easily inhibited either just before or just after cytoplasmic division.

Since most of the filaments described here give rise to a normal-appearing colony when plated on nutrient agar, the effect measured by the microscopic technique is more correctly described as an extended division delay rather than a permanent inhibition. The colony formation is thus a result of a recovery of the ability to divide superimposed upon the original inhibition. The extent of recovery depends on the plating conditions. The initial inhibition seems to be very specific, and DNA, RNA, and protein synthesis continues at the usual rate (*11*).

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Respiratory Competence in the Diagnosis of Gene-Controlled Phenotypes in Saccharomyces

Respiratory deficiency (*1–6*) in yeast diminishes the phenotypic expression of many genes controlling the synthesis of enzymes. Comparison of the gene-controlled, adaptive fermentation of different sugars by respiration-deficient (aer) and respiration-sufficient (AER) strains indicates a slowed or delayed expression of fermentative ability. Studies of the adaptation of yeast to galactose in defined atmospheres suggest dependence of the rate of adaptation on the availability of oxygen. Thus, both a functioning respiratory mechanism and aerobic conditions are implicated in the rapid synthesis of enzymes. Respiration-deficient yeasts grow slowly on the surface of complete nutrient agar containing 2 percent of glucose and even more slowly on synthetic nutrient agar. When respiration-deficient strains carrying the appropriate dominant genes for fermentative ability are tested by replica plating or streaking on a synthetic medium for ability to ferment melezitose or galactose, or to synthesize adenine, they often resemble cultures carrying the recessive gene when they are scored after the usual period of from 1 to 2 days required for the respiration-sufficient dominant strain to form confluent growth. An example of the effect of respiratory competence on the diagnosis of phenotypes follows.

A hybrid (15177-uv4 × 8256) was made between two cultures, both of which were apparent nonfermenters of melezitose (one had been obtained by ultraviolet irradiation). The diploid hybrid was a rapid fermenter of melezitose, capable of rapid growth on solid synthetic medium or in broth tubes containing melezitose. This phenomenon might have been the result of the synergistic effect of two pairs of complementary genes controlling melezitose fermentation. Four tetrads segregated 2 AER melezitose-fermenters : 2 AER melezitose-nonfermenters. Further tests showed that the apparent nonfermenter parent (15177-uv4) was respiration-deficient, carrying the dominant gene controlling melezitose fermentation, while the other (8256) was respiration-sufficient, carrying the recessive allele for melezitose fermentation. It is clear that synergistic or complementary melezitose genes were not involved. Only a single pair of genes (*7*) controlling melezitose fermentation was concerned, the diminished fermentative ability of one parent being under cytoplasmic control. The types of progeny from the hybrid depend on whether or not the respiratory deficiency is under cytoplasmic or genic control (*8*). The effect of respiratory damage in obscuring the genotype of cultures is particularly significant, since many biochemical mutants used in genetic studies are derived from ultraviolet radiation, which is an inducer of respiratory deficiency in yeast (*9*).

It is reasonable to suppose that many different kinds of defects, both in genes and in cytoplasmic granules, may lead to respiratory deficiency. It is possible that (in addition to *bona fide* recessives) defects expressed by the slowing of adaptation to galactose- or maltose-fermentation which segregate regularly may be attributable to gene-controlled respiratory defects. This is especially true of delays in Durham-tube fermentation involving periods of less than 5 days (*10*) (Table 1). Delays of more than 5 days often involve mutation and selection. Reports of isomeric genes con-

Table 1. The effect of differences in respiratory ability of fermenters (GA) and nonfermenters of galactose (ga). AER signifies the phenotype for respiratory sufficiency; aer signifies the phenotype for respiratory deficiency. GA and ga indicate genotype. Plus sign denotes rapid growth of cells and gas evolution in inserts of Durham tubes and confluent growth of cells when streaked on the surface of agar. Minus sign denotes no appreciable growth and a lack of gas evolution in inserts of Durham tubes and no appreciable growth of cells streaked on the surface of agar. Mutant colonies usually are detected at frequencies of from 10^{-6} and 10^{-10} nonfermenter cells plated.

Genotype	Respiratory phenotype	Galactose Durham tube		Galactose agar streak	
		2 days	4 days	2 days	6 days
GA	AER	+	+	+	+
ga	AER	−	−	−	Mutant colonies
GA	aer	−/+	+	−	+
ga	aer	−	−	−	Mutant colonies

Table 2. Genotype and phenotype of adenine-requiring yeasts. AD and ad signify genes controlling adenine synthesis. AER and aer signify respiratory phenotype.

Genotype	Respiratory phenotype	Synthetic adenineless media	
		Agar 2 days	Broth 2 days
AD	AER	+	+
AD	aer	− or +/−	+
ad	AER	−	−
ad	aer	−	−

trolling the rates of carbohydrate fermentation in yeasts should be clarified by tests of the competence of the respiratory mechanisms of the segregants involved.

Similar complications are involved in the diagnosis of phenotype and genotype in adenine-requiring (ad) yeasts. The pinkish-red pigmentation of these yeasts is controlled by (i) genes, (ii) extrachromosomal factors, and (iii) environment (*11*). Both pink and brownish cultures give rise to adenine-requiring white colonies spontaneously, with relatively high frequencies. The pink color is restored on outcross to normal, and genetic tests revealed that gene mutation was not involved in the spontaneous change from red to white. In the absence of contact with air, such cultures form little or no pigment. Certain adenine-requiring, respiration-deficient pigmented strains form white colonies on 0.1 percent glucose agar (*12*), brownish colonies on 2 percent glucose agar, and deep red colonies on 8 percent glucose agar. Other nutritional factors (the concentration of yeast extract) influence color. In several strains, adenine-requiring, respiration-deficient clones can be distinguished from adenine-requiring, respiration-sufficient clones on 2 percent agar, on which the former are brown, while the latter are pink (*13*).

For some unknown reason, the phenotype of adenine-independent (AD), respiration-deficient yeasts is different in synthetic broth and on synthetic agar (Table 2) when scored over the usual period of from 1 to 2 days. Such cultures do not develop the positive phenotype (confluent growth) on adenineless synthetic agar plates, although they are phenotypically adenine-independent (show rapid growth) in broth. If an adenine-independent, respiration-deficient culture is diagnosed erroneously as adenine-dependent (because of the phenotype on agar), the vigorous growth which the hybrid with an adenine-dependent AER culture (AD aer × ad AER) displays on agar might be interpreted incorrectly to indicate the presence of two nonallelic genes controlling adenine-synthesis.

In certain matings (*8*) between (i) adenine-dependent mutants of strain 8256 which had been altered from pink to brown by ultraviolet irradiation and (ii) adenine-dependent mutants of strain 8282 which had changed spontaneously from pink to brown, the hybrid was pink—that is, brown × brown produced pink—suggesting the presence of nonallelic, synergistically acting genes. Subsequent crosses showed that the brown parents were both respiration-deficient and that the hybrid was respiration-sufficient. In 14 tetrads from three such hybrids, segregation was 2 ad AER pink: 2 ad aer brown. Tetrad analysis showed that respiratory competence was under genic control in one parent and cytoplasmic control in the other, and that the "nonallelism" was attributable to the restoration of respiratory competence in the hybrid by the complementary action of gene and cytoplasm.

A few respiration-deficient progeny may be expected from any AER × AER hybrid. For example, 31 tetrads from such a mating yielded the following:

AER	AER	AER	AER	22
AER	AER	AER	aer	2
AER	aer	aer	aer	2
aer	aer	aer	aer	5

It may be inferred that the respiratory granules are often lost during the process of cutting out the spore plasm from the epiplasm.

In view of these results, a fundamental ambiguity prevails in certain genetical analyses of the inheritance of slow fermentation, adenine-synthesis, and pigmentation. This ambiguity arises from the absence of information concerning the respiratory competence of hybrids and segregants. Routine tests of segregants for their respiratory phenotypes are an essential complement to any genetical analysis, regardless of the respiratory competence of the parents.

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References and Notes

1. A number of experimental criteria have been employed in the diagnosis of respiration deficiency (*2*). In our laboratory a respiration-sufficient (AER) strain is defined operationally in terms of (i) $Q_{O_2}(N)$ about 1000 with glucose as substrate; (ii) growth on lactate agar (*3*); (iii) intensely colored colonies after overlay with 2,3,5-triphenyl tetrazolium chloride (TTC) (*4*); and (iv) alkali production in an acetate medium (*5*). A respiration-deficient (aer) strain is defined operationally in terms of (i) $Q_{O_2}(N)$ 0–50 with glucose as substrate; (ii) inability to grow on lactate agar (*3*); (iii) uncolored or faintly colored colonies after overlay with TTC (*4*); and (iv) no alkali production in an acetate medium (*5*).
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"Lethal" Variant of Candida albicans, a "Petite Colonie" Mutant

Two spontaneous and irreversible hereditary variants may be easily distinguished in the pathogenic yeast *Candida albicans* (*1*). The colonies of the normal (wild) form, strain 582, are smooth and are built up by typical yeast cells ("Y" form). One variant, strain 806, gives wrinkled, hard, rough colonies, and, microscopically, it is filamentous or mycelial ("M" form or variant). The second variant was called "lethal," and it is characterized by smaller sized colonies that perish rather rapidly.

Nickerson carried out studies with strains 582 ("Y" form) and 806 ("M" variant), and, in a recent paper, Nickerson and Falcone (*2*) state that the "M" variant is deficient in a protein-disulfide reductase that is apparently essential for cell division but not for growth.

We have studied the biochemical behavior of the "lethal" variant (strain 807), and our results show that the respiration of the mutant is nearly abolished. In manometric studies in a Warburg apparatus (yeast, 4 mg; buffer, 0.05M KH_2PO_4; substrate, 100μM glucose; temperature, 30°C; atmosphere, air or N_2) the normal form gave: $Q_{CO_2}{}^{N_2} = 13.2$ (μl/mg of yeast per 10 minutes); $Q_{CO_2}{}^{air} = 8.9$; $Q_{O_2}{}^{air} = 6.8$; respiratory quotient = 1.3; and Pasteur effect = 33 percent (inhibition). On the other hand, the following results were obtained with the "lethal" variant: $Q_{CO_2}{}^{N_2} = 12.4$; $Q_{CO_2}{}^{air} = 12.5$; $Q_{O_2}{}^{air} = 0.24$; respiratory quotient = 53; and Pasteur effect = 1.6 percent (inhibition).

Cytochrome-*a* and -*b* bands were absent from the absorption spectrum of the "lethal" mutant. These bands were present in the spectrum of the normal form. The α and β bands of cytochrome *c* were