THE PROCESS OF SPONTANEOUS EXTRANUCLEAR MUTATION IN YEAST

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Received June 17, 1957

CONVINCING evidence has been provided to show that respiratory mutants of yeast can arise as a result of extranuclear, as well as nuclear change (EPHRUSSI, HOTTINGUER and TAVLITZKI 1949). The extranuclear mutants are known as "vegetative petites." At least two classes of them exist (EPHRUSSI *et al.* 1955). They can be induced by means of specific mutagenic agents, such as the acridine dyes (EPHRUSSI and HOTTINGUER 1950) and radiations (RAUT 1954). When such agents are used, the mutation appears to involve the destruction or inactivation of heritable cytoplasmic particles. Extranuclear variants can also arise spontaneously, and it has been shown that the rates at which these are produced can be affected by the genic constitution of the cells (EPHRUSSI and HOTTINGUER 1951) and by temperature (YCAS 1956). However, the spontaneous process has received less attention than has the induced, and so far, investigations have permitted the underlying mechanism to be explained in general terms only.

This report is concerned with the process by which vegetative petites are produced in an unstable strain of yeast. The interpretation of strain behavior is based on the premise that mutation results from the depletion of essential cytoplasmic factors, and the data are applied to an elucidation of the mechanism by which this depletion occurs. It would, however, be difficult to interpret the data in a way that did not involve such particles, and the results of the investigation can be regarded as providing yet further evidence of their existence. Alternative to depletion, interpretations of the mutation process in terms of functional inactivation are possible. However, as the present inquiry is pursued, it will be seen that these become increasingly untenable.

The loss of cytoplasmic particles from a cell could be a single and sudden event resulting from the "chance" breakdown of some mechanism controlling the orderly distribution of particles by the mother to the daughter cell. If so, the probability of petite production would be the same for all cells of the strain. Alternatively a mechanism for determining the precise distribution of particles may not exist. In this case the final loss may be simply a result of imprecise assortment. If so, vegetative petites would more commonly occur where a partial reduction in particle number had already taken place.

To discriminate between these two possibilities, sudden loss and loss preceded by decline in numbers, a technique was devised by means of which variations in

stability of individual cells could be detected. Evidence is presented that a mechanism does not exist for the equal distribution of cytoplasmic particles at cell division. Evidence is provided also that the multiplication rate of the particles may, under some conditions, be at least partially independent of the multiplication rate of the cells.

MATERIAL AND METHODS

The unstable strain, number 4572, used in this investigation, was derived from an inbred of *Saccharomyces cerevisiae*. Its instability did not become apparent until inbreeding had progressed through 14 generations.

Experimental methods were routine with one exception—rates of change in the diameters of microcolonies were used to estimate the relative degree of stability of isolated cells and also to compare rates of cell division. In applying this technique, isolated cells were transferred by micromanipulator to individual agar droplets (approximately 0.1 ml in volume) formed on microcoverslips. These were then inverted over Van Tieghem cells each containing a few drops of water. The cells were sealed with Vaseline and incubated at 33.5° C. The resulting clones assumed a circular outline when only a few hundred cells had been produced. At this stage periodic measuring of the colony diameters could be commenced. This was done in two directions, using a micrometer scale in conjunction with a ×15 microscope eyepiece and ×10 objective. Readings were taken to the nearest 0.005 mm and averaged. Using a fortified dextrose medium, cell counts of microcolonies, by Neubauer chamber, indicated that, for colonies up to 1.0 mm in diameter, (5×10^5 cells) the logarithm of the diameter is directly proportional to the logarithm of the number of cells. It was further found that growth remains in the logarithmic phase throughout 18 generations. A plot of the logarithm of diameter against time thus yields a linear curve for stable strains. Since the present report is concerned with relative rather than actual growth rates, conversion from diameter of clone to cell number has not been carried out when describing results. The efficacy of the technique is evident from the graphs. However, it must be emphasized that this success was largely due to the use of a strain of yeast which produced microcolonies of extraordinarily regular outline.

Throughout the investigation the same batches of two types of media, one fortified, the other minimal,* were used for growth measurements. They were kept refrigerated in 10 ml aliquots. No effect of ageing could be detected over a period of several months.

* The fortified medium had the following composition: Bacto-peptone, 5.0 gm; yeast extract, 2.5 gm; $(NH_4)_2\ SO_4$, 6.0 gm; Mg $SO_4 . 7H_2O$, 1.0 gm; KH_2PO_4, 2.0 gm; $CaCl_2 . 2H_2O$, 1.5 gm; carbohydrate, 20.0 gm; Bacto-agar, 40.0 gm; distilled water, 1 L.

The minimal medium had the following composition: asparagine, 0.1 gm; sodium succinate, 1.5 gm; NH_4Cl, 1.0 gm; KH_2PO_4, 2.0 gm; Mg $SO_4 . 7H_2O$, 0.25 gm; $ZnSO_4$, 1.0 mg; $FeCl_3$, 5.0 mg; $CuSO_4 . 5H_2O$, 1.0 mg; $CaCl_2 . 2H_2O$, 1.5 gm, β-alanine, 0.5 mg; thiamine HCl, 20.0 μg; pyridoxine HCl, 20.0 μg; biotin, 1.0 μg; uracil, 20.0 mg; carbohydrate, 20.0 gm; Bacto-agar, 40.0 gm; distilled water, 1 L.

All crosses were made between lines of the same strain, and markers were consequently not available for purposes of verification. This difficulty was partly overcome by replication of crosses and by the use of a mating technique in which the zygotes, resulting from the individual mating of paired haploids, were isolated.

To determine the spectroscopic characteristics of cultures the method described by RAUT (1953) was adopted, using a Zeiss direct vision hand spectroscope.

RESULTS AND DISCUSSION

Instability and the production of lethal cells

Three types of cell were encountered in this study: "lethal," "petite" and "normal." Lethal cells were incapable of growth on the fortified medium. Petite cells formed slow-growing colonies that were respiratory-deficient. Normal cells produced colonies of widely varying sizes.

Serial transfer from unselected groups of colonies: The instability of strain 4572 was first suspected from the morphology of its older colonies on fortified dextrose medium. The outlines of these were scalloped, an indication that cells of reduced growth rate were appearing with considerable frequency within them. Instability of the strain was verified when populations were maintained by repeatedly transferring the diluted washings from 24 hour plates; the plates of later transfers were heavily interspersed with petite colonies.

Two other unusual features of strain behavior were revealed by this consecutive transfer method:

(1) There was a discrepancy between expected and actual colony counts (number of cells in the inoculum compared with number of colonies visible to the naked eye). It was this feature which first suggested that lethal variants were being produced in addition to petites. The discrepancy was greatest in the third transfer when, in the course of four experiments, on an average, only 53 percent of cells produced visible colonies. In subsequent transfers the discrepancy was reduced or absent. Later, it became evident that this shift was due to the accumulation of stable petite cells to the point where they outnumbered the normal.

(2) Among the earlier transfers, an increase in variability of "normal" colonies was accompanied by an obvious decrease in their average diameter. From this it was apparent that clones derived from normal cells were themselves becoming heterogeneous in character. The development of variability was not unexpected, since it was considered likely that the inbred line was unadapted to the medium. However, a decrease in average diameter and an increase in the frequency of lethal variants are findings contrary to those expected as a result of selection pressures. An explanation both for the presence of nonreproducing cells and for the reduction in colony diameter might be found in a hypothesis that growth of normal cells is inhibited by respiratory-deficient cells. However, it seemed more likely that lethal variants were, in fact, being produced and that the instability of the strain was increasing with each transfer.

Serial single-cell isolations from normal colonies: The simple plating technique described above was inadequate for obtaining reliable estimates of the proportion of either respiratory-deficient or nonreproducing cells within a population. Serial transfer could not be continued, to any useful purpose, beyond the third, when variability developed among petite colonies. Furthermore, it was apparent that uncontrolled environmental factors were contributing to the vagaries of strain behavior.

The single-cell technique was adopted because it permitted a detailed investigation of nonreproducing cells. Further, it was considered that shifts in stability could be more readily investigated by a comparison of the behavior of individual clones from a population than by a study of population behavior as a whole. Finally, the technique made a rigid control of environmental conditions possible.

An experiment was first performed to determine if inherent differences in rates of reproduction of isolated cells could be detected as differences in rate of change in diameter of microcolonies. Not only was the technique successful in detecting such differences, but the results of the experiment, described below, provided basic information on the nature of the instability of the strain.

Twenty-four isolations were made from a stock slant that was started from a single colony four days previously. The growth of these isolates (a single measurement was made when growth was very rapid) is recorded in Figure 1. Clonal heterogeneity is obvious. Although the majority of colonies had a diameter of 1 mm at 24 hours, three were unmistakably retarded.

Figure 1 also illustrates the mode of production of lethal cells. One of the retarded colonies stopped growing after two days and its diameter had not exceeded 0.06 mm when, at four days, its cells began to disintegrate.

Two of the large colonies and one of the retarded colonies referred to above were sampled after 24 to 25 hours of growth. The growth patterns of clones derived from the large colonies (numbers 2 and 18) are shown in Figure 2. Here, eight of 14 clones are unmistakably retarded. The behavior of the cells isolated from the retarded colony, number 19, was in sharp contrast; these cells produced only lethals. A total of 26 cells was produced, the number per isolate ranging from zero to seven.

In a third series of transfers, an attempt was made to maintain the normal population by sampling from the large colonies numbers 16 and 26 of Figure 2 after 25 to 26 hours of growth. This attempt met with complete failure, as all isolates produced abortive clones. From eight isolates of number 26, the faster growing of the two colonies, only 815 cells were produced after five days incubation; the number of cells produced by each isolate ranged from 2 to 217. Eight isolates from number 16 produced a total of only 56 cells, 2 to 20 cells arising from each isolate.

Also, petite colonies resulted from the growth of some of the single cell isolates. Colony number 4, one of those that showed renewed growth after considerable delay (Figure 2), was found to have become a stable variant. On transfer, cells

from it grew at less than half the rate of the fastest of normal clones. Spectroscopic analysis showed this, and all other colonies of similar origin examined later, to be respiratory-deficient; the bands for cytochromes **a** and **b** were invariably absent from their spectra. The distinctive growth curves of respiratory-deficient colonies were used for purposes of identification in later studies. Although variants of increased growth rate were encountered occasionally among petites, the growth of these did not differ sufficiently to cause confusion.

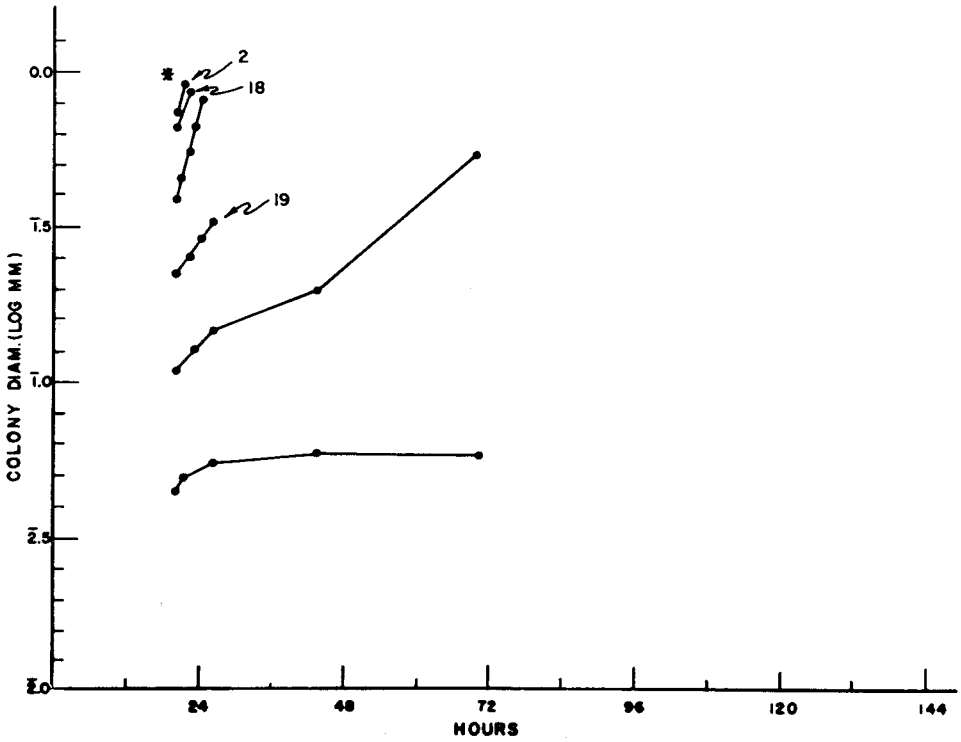


FIGURE 1.—Growth curves of single cell isolates of strain 4572, derived from rate of change in diameter of micro-colonies.

* In 18 instances colony diameters fell between 0.7 and 1.0 mm at twenty hours. These colonies were not measured subsequently.

It is evident that the cells of strain 4572 do in fact produce "normal" clones of strikingly different growth rates. It is also evident that the strain produces lethal variants as well as petites. Certainly, the production of abortive colonies cannot be attributed to the inhibition of normal cells by respiratory-deficient variants, since many of the abortive colonies were completely free of such variants. Indeed, had it not been for the production of petites, the whole of the small sampled population would have died.

"Petites" as mutants of nonreproducing cells

The origins of petite and lethal cells were determined from the results of appropriate crosses. The petites will be considered first. Of the two variant types, these can be more readily analysed by direct genetic means, but even here the scope of genetic tests is somewhat restricted because of the well known fact that such variants have not been induced to sporulate. The difficulty can, however, largely be overcome, as EPHRUSSI *et al.* (1949) have shown, by using variants which have arisen during the haploid phase. This method was applied in the present investigation after extended tests had shown that haploid segregants of strain 4572 were apparently identical with the diploid strain as regards stability.

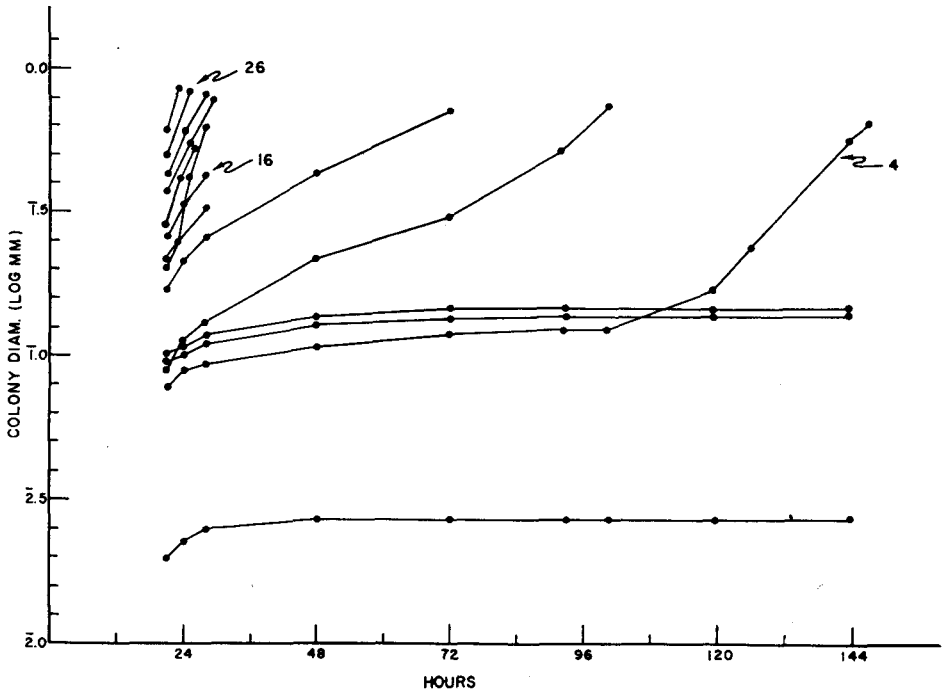


FIGURE 2.—Growth curves of isolates from colonies No. 2 and No. 18 of Figure 1.

The crossing tests of the petites indicated that the respiratory deficiency is not associated with mutation of a chromosomal gene. In these tests, five independent crosses, normal to petite were made, each petite being of independent origin. All five of the resulting diploid zygotes produced normal clones, as was ascertained from their growth rates. Two asci from each of these five diploids were dissected. The four segregants from each ascus produced normal clones and extranuclear inheritance was inferred from this outcome. It should be noted that no backcrosses were made and that therefore multigenic inheritance cannot be rigorously excluded. However, it was considered that the results of the tests were sufficiently

indicative of extranuclear inheritance to identify the variants with more thoroughly tested petites obtained as vegetative mutants by other investigators.

As regards abortive cells, the characteristic way in which they appear in the population suggested that they also were respiratory-deficient. Certainly, the frequency with which abortive colonies were produced suggested that an extranuclear change was responsible for their occurrence. Furthermore, it seemed likely that abortive cells were the progenitors of petite colonies, since the latter appeared almost exclusively as sporadic outgrowths from abortive colonies. This latter observation suggested that there is a genic difference only between cells of abortive and petite colonies. And since the respiratory-deficiency of petites is of extranuclear origin, it would appear that abortive cells are themselves respiratory-deficient.

Unfortunately, neither the assumption that abortive cells are respiratory-deficient nor the assumption that a gene difference exists between the two variants, is open to direct test. Little reliance can be placed on the results of spectroscopic analysis of abortive cells since a positive reading could be attributed to the presence of normal cells and a negative reading to an irrelevant physiological condition of the moribund cells. The hypothesis of a gene difference between the two variant types cannot be tested directly by a cross since abortive cells can only be recognized as such by their dying. Furthermore, neither the apparent absence of "petite" cells from normal colonies nor their obvious presence in abortive colonies provides really good evidence that they do not originate from normal cells. Their absence in samples of normal colonies could be due rather to scarcity resulting from selection pressures than to the fact that they are not produced in such colonies. In abortive colonies, on the other hand, they might well originate among the few remaining normal cells over which they would have a competitive advantage.

It was reasoned, however, that information relevant to the genetic constitution of abortive cells might be obtained from the segregants of the cross petite × normal. If the cells of petite colonies do contain a mutant gene which distinguishes them from abortive cells, then the normal diploids produced by the above-mentioned cross must be heterozygous for that gene. On segregation, such diploids should yield two types of haploid. One type, mutant free, would be a typical unstable line. The exact behavior of the other type is unpredictable, but certainly it should not produce abortive colonies.

The crossing procedure that was followed is diagrammed in Figure 3. The distinguishing characteristics of four segregants of an ascus of strain 4572 were determined. Each was found to be respiratory-sufficient (normal). On transfer each produced abortive clones and, subsequently, petite colonies. "Brother-sister" matings between respiratory-sufficient cells and cells of petite colonies were then made. The resulting diploids were supposedly heterozygous for the above mentioned mutant gene. These were sporulated. All four segregants from each ascus were found to be respiratory-sufficient. Each segregant clone was then tested

further by transferring isolated cells. The results obtained with four asci of three diploids (each of which involved a petite of independent origin) are presented in Table 1. Two segregants from each ascus were typical unstable strains; they produced abortive clones some of which subsequently produced petite colonies. The other two segregants produced no abortive clones; growth patterns were those of normal or, in a few cases, petite colonies. There was no indication from their growth curves that the petites had originated as abortive clones.

TABLE 1

Segregants from crosses of normal to petites

Cross		4691				4693								4665			
Segregants		a	b	c	d	1a	1b	1c	1d	2a	2b	2c	2d	a	b	c	d
Clone diameter (mm) at 23 hrs.		.30	.25	.43	.42	.24	.23	.46	.34	.29	.30	.53	.53	.12	.14	.29	.27
Frequency of colony types* produced by segregants on transfer	Normal	10	10	10	7	7	7	1	1	10	10	10	7	20	29	2	2
	Abortive	0	0	3	4	0	0	11	10	0	0	3	4	0	0	11	13
	Petite	0	2	0	0	4	3	0	0	0	2	0	0	10	3	0	0

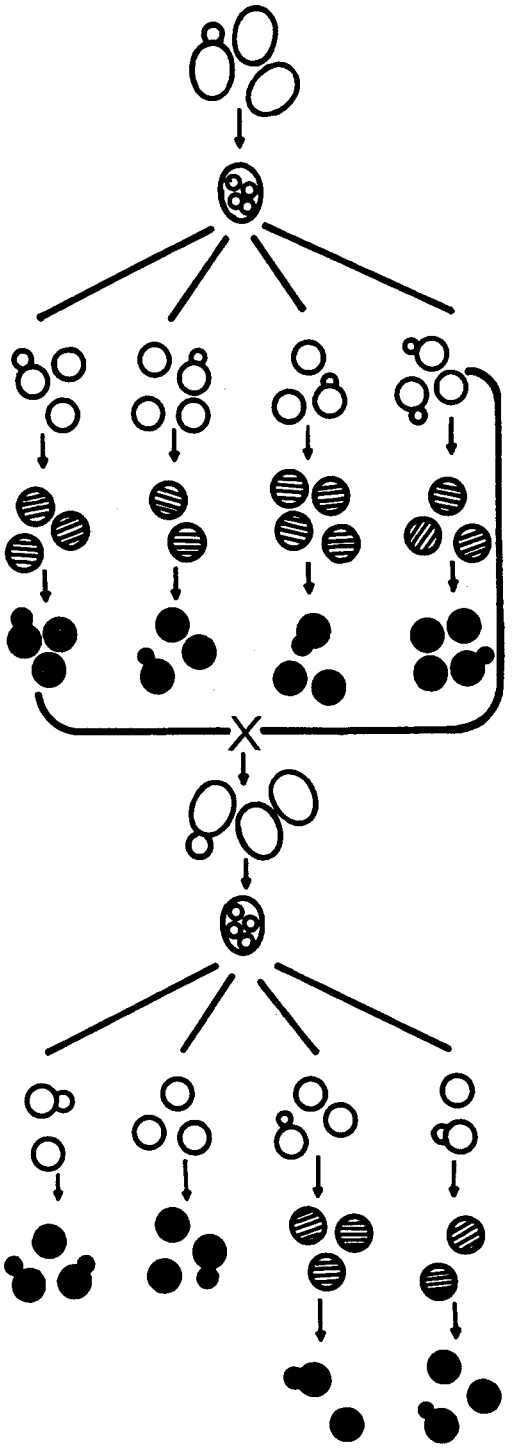
* Normal—diameters reach 1.0 mm within 36 hours.
Abortive—growth curves show obvious declines.
Petite—growth curves are depressed but linear.

The data provide good evidence that (1) the instability of strain 4572 results in the production of a single variant type which is respiratory-deficient, and that (2) respiratory-deficiency is lethal in the absence of a mutant gene. They do not imply that the gene mutation under consideration does not, on occasion, precede the occurrence of respiratory deficiency. The rarity of such mutants among normal cells can be interpreted in terms of selection pressures.

The mutant segregants possessed another distinguishing property in being more stable than normal segregants. This was suggested by the data from all four asci, but was confirmed for the segregants of number 4665 when an attempt was made to maintain the haploids by serial transfers of single cell isolates. For the two normal segregants, this attempt met with complete failure; they could not be maintained beyond the second transfer. The behavior of the two variant segregants was in sharp contrast; only 2 of 18 isolates from one, and 6 of 16 from the other produced petite colonies at the third transfer. It is apparent that mutant genes which prevent death of respiratory-deficient cells can also confer increased stability upon respiratory-sufficient ones.

There is good evidence that mutations to stability are not identical in all cases. Thus, plating of the heterozygotes indicated that the mutant gene was dominant

FIGURE 3.—Relationship of abortive to "petite" cells as determined by genetic analysis. White, respiratory-sufficient cells; shaded, abortive cells; black, "petite" cells.



for stability in two of the strains (numbers 4665 and 4693), and recessive in the third.

Mutation rate as a function of prior partial loss of particles

The most striking feature of strain behavior as shown in Figures 1 and 2 is the decline in growth rate of individual clones. The rate of this decline is obviously not the same in all cases. On the contrary, colonies can produce an array of growth curves. At one end of the range are those colonies in which no decline is evident; at the other extreme are those in which the decline culminates in complete cessation of growth. Although these declines may be due, in part, to a general and uniform reduction in rate of cell division, they must result largely from the accumulation of abortive lines. This is evident from the fact that isolates sampled from a single colony again produce an array of curves. It is apparent that rate of decline depends on the rate at which abortive lines are accumulated, and that extreme differences can exist between clones in regard to these rates of accumulation.

A related feature is suggested by a comparison of the growth curves in the two figures. The diameters of 21 of the 24 colonies represented in Figure 1 exceeded 1 mm at 36 hours, whereas none of the 14 colonies in Figure 2 attained this diameter in an equivalent length of time.

Since colony size reflects the rate at which abortive lines are accumulated, the increasing reduction in their diameters (at 36 hours growth) with each successive transfer suggests that this rate of accumulation is increasing throughout the sampled population. The presence of this trend is confirmed by the collected data given in Figure 4. Here, the logarithms of the diameters of individual clones are

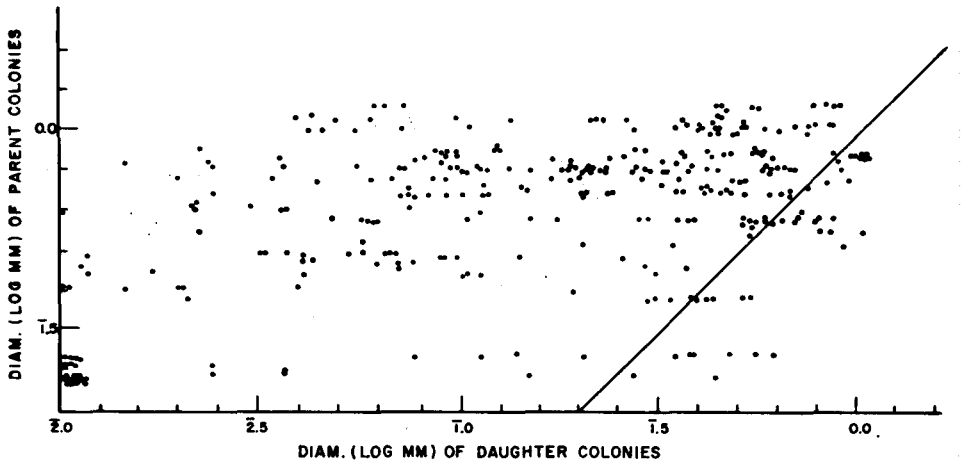


FIGURE 4.—Correlation of the growth of colonies with the growth of isolates from them. The data are colony diameters at 23 hours. Points to the left or right of the diagonal line represent instances respectively, where the diameters of parent colonies exceeded, or were exceeded by, those of their daughters.

plotted against the logarithms of the diameters of the colonies from which the cells were isolated. Of 328 isolates from 51 colonies, 302 (92 percent) produced colonies smaller than those from which they were taken. Clearly, there is a tendency for abortive cells to accumulate at an increasing rate in a sampled population. Paradoxically, a general and continuous increase in rate of accumulation of abortive cells cannot occur in the population taken as a whole, for serial plating experiments have consistently failed to produce a population free of respiratory-sufficient cells.

Two different hypotheses can explain the mechanism of variant production. One of these assumes that respiratory-deficiency is initiated by a single, though frequent, event. It further assumes that there is a lag of considerable duration between this event (mutation) and its phenotypic expression. On the basis of this hypothesis, a declining growth curve is merely an expression of this lag, and rate of decline a measure of the residual growth potential of an isolated cell. The second hypothesis assumes that the induction of respiratory deficiency occurs as a multiple process in each step of which the instability of the cell increases. Applying this hypothesis, rate of accumulation of abortive lines is a measure of how far the process has advanced.

It was considered that the validity of the two hypotheses could be tested if environmental conditions could be found in which the strain was stable. Under such conditions one might determine whether a cell which produced a colony of declining growth rate (under the usual growing conditions) was genetically normal. If the progeny of the "unstable" cell were stable in the changed environment it would then be evident that the cell was genetically normal rather than mutant. From this it would follow that the production of lethals is a multiple process.

Conditions conducive to stability were obtained by using a minimal medium. On plates of this medium, the outlines of respiratory-sufficient colonies did not become scalloped. The increase in stability was confirmed from observations on microcolonies; in the course of seven consecutive series of transfers, involving 225 cells isolated from 18 unselected colonies, only two respiratory-deficient clones were obtained. It is convenient to note here that these variants are not abortive on the minimal medium. They are, however, equivalent to lethals and not to viable petites, and their growth rate is distinctly reduced compared with that of respiratory-sufficient cells.

In the knowledge that strain 4572 is relatively stable on a minimal medium, an experiment was performed to determine whether an "unstable" isolate was genetically normal or mutant. Cells from colonies incubated for 24 hours on a fortified medium were transferred both to this and to minimal media. On the fortified medium the growth curves of 24 of 53 isolates showed obvious declines (eight clones were completely abortive). In contrast, on minimal medium only 7 of 53 isolates produced respiratory-deficient colonies (abortive on complete medium). The ratios differ significantly. It is evident that not all cells whose colonies pro-

duce declining growth curves are genetically respiratory-deficient. It follows that the induction of respiratory-deficiency is, in all likelihood ,a multiple process.

From the foregoing results it is clear that differences in rates of accumulation of abortive lines do, in fact, reflect differences in instability between isolated cells. In terms of cytoplasmic factors, the interpretation is obvious: degree of stability is dependent on the number of particles present in the cell.

It should be noted that both acriflavine-induced and gene-induced instabilities have also been attributed to a diminished number of cytoplasmic particles per cell (EPHRUSSI and HOTTINGUER 1951). In the case of strain 4572 on a fortified medium, it is evident that the degrees of instability of the cells of a single clone can cover a wide range. This heterogeneous condition of related cells strongly suggests that distribution of particles at cell division is inherently imprecise.

In addition, it is clear from the foregoing results that there is a general, if somewhat disorderly increase in instability throughout sampled lines. The original thesis, derived from serial platings, that shifts in stability occur within populations is thus confirmed. It is evident that there is a general tendency for the number of particles per cell to decrease.

The phenomenon of a progressive decrease in particle number within a line admits of a simple explanation: the rate of division of cells (and chromosomes) is higher than that of the cytoplasmic factors; as a result the average number of particles per cell is gradually lowered. It is noteworthy that selection for a fast rate of growth was maintained throughout the inbreeding program involving strain 4572. Such an explanation would find support in evidence that the restoration of comparative stability accompanying genic and environmental changes is correlated with a reduced rate of cell division.

Estimates of the rate of cell division necessary for the compilation of such evidence, can be derived from the rate of increase in colony diameter. In the case of the unstable strain rate of cell division may be underestimated, because even the fastest growing colonies may be producing abortive cells. Nevertheless, a reduced rate of cell division was found to be correlated with increased stability of the strain in each of three independent instances:

(1) The fact that strain 4572 grows at a reduced rate on stabilizing minimal medium is obvious from comparison of plated colonies; on the fortified medium, many colonies are visible at 24 hours, whereas on a minimal medium none of the colonies is visible before 48 hours. Upon the addition of either Bacto-peptone or yeast extract to the minimal medium, colonies appear sooner and again show instability, as judged by their scalloped outlines.

(2) In the second instance, advantage was taken of the mutant genes conferring stability on haploids. Here, a reduced rate of cell division is not as obvious as in the foregoing, but is nevertheless demonstrable when the segregants of heterozygotes are compared with each other. The diameters of the clones produced by spore isolates are given in Table 1. Each of the two mutant spores from each ascus produced fewer cells at 23 hours than did either of the non-

mutant spores from the same ascus. The probability that such a distribution of diameters occurred by chance is less than one in a thousand.

(3) In the third instance, stability of the strain was investigated under conditions that were known to reduce rate of cell division. Strain 4572 is "galactose negative," being homozygous recessive at a locus g_4 (so termed in this laboratory). Plating experiments had already suggested that galactose negative strains grow more slowly on a galactose than on a dextrose medium. To confirm this, cells isolated from colonies grown on fortified galactose medium were taken alternately to fortified galactose and fortified dextrose media. The distributions of diameters at 21 hours are shown in Table 2. Twenty-six of 52 isolates on the

TABLE 2

Effect of carbohydrate on growth rate of strain 4572

	Distribution of colony diameters (mm) at 21 hours									
	.1	.2	.3	.4	.5	.6	.7	.8	.9	Total
Galactose	2	12	17	13	8	0	0	0	0	52
Dextrose	3	4	5	5	9	5	11	7	3	52

dextrose medium produced larger colonies than any of 52 isolates on the galactose medium. It is apparent from this that the rate of cell divisions is lower on a galactose medium than on a dextrose medium.

Increased stability of strain 4572 on galactose medium was indicated by the smooth outlines of its colonies and was confirmed by serial transfer of isolated cells from 24 hour colonies. Here, eight consecutive series of transfers were made. Of 212 isolations from 25 unselected colonies only 30 produced petite colonies. It should be noted that on galactose medium, as on minimal, respiratory-deficient cells are able to reproduce. Their reproduction is completely arrested, however, when the colonies have attained a diameter of about 0.6 mm.

The evidence that each of these stabilizing influences reduces the rate of cell division does not, of course, eliminate the likelihood that multiplication of cytoplasmic factors is under genic or environmental control as has been postulated by EPHRUSSI (1952) to account for gene-induced cytoplasmic instability with respect to petite formation. As regards strain 4572, it is possible particles are in competition with the remainder of the cell for substrate materials. If so, a reduced rate of cell division may well be accompanied by an increased rate of division of the particles. Nevertheless, cells and their cytoplasmic factors show a surprising independence in regard to multiplication. It seems that there is no mechanism compelling a synchronization of the two rates. As a result, strain 4572 is placed in an "unfortunate" position; a mutation to a more rapid rate of cell division will quickly replace a stable population, after which the strain is subject to the hazards accompanying a progressive loss of its cytoplasmic particles.

Since the evidence in hand is consistent with the interpretation that the cells of strain 4572 outstrip their cytoplasmic factors in multiplication, it is pertinent

to question how the strain manages to maintain itself on a fortified dextrose medium. A satisfactory answer is supplied in the data shown in Figure 4. It can be seen that eight percent of isolated cells produced larger colonies than were those from which they were taken, a fact which suggests that the progressive decline in stability can, on occasion, be reversed. By sampling such clones it was found that the increase in stability is transitory. Nevertheless it seems likely that the sporadic appearance of these exceptional colonies can account for the survival of the strain. Their occurrence can be attributed to the unequal assortment of particles at cell division. Survival of the strain would also, of course, be dependent on the maintenance of population size at or above a minimum.

It should be pointed out that since instability results from the addition of peptone or yeast extract to a minimal medium one may consider the mutants of strain 4572 to be nutrient induced rather than of spontaneous origin. However, from the evidence of unequal assortment of particles at cell division in this strain, it may be inferred that imprecision in the distribution of particles is a general characteristic of yeast behavior. It would therefore seem that the spontaneous appearance of a vegetative mutant in a "stable" line results from a sequence of chance events and not from the breakdown of a system concerned with orderly assortment of particles.

SUMMARY

The mode of production of spontaneously occurring respiratory-deficient mutants, extranuclear in origin, was investigated in an unstable strain of yeast.

The relative degrees of stability of individual cells were determined by the timed measuring, by microscope, of microcolony diameters.

The mutation to respiratory deficiency was found to be lethal, but death can be averted by a gene mutation. Cell populations were found to be heterogeneous as regards stability, and mutation was shown to be the final step in a predisposing sequence of events.

The effects on stability of genic and nutritional changes were investigated, and it was shown that stability is correlated with the rate of cell division.

It was concluded that (1) a mechanism for determining the precise distribution of essential cytoplasmic particles at cell division does not exist, and that (2) the multiplication rate of the particles is not wholly dependent on the multiplication rate of the cells (and chromosomes).

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