SUPPRESSIVENESS: A NEW FACTOR IN THE GENETIC DETERMINISM OF THE SYNTHESIS OF RESPIRATORY ENZYMES IN YEAST

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In a series of papers from this laboratory[1] published since 1949, it was shown that clones of baker's yeast (*Saccharomyces cerevisiae*), whether diploid or haploid, constantly give rise during their growth to mutants ("vegetative 'petites' ") which are stable in vegetative reproduction and are characterized by a reduced colony size on media in which sugar is the limiting factor. The reduced colony size was shown to be due to a respiratory deficiency of the mutant cells,[2] due in turn to the lack of several enzymes (including cytochrome oxidase) bound, in normal yeast, to particles sedimentable by centrifugation.[3] It was suggested that, from the genetical point of view, the mutation resulting in the production of vegetative mutants consists in the loss or irreversible functional inactivation of a particulate cytoplasmic autoreproducing factor, required for the synthesis of respiratory enzymes.[4] This interpretation was based on the results of crosses which will be recalled below. It will suffice to say at this point that a single strain of vegetative mutants was utilized in the earlier detailed genetic study;[5] thus, until more recently, no systematic attempts had been made to compare the genetic behavior of a number of such mutants of independent origin. It is such a study, undertaken three years ago, that has now revealed that there exist at least two types of vegetative respiratory mutants which are, so far as we know, identical biochemically but which can be distinguished by their behavior in crosses with normal yeast. The purpose of this paper is to present a brief summary of the evidence for the existence of these two types of vegetative mutants, which, for reasons to appear below, will be referred to, respectively, as "neutral 'petites' " and "suppressive 'petites' " (the one previously studied[5] belonging to the former class).[6]

EXPERIMENTAL PROCEDURE AND SUMMARY OF RESULTS

A model experiment revealing the occurrence of two genetically different classes of vegetative "petites" and showing their differential characteristics is schematically represented in Figure 1, which will be referred to in the ensuing description.

A haploid strain, normal with respect to respiration (*N*) and carrying the recessive mutant genes *tr* and *his*, causing, respectively, tryptophan and histidine requirements, is plated out on complete medium (C.M.) in a Petri dish. Among the colonies produced, two small colonies are selected and subcultured; tests are performed on the resulting clones to ascertain that they represent stable respiration-deficient mutants. It will now be assumed that the two selected mutants happen to belong to the two extreme classes of vegetative mutants, i.e., that one of them is a neutral "petite" (*n*) and the other highly suppressive (*s*). We shall now examine the results of the crosses of these two mutants with a single strain of normal yeast of opposite mating type (the mating types of the strains involved are indi-

cated by the symbols a and α) carrying the recessive genes *met* and *ur* (methionine and uracil requirements).

The crosses are performed by the mass-mating technique,[7] i.e., by mixing a few drops of two cultures in a small volume of fresh complete medium contained in a test tube.

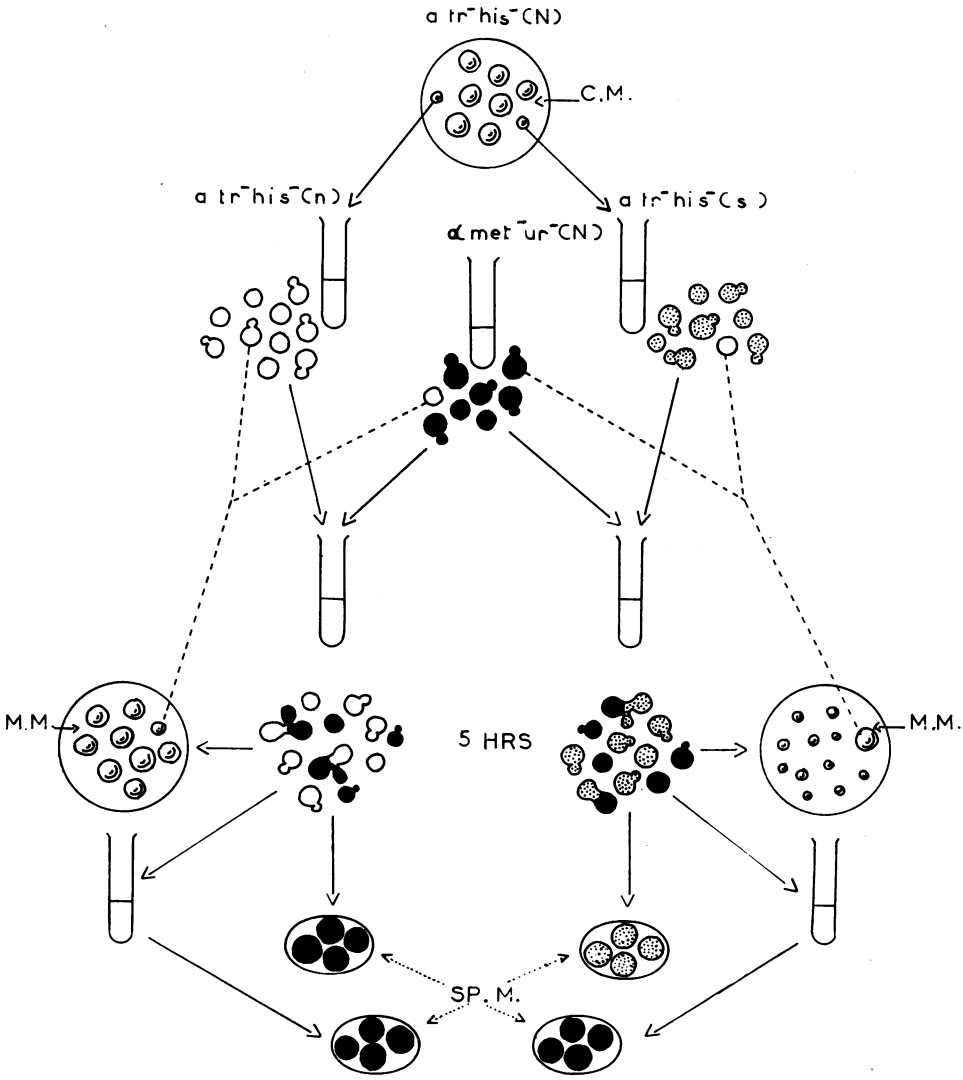

FIG. 1.—Design of a model experiment for the demonstration of the behavior of neutral and suppressive "petites" in crosses with a normal yeast. (*Black:* normal yeast cells; *strippled:* suppressive "petites"; *white:* neutral "petites.") Further explanations in text.

Five hours after the crosses are made, the mass-mating mixtures consist of haploid (parental) cells which have not yet mated and of zygotes, some of which are in process of producing a first diploid bud. However, no free diploid cells (detached from the zygotes which produce them) are to be found as yet.

The following three operations are performed at this time:

A. A portion of each mating mixture is diluted and plated in Petri dishes on minimal medium (M.M.), where only prototrophic cells are able to proliferate. Therefore, each of the colonies formed on these plates represents the vegetative progeny (clone) produced by a zygote. The size of the colony indicates the normal or mutant (with respect to respiration) character of the clone, which is verified by spectroscopic examination.[8]

B. A large portion of each mating mixture is placed on sporulation medium (Sp.M.).

C. One or two drops of each mating mixture are inoculated into tubes of complete medium and incubated overnight, during which period additional zygotes and diploid cells are produced; the latter undergo a maximum of six generations. Thereafter, the cells are transferred to sporulation medium.

The asci produced in the few days following operations B and C are dissected, and ascospores are isolated in the microdissection chamber in droplets of complete medium, where they are allowed to give rise to haploid clones. These clones are plated out on complete medium. As in operation A, the size of the colonies formed on the plates is taken as an indication of the normal or mutant (respiratory) character of the clone, and this is verified by spectroscopic examination.[8] The presence of the different marker genes is tested by platings on appropriately supplemented synthetic media.

The results of the three operations described above are given in Figure 1 and may be summarized as follows:

A. The zygotes of the cross involving a neutral "petite" (cross $N \times n$, left side of Fig. 1), plated on minimal medium, give rise to a great majority of colonies of normal size. The percentage of small colonies is equal to the percentage of "petites" in parent-strain N, established by a control plating of the latter on complete medium (not shown in Fig. 1).

Under the same conditions, the zygotes of the cross involving a highly suppressive "petite" (cross $N \times s$, right side of Fig. 1) give the reverse picture: the great majority of colonies produced are of small size.

Spectroscopic examination of cultures derived from the large and from the small colonies of the two crosses confirms that the latter are composed of mutant (respiration-deficient) cells, the former of normal cells.

B. When the mating mixtures are placed on sporulation medium immediately after zygote formation, the types of asci produced by the two crosses are different in the following ways: (1) All, or almost all, asci of the cross involving a neutral "petite" ($N \times n$) contain four spores which give rise to clones that are normal with respect to respiration (asci 4:0). (2) All, or almost all, asci of the cross involving a highly suppressive "petite" contain, on the contrary, four spores which give rise to respiration-deficient clones (asci 0:4). Further crosses of segregants from these asci, thus far performed on a limited scale, show that all of them belong to the class of suppressive "petites."

In the asci of both crosses, the marker genes undergo 2:2 segregation.

C. If, prior to being placed on sporulation medium, the mating mixtures are subjected to a transfer, the asci of both crosses are of the 4:0 type, i.e., they contain

only spores which give rise to normal clones. Here again, the segregation of the marker genes is 2:2 in every ascus.

INTERPRETATION

I. *Cross $N \times n$.*—The fact that all four spores of the great majority (or all) of the asci of this cross give rise to normal clones indicates that the two parental strains are not differentiated by a single Mendelian gene. This observation corroborates the results of earlier work,[5] in which the cross $N \times n$ (involving a different "petite" strain) was followed by a series of four backcrosses to the mutant parent. It may be recalled that the results of these backcrosses were similar to those of the F_1, i.e., the backcross asci contained only spores giving rise to normal clones.[9] It was concluded that the respiratory deficiency is most probably not transmitted in a Mendelian fashion, and, instead, the following interpretation was suggested, which will be adopted here in view of the similarity of the present results with those previously published.

It is assumed that the normal yeast cell contains in its cytoplasm a particulate autoreproducing factor, to be referred to below as the "normal cytoplasmic factor," required for the synthesis of respiratory enzymes, and that the mutation $N \rightarrow n$ is the result of the loss of this factor or of its mutation to a functionally inactive form. When the two cell types (N and n) are crossed, zygote formation is accompanied by the mixture of the cytoplasms of the fusing cells. As a result, practically all diploid cells produced by the zygotes contain the normal cytoplasmic factor. Hence, plated on minimal medium (operation A), the zygotes give rise to colonies of normal size. The production on the plates of a few small colonies is ascribed to the presence in the mating mixture of some mutant zygotes resulting from copulations between cells of the mutant parent with the few similar cells ("petites") always present in populations of normal yeast. Finally, when the "hybrid" diploid cells sporulate, some particles of the normal factor are included in each of the spores. Each of these spores, therefore, gives rise to a normal clone.

If this interpretation is accepted, it appears that, from the point of view of the character considered (respiratory deficiency), the behavior of a neutral "petite" in a cross with normal yeast is that of a purely neutral element which contributes nothing to the zygote. This point is emphasized here because, as will be seen in the next paragraph, it is in this respect that neutral and suppressive "petites" are strikingly different.

II. *Cross $N \times s$.*—In interpreting the results of this cross, we shall begin by considering its first characteristic feature: the high proportion of small colonies produced on plating the mating mixture on minimal medium (operation A), i.e., the high proportion of zygotes giving rise to clones of mutant diploid cells. The simplest hypothesis which would account for this fact consists in ascribing these zygotes to preferential mating between cells of the mutant strain (s) and the few "petites" always present in the normal strain (N). However, experiments especially designed to this effect (pair matings in the microdissection chamber) definitely disprove this assumption. Therefore, the predominant occurrence of zygotes giving rise to mutant diploid clones must be regarded as the result of an *active process of suppression* by the mutant parent (s) of the normal cytoplasmic factor con-

tributed to the zygote by the normal parent (N). In other words, in contrast to neutral "petites," the suppressive "petites" may not be regarded as purely neutral elements. They contribute to the zygotes something which suppresses the perpetuation of the normal cytoplasmic factor contributed by the normal parent, by destroying it or by interfering with its multiplication or its distribution into the diploid buds. This something will be referred to as the "suppressive factor."

The production by the same cross of a few zygotes which give rise to normal diploid clones may be ascribed to the occurrence in the suppressive strain (s) of some neutral "petites" (n) which owe their origin to mutations $s \rightarrow n$ and which, in the cross with N, behave like the neutral "petites" discussed above. The occurrence of mutations $s \rightarrow n$ (which may consist in the loss or mutation of the suppressive factor) is confirmed by the isolation from suppressive clones of purely neutral subclones.

Turning now to the interpretation of the results of the analysis of the asci of the cross $N \times s$, it may be recalled that the facts to be accounted for are (a) the occurrence, incompatible with a Mendelian segregation in a diploid, of asci of both 4:0 and 0:4 types and (b) the dependence of the type of asci observed on the time at which sporulation is induced. The following interpretation is based on the assumptions that (a) the two types of asci are derived, respectively, from the two types of zygotes described above and (b) the peculiar time relationship is due to a delay in the effect of the suppressive factor, assumed to be cytoplasmic and autoreproducing.

The cross $N \times s$ produces a majority of zygotes containing the suppressive factor. If this factor immediately converted all these zygotes into "petites," their diploid vegetative progeny could not sporulate (because "petites" are unable to sporulate). It is therefore assumed that the effect of the suppressive factor is not immediate and that the diploid cells produced by this class of zygotes, placed on the sporulation medium immediately after their formation (operation B), are still able to sporulate, because they still contain the normal cytoplasmic factor or, at least, the preformed respiratory enzymes. However, all the ascospores thus produced carry the suppressive factor and therefore give rise to mutant clones. Under the same conditions, asci of the 4:0 type are rarely observed because of the rarity of the class of zygotes producing normal diploids.

When, on the other hand, prior to being placed on sporulation medium, the mating mixture is subjected to a transfer (operation C), the suppressive factor is granted the time to convert the diploid cells into "petites" unable to sporulate (the preformed enzymes must be "diluted" in the course of the cell multiplication which takes place during this period). Therefore, the only asci produced after the transfer are those derived from the zygotes which have received no suppressive factor, i.e., from the zygotes resulting from copulations of cells of the normal strain with the few neutral mutants contained in the suppressive strain. All the asci observed in this case are therefore of the 4:0 type.

The proposed hypothesis thus accounts, in a rather simple fashion, for the results of the experiments described thus far, including the peculiar time sequence in the appearance of the two types of asci. It is supported by the already mentioned isolation from suppressive strains of purely neutral subclones and by the evidence that all spores of the 0:4 asci give rise to suppressive clones. An additional problem is, however, posed by the existence of "petite" strains exhibiting various "degrees

of suppressiveness," i.e., of strains intermediate in their behavior between the two extreme types deliberately selected above for the description of the model experiment.

The occurrence of such strains can be demonstrated, and their degree of suppressiveness measured approximately, by the percentage of zygotes giving rise to mutant diploid clones following a cross with a normal strain (i.e., by the percentage of small colonies in operation A). If a number of "petite" strains are studied in this manner, it is found that they exhibit a variety of degrees of suppressiveness, ranging from 0 to nearly 100 per cent.[10]

The degrees of suppressiveness characteristic of different strains are relatively stable; they tend to be perpetuated as such in the course of vegetative reproduction. A decrease of suppressiveness can, however, occur spontaneously and can be experimentally induced. Thus a highly suppressive strain can eventually become neutral. Changes in the opposite direction either do not occur or are sufficiently rare to escape detection at the population level.

Concerning the significance of the different degrees of suppressiveness, two extreme hypotheses can be formulated:

1. There exist *at the cell level* only two types of "petites": a neutral and a completely suppressive type. Suppressive *strains* are mixed populations of the two cell types, coexisting in proportions which assume different values in different strains: the smaller the proportion of neutral "petites," the higher the degree of suppressiveness of the strain.

2. The cells of a suppressive strain (with the exception of the few neutral mutants present in the latter) all belong to a single type characterized by a certain *probability (i.e., efficiency) of suppression* which has different values in different strains. This implies that there may exist as many cell types as there are degrees of suppressiveness.

Experimental evidence bearing on these hypotheses and on the mode of action of the suppressive factor will be published shortly.

SUMMARY

The occurrence of two different classes of vegetative, respiration-deficient mutants of yeast is described. The behavior of the mutants of the two classes, referred to as "neutral 'petites'" and "suppressive 'petites,'" in crosses with normal yeast is illustrated by a model experiment.

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[1] B. Ephrussi, in *Unités biologiques douées de continuité génétique* (Paris: Edition du C.N.R.S., 1949); B. Ephrussi, H. Hottinguer, and A. M. Chimènes, *Ann. Inst. Pasteur*, **76**, 351–364, 1949; J. Tavlitzki, *Ann. Inst. Pasteur*, **76**, 497–509, 1949; S. Y. Chen, B. Ephrussi, and H. Hottinguer, *Heredity*, **4**, 337–351, 1950.

[2] P. Slonimski, *Ann. Inst. Pasteur*, **76**, 510–530, 1949.

[3] P. Slonimski and B. Ephrussi, *Ann. Inst. Pasteur*, **77**, 47–63, 1949; P. Slonimski, *Recherches sur la formation des enzymes respiratoires chez la levure* (Paris: Masson & Cie, 1952).

[4] B. Ephrussi, P. L'Héritier, and H. Hottinguer, *Ann. Inst. Pasteur*, **77**, 64–83, 1949; P. Slonimski and B. Ephrussi, *op. cit.;* B. Ephrussi and H. Hottinguer, in *Cold Spring Harbor Symposia Quant. Biol.*, **16**, 75–84, 1951.

[5] B. Ephrussi, H. Hottinguer, and J. Tavlitzki, *Ann. Inst. Pasteur*, **76**, 419–450, 1949.

[6] Preliminary reports on this work have been presented at the Eighth International Congress of Genetics (Bellagio, 1953) and at the International Congress of Botany (Paris, 1954).

[7] C. C. Lindegren and G. Lindegren, these PROCEEDINGS, 29, 306–308, 1943.

[8] The spectrum of "petite" yeast differs from that of normal yeast by the absence of the bands of cytochromes a and b (P. Slonimski and B. Ephrussi, *op. cit.*).

[9] Two further backcrosses gave similar results (B. Ephrussi and H. Hottinguer, unpublished).

[10] The degree of suppressiveness of a strain is measured by the percentage of zygotes giving rise to mutant clones following a cross with a normal strain *minus* the percentage of "petites" in the latter.

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*A CYTOGENETIC STUDY OF THE X CHROMOSOME OF DROSOPHILA BUSCKII AND ITS RELATION TO PHYLOGENY**

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Drosophila busckii, the only known representative of the subgenus *Dorsilopha*, has a number of genetic and cytologic peculiarities that shed special light on the probable phylogeny of the chromosomal groups in the genus *Drosophila*, no less on other problems of general genetic interest. Some of these features have been touched upon in earlier papers on comparative genetics,[1, 2] especially those on the cytogenetics of the Y chromosome of *D. busckii*.[3, 4] The present paper is concerned chiefly with new cytogenetic data that bear on the structure of the general chromosomal complement of this species, and especially upon the structure of the X chromosome.

According to current accounts, two chief chromosomal types of *D. busckii* exist in nature. In one there are four pairs of chromosomes: a pair of rod's, two pairs of V's, and a pair of dotlike chromosomes.[5] In the other chromosomal type there are said to be but three pairs of chromosomes, there being no dotlike elements. Furthermore, this dotless form is said to possess three morphologically different sorts of X chromosome, namely, a simple rod-shaped X,[1, 4, 6, 7] a rod-shaped X that terminates proximally in a spherical satellite,[8] and finally a J-shaped X chromosome.[9, 10]

In view of the conflicting accounts, the current investigation was undertaken with the aim of reinvestigating the chromosomal set of *D. busckii* and was carried along in three directions: (1) a cytological analysis of the mitotic and polytene chromosomes of *D. busckii* from geographically remote populations; (2) a cytogenetic analysis of certain mutant strains; and, finally, (3) an analysis of the homology of the X and Y chromosomes.

EXPERIMENTAL DATA AND RESULTS

The lines that were cytologically analyzed are listed in Table 1. In all lines the chromosomal complement was the same, consisting of three pairs of chromosomes. The rod-shaped pair is that of the sex chromosomes, and the two V-shaped pairs are autosomes. No evidence of gross dissimilarities in chromosomal complement was discerned in any of the strains.