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Mitochondrial genetics, circular DNA and the mechanism of the *petite* mutation in yeast

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SUMMARY

We propose a general hypothesis involving properties of circular DNA which can explain such phenomena as the *petite* mutation, suppressiveness, and the polarity observed in mitochondrial recombination in the yeast *Saccharomyces cerevisiae*. This hypothesis involves excision and insertion events between circular DNA molecules as well as structural rearrangements in the DNA generated by these events. The special properties of circular DNA have been considered in analysing recombination, and a number of results are obtained which are not intuitively apparent.

This hypothesis can be applied to any situation involving circular DNA such as bacterial plasmids and cytoplasmic circular DNAs, where the opportunity exists for recombination and rearrangement events.

In biochemical and genetic studies of cytoplasmic inheritance, considerable attention has focused on the yeast *Saccharomyces cerevisiae* because of the well-characterized cytoplasmically inherited *petite* mutation and the existence of many genes located in mitochondrial DNA. However, several areas of research into the mechanism by which *petite* mutants are formed, the suppressiveness of *petites* and the polarity of recombinant types observed in mitochondrial genetic crosses, are contentious. In this article we examine these problems in terms of the behaviour of circular DNA molecules, and propose a hypothesis which can account for these seemingly diverse phenomena. The hypothesis invokes excision and insertion events within and between circular mitochondrial DNA molecules.

The *petite* mutation resulting in respiratory deficiency occurs spontaneously at the high rate of around 1 % per generation (Ephrussi, 1953; Nagai, Yanagishima & Nagai, 1961). It is irreversible, cytoplasmically inherited (Ephrussi, 1953; Wright & Lederberg, 1957) and located on a factor termed ρ which is now identified as a mitochondrial DNA (Sherman, 1963; Goldring *et al.* 1970; Nagley & Linnane, 1972). The *petite* phenotype also arises by mutation in any of a number of nuclear genes but these 'segregational' mutations occur at a much lower frequency than the cytoplasmic mutation (Sherman, 1963; Chen, Ephrussi & Hottinguer, 1950; Hartwell, 1970).

Several authors have drawn attention to the high spontaneous appearance of the

petite 'mutation', a process which is all the more surprising in view of the claim that there can be 50–100 ρDNA* molecules in a respiratory competent cell (Williamson, 1970; Nagley & Linnane, 1970). This high rate of *petite* formation does not therefore appear to be due to gene mutation processes such as transitions, transversions or frame shifts which occur at a far lower spontaneous frequency.

Previous hypotheses for the formation of *petites* have involved the loss of a cytoplasmically occurring particle (Ephrussi, 1953), a master template of ρDNA associated with the nucleus (Maroudas & Wilkie, 1968), an altered ρDNA polymerase (Slonimski, 1968; Carnevali, Morpurgo & Tecce, 1969), the accumulation of point mutations in ρDNA (Borst & Kroon, 1969) and the dominance of a mutated ρDNA product (Williamson, 1970). However, these hypotheses have proved to be inadequate since they fail to satisfactorily account either for the high spontaneous frequency of *petites* or for phenomena such as suppressiveness.

1. PETITE FORMATION

We propose an alternative hypothesis for the generation of *petite* mutants. In this hypothesis we suggest that it is primarily the circular nature of the ρDNA which provides a framework for understanding the production of *petites*, as well as many of the phenomena that have been described in the mitochondrial genetic system in yeast. We envisage that an essential step in *petite* formation is the generation of circular molecules smaller than the complete genome. The generation of these incomplete genomes is considered in detail later. Since our proposal involves structural rearrangements within and between circular DNA molecules, rather than involving mutations in single genes, as most of the previous hypotheses have done, it can account for the high spontaneous frequency of *petites*.

Once an incomplete genome is generated, two processes are probably involved in the appearance of buds which lack any complete mitochondrial genomes. The first of these can be a passive process depending upon the chance segregation into a bud of a number of circular molecules which together lack the necessary information for the expression of a complete mitochondrial genome. The active process of *petite* generation may further be the result of two processes. The smaller circular molecules may be at a replicative advantage or they may be involved in forming further defective molecules by inserting into complete genomes. The formation of further defective molecules from these hybrid molecules containing duplications is considered at length in the section on suppressiveness, but essentially we view the hybrid molecules as being more unstable than monomers, owing to their increased size and their increased internal homologies. Thus it can be envisaged that once a defective ρDNA molecule arises in a yeast cell that cell is a potential pro-genitor of *petites*.

* The term ρDNA is used to denote the mitochondrially located DNA of buoyant density 1·684 g/cm^3. The possibility that other cytoplasmically occurring DNA species are associated with mitochondria has not been excluded (Clark-Walker, 1972; Clark-Walker & Miklos, 1974).

2. CIRCULAR DNA AND THE FORMATION OF SMALLER CIRCLES

In this section we describe some possible ways of generating these molecules and subsequently we evaluate the important structural features of ρDNA that may facilitate these events.

The mitochondrial DNA of diverse species of fungi has been found to occur in the form of covalently closed circular DNA varying in size from 14 to 25 μm (Hollenberg, Borst & Van Bruggen, 1970; Clayton & Brambl, 1972; Agsteribbe, Kroon & Van Bruggen, 1972; Clark-Walker & Gleason, 1973). In respiratory

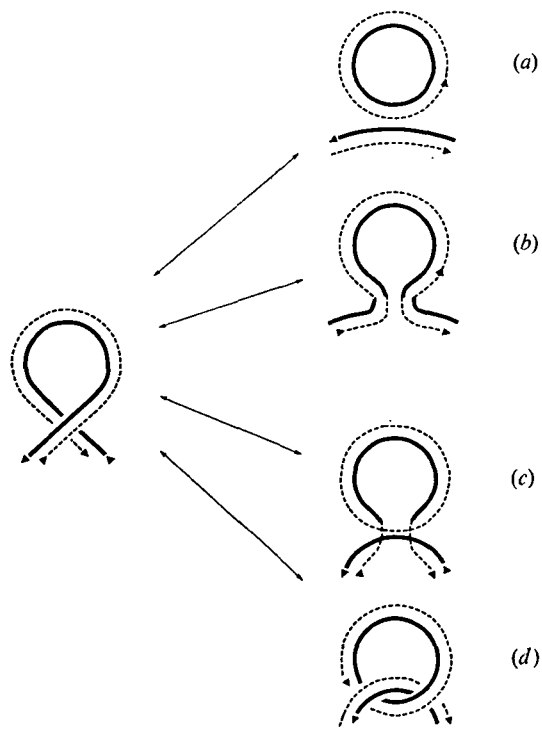

Fig. 1. Structural rearrangement events in double-stranded DNA to generate: (*a*) excision, (*b*) inversion, (*c*) fused circles, and (*d*) catenane.

competent yeast the ρDNA is circular and 25 μm in length; moreover it can also exist in a circular form in *petites* but the circles are of much smaller size. At least some of the ρDNA of three different *petites* has been shown to exist as covalently closed circular molecules varying in size from 0·05 to 1·6 μm (Clark-Walker & Miklos, 1974; Bernardi *et al.* 1968; Hollenberg, Borst & Van Bruggen, 1972). The existence of these small circular molecules in *petites* has led us to inquire into the processes of their formation and into the mechanism of the *petite* mutation.

Circular incomplete genomes could be formed from either monomeric or dimeric molecules. In a monomeric circular molecule a number of different reunions of the strands may occur in particular regions of the DNA (Fig. 1). The excision of the small circular molecules in Fig. 1 (*a*) is analogous to the well-documented excision

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of λ phage from the *E. coli* chromosome (Campbell, 1969). In the other possible events illustrated in the figure (1*b–d*), the reunions would produce an inverted portion of the genome, two fused circles and a catenane.

The rearrangements resulting in structures such as fused circles and catenanes have been described previously by others (Hudson & Vinograd, 1967; Clayton

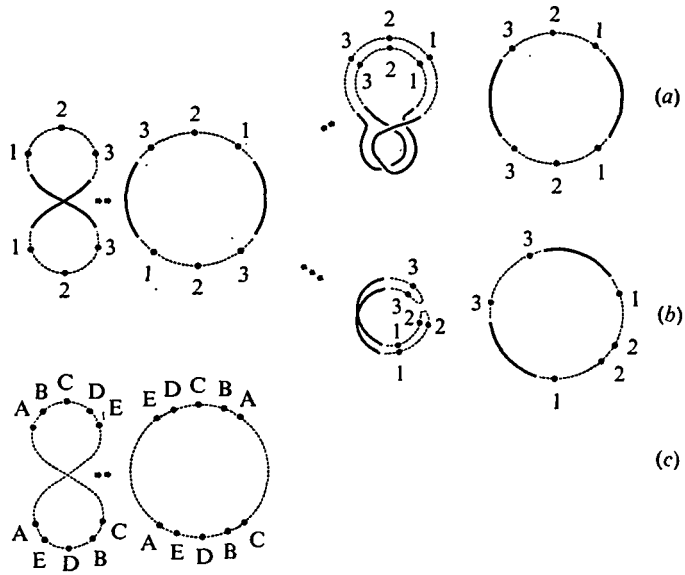

Fig. 2. Formation of rearranged dimers. Regions of partial homology are indicated by the solid lines. (*a*) The generation of an inverted dimer from a tandem dimer by a single cross-over event in a region of partial homology which has been paired in a reversed way. (*b*) Formation of an inverted dimer by a reversed reunion event following homologous self-pairing. (*c*) Formation of a dimer having a transposition by insertion between two monomers, one of which carries a transposition for the ED region.

Davis & Vinograd, 1970). Transitions between the various structures could also happen; insertion of two circles may occur as a reversal of the excision process and insertion in the opposite direction results in an inverted region. If after excision the excised portion inserts at a different site then a transposition will be formed. These excision and insertion events could preferentially occur at sites of partial base sequence homology. An indication that ρDNA contains such regions is apparent from the structural data on this molecule which are discussed later.

Incomplete genomes can also be formed from dimeric molecules in a number of different ways, but in contrast to the restricted excision from monomeric molecules, homologous pairing of duplex strands in a dimer could produce excision from anywhere in the genome.

The formation of dimers may be most probable during or just after replication, since the two monomers will still be in close proximity as part of a replication complex. Alternatively, dimers may be formed after two monomers have undergone

homologous pairing followed by a single insertion. Defective molecules cannot be formed from tandem dimers following homologous pairing since excision events yield only complete genomes or catenanes. On the other hand, defective molecules can be produced by homologous pairing from dimers containing an inverted or transposed region.

An inverted dimer or one containing an inverted or transposed region can be generated in the following ways. An inverted dimer can be formed by a reversed insertion between two monomers (analogous to Fig. 1*b*) or a reversed reunion

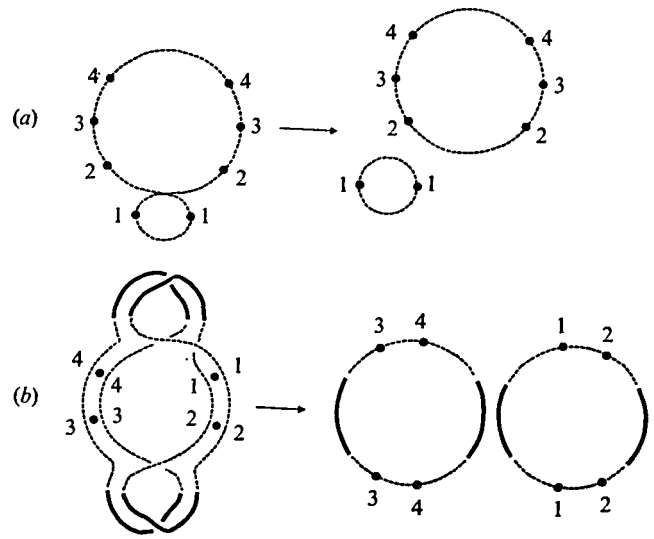

Fig. 3. Formation of defective genomes. (*a*) Generation of two duplicated defective molecules by a single excision event following homologous pairing in an inverted dimer. (*b*) Formation of two duplicated defective molecules by a double cross-over event from a tandem dimer paired in an inverted way in two regions of partial homology.

event in a tandem dimer (Fig. 2*b*). An inverted region in a dimer can be formed either by an inversion occurring in an already formed tandem dimer or by insertion between two monomers, one of which contains an inversion. A dimer containing a transposition could be generated in a similar way. Furthermore, an inverted dimer can be formed by a single insertion in a region of partial homology in a tandem dimer paired as shown in Fig. 2(*a*).

Once a dimeric molecule is formed, which is inverted or contains an inversion or a transposition, it can, following homologous pairing, undergo excision to yield defective molecules (Fig. 3*a*). A further mechanism for the production of defective molecules is a double exchange event in a tandem dimer which is paired in two or more regions of partial homology. These events could produce either catenanes or two separate defective genomes (Fig. 3*b*).

The actual occurrence of dimeric molecules in the mitochondrial DNA of many organisms is well documented, the proportion of dimers to monomers usually

being 1–10% (Hudson & Vinograd, 1967; Nass, 1969). The sedimentation studies of ρDNA in yeast indicate that here too dimers occur in significant frequency (Blamire *et al.* 1972).

3. INFORMATION DECAY AND FIXATION IN *PETITES*

Once an excision event has produced a defective ρDNA molecule and a *petite* cell has been formed as discussed earlier, the ρDNA in this *petite* need not necessarily be stable and may undergo further changes. We view the changes in ρDNA that occur from initial *petite* formation to final stability as arising from similar excision–insertion events as are involved in the formation of the original defective molecule.

Genetic analyses have indicated that *petites* can progressively lose ρDNA encoded antibiotic resistance genes either spontaneously or during further ethidium bromide treatment (Linnane *et al.* 1968; Michaelis, Petrochilo & Slonimski, 1973). Molecular studies have shown that the ρDNA can vary considerably in buoyant density in different spontaneous *petites*, implying that different sequences have been retained (Mounolou, Jacob & Slominski, 1966). This finding is supported by hybridization studies involving mitochondrial encoded t-RNAs, where it has been found that different *petites* vary in their retention of different t-RNA sequences (Cohen *et al.* 1972; Carnevali *et al.* 1973).

Sequence homology between ρDNAs from *petites* and ρ^+ strains has also been studied by hybridization techniques. Sanders *et al.* (1973) find no sequence homology between ρDNAs from three different *petites* which have been grown for many generations but nevertheless all three hydridize to ρDNA from ρ^+ cells.

Besides progressive loss of information from *petite* ρDNAs, certain segments of the genome can undergo amplification to produce tandem repeats of a particular segment (Sanders *et al.* 1973; Faye *et al.* 1973). Such a molecule would be prone to multiple insertion and excision events and this may account for the intertwined and entangled DNA structures termed 'fishnets' seen in *petites* (Hollenberg *et al.* 1972).

4. MITOCHONDRIAL 'RECOMBINATION'

We have considered that ρDNA undergoes excision and insertion events to generate smaller defective genomes, and the frequency of these events may be indicated by the mitochondrial genetic 'recombination' data discussed below.

The first demonstration of recombination between mitochondrial genetic markers in respiratory competent strains was made by Thomas & Wilkie (1968). These authors briefly commented on the possibility of circularity in the mitochondrial genome and on the problems of dominance and recessiveness of the antibiotic resistance genes. Later authors have verified and extended these studies on 'recombination', but have not included in their interpretations some of the consequences involved with circular rather than linear genomes and dominance or recessiveness of the particular genes (Coen *et al.* 1970; Rank, 1973). We wish to

show that if circular mitochondrial genomes are considered, a number of results can be obtained which are not intuitively apparent. In our opinion, 'recombination' of mitochondrial genetic markers occurs by insertion of one genome into another by way of homologous pairing to produce a dimer. A more complex situation is when insertion occurs in preferred regions of partial homology.

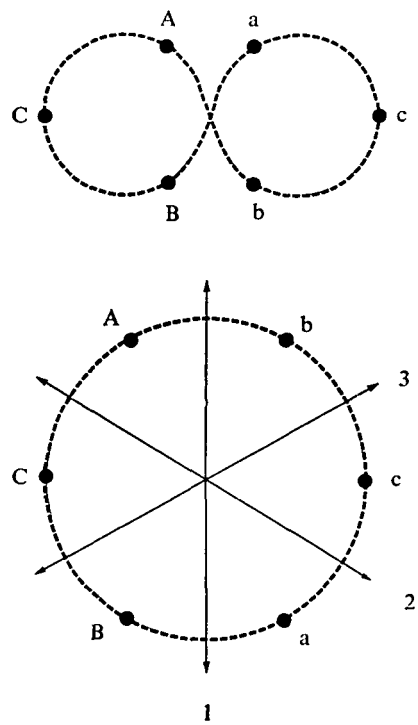

Fig. 4. The generation of reciprocal recombinant classes from excision events in any of three regions following the formation of a dimer by insertion between A and B. The formation of further reciprocal recombinant classes by insertions between B–C and C–A is presented in Table 1.

After a dimer is formed, excision can occur to produce two complete but genetically different molecules. For example, Fig. 4 illustrates a case in which three genes are equidistantly placed on a circular genome and it is envisaged that insertion can occur with equal probability in any of three regions A–B, B–C and C–A. In the hybrid dimers, excision can also occur in any of three regions, resulting in a total of nine possible pairs of gene combinations. As illustrated in Table 1, all possible 'recombinant' types are obtained, but the parental class occurs in higher frequency than any of the three 'recombinant' classes. If the markers occur asymmetrically on the circular genome, then the parental class will occur with an even higher frequency.

Another factor which has to be considered in dealing with crosses involving multiple copies of ρDNA, is the ratio of the two mixing mitochondrial genomes in

the resulting zygote. Hoffman & Avers (1973) have shown that yeast cells may have only a single multibranched mitochondrion, and if this is the case, mitochondrial fusion may involve only two mitochondria bringing all the ρDNA genomes in a zygote into a common pool. Furthermore since various strains of yeast are known to vary in their content of ρDNA (Williamson, 1970; Fukuhara, 1969), fusion of two cells having different amounts of ρDNA will alter the ratio of the parental genome types. For instance, in the following example we consider a cross where

Table 1. *Formation of recombinant classes following random insertion and excision events between ABC and abc molecules*

Region of insertion	Region of excision	Resulting genomes
A–B	B–a	ABC:abc
	a–c	aBC:Abc
	c–b	AbC:aBc
B–C	C–b	ABC:abc
	b–a	AbC:aBc
	a–c	ABc:abC
C–A	A–c	ABC:abc
	c–b	ABc:abC
	b–a	aBC:Abc

Class	Genotypes	Frequency
Parentals	*ABC:abc*	3
'Recombinant' 1	*aBC:Abc*	2
'Recombinant' 2	*ABc:abC*	2
'Recomblnant' 3	*AbC:aBc*	2

three markers are asymmetrically arranged so that distances between the genes represent $\frac{1}{10}$ (A–B), $\frac{5}{10}$ (B–C) and $\frac{4}{10}$ (C–A) of the circumference, and the ratio of the parental genomes in the zygote is 3 (ABC): 1 (abc). The resulting parental types will have a frequency of 64% (ABC) and 14% (abc), whereas the three 'recombinant' classes will occur with a frequency of 3% (Abc, aBC), 15% (ABc, abC) and 4% (Abc, aBC) in a cross involving random interactions and 'recombinations' of all the molecules.

These figures will be modified if only some of the ρDNA molecules in the zygote are able to engage in 'recombination'. In such a case the frequencies of the parental types will more closely reflect the input proportions of parental ρDNAs and in genetic mapping studies the true distance between markers will be underestimated.

The foregoing considerations offer an explanation for the polarity observed by many authors in the different frequencies of parental types resulting from a cross. However, polarity has also been found between 'reciprocal recombinant' types (Coen *et al.* 1970), and in some strains of yeast these types deviate markedly from a one-to-one ratio (Wilkie & Thomas, 1973; Howell *et al.* 1973).

We feel that polarity between 'reciprocal recombinant' types may be explained

by a consideration of structural rearrangements within the ρDNA of different strains. These structural rearrangements involve the transposition or duplication of a portion of the genome. Transpositions or duplications will have a profound influence on the frequency of 'reciprocal recombinant' types when strains carrying them are mated to ones lacking these rearrangements. In a dimer carrying a transposition (Fig. 2*c*), a proportion of excisions following homologous pairing will produce molecules containing less than and greater than a complete genome. The phenotype of a cell containing the ρDNA having the duplication will depend on the dominance relationships of the alleles involved. The cell containing the deficient molecule on the other hand will be lost from the analysis of 'recombination' events through *petite* formation. Thus one of the 'reciprocal recombinant' types can predominate.

Polarity can also result from a monomer containing a duplication. If such a molecule undergoes 'recombination' the resulting dimer will contain a triplicated region. Excisions from this structure will ultimately generate defective molecules in the amplified portion of the genome, preferentially removing genes in this region from the analysis.

If 'recombination' preferentially involves insertion and excision events at regions of partial homology, then polarity may also result from a strain that has ρDNA with a deletion of a non-essential region of partial homology. When dimeric molecules containing such a deficiency are formed by 'recombination', only a restricted range of excision recombinant types is favoured.

In summary, the degree of influence of transpositions, duplications and deficiencies will depend on whether the genetic markers in question are located within or distant to the rearranged region, on the size of the region itself and on the relative positions of the insertion and excision sites.

5. SUPPRESSIVENESS

Besides these investigations involving ρ^+ by ρ^+ crosses several authors have examined aspects of ρDNA in ρ^- by ρ^+ crosses. If such a cross produces a high frequency of *petites*, the haploid *petite* strain is termed 'suppressive' (Ephrussi, De Margerie-Hottinguer & Roman, 1955); if it produces no increase over the background frequency it is termed 'neutral'. Neutral *petites* generally contain no detectable ρDNA, whereas 'suppressive' *petites* contain some ρDNA which can be of altered buoyant density. There does not appear to be a correlation between buoyant density and the degree of suppressiveness (Mounolou *et al.* 1966; Michaelis *et al.* 1970). Furthermore, in a cross of a suppressive *petite* having ρDNA of altered buoyant density by a normal respiratory competent strain, the resulting diploids can have ρDNA of intermediate buoyant density (Carnevali *et al.* 1969; Shannon *et al.* 1972).

We view suppressiveness as insertion of two circular molecules followed by subsequent excision from the hybrid molecule to produce defective genomes. In our mechanism, a single insertion event following homologous pairing in a ρ^- by

ρ^+ cross, does not result in loss of information from the ρ^+ genome, since a partially duplicated molecule, still containing complete information, is produced. Furthermore, this molecule must be of intermediate buoyant density, a density which depends on the relative sizes and densities of the ρ^- and ρ^+ genomes and on the number of insertions. Additionally we view suppressiveness as having two components; the first is related to the amount of insertion that occurs between ρ^- and ρ^+ genomes, and the second depends on the frequency of excisions from the partially duplicated molecules. Therefore the degree of suppressiveness of a ρ^- by ρ^+ cross is not necessarily a reflexion of the amount of genetic 'recombination' found in that cross.

We think then that suppressiveness is not only related to the amount of insertion but is also a reflexion of excision events which would depend on the size and genetic complexity of the ρDNA of the particular *petite* strains involved.

6. *PETITE* NEGATIVE YEASTS AND THE STRUCTURE OF ρDNA

Some yeasts do not yield cytoplasmically inherited respiratory deficient phenotypes and these are termed *petite* negative yeasts. Nevertheless, genetic studies have shown that the respiratory-deficient phenotype is possible in some *petite* negative yeasts such as *Schizosaccharomyces pombe*, *Kluyveromyces lactis* and *Hansenula wingei* which cannot utilize non-fermentable substrates. All these mutants are chromosomally inherited (Heslot, Louis & Goffeau, 1970; Wolf *et al.* 1971; Herman & Griffin, 1968; Crandall, 1973). If the mechanism of generation of the *petite* mutation in *petite* positive yeasts is general, why is it not possible to obtain cytoplasmically inherited respiratory deficient mutants in the *petite* negative yeasts discussed above?

We wish to consider two possible explanations, the first being that the mitochondrial DNA of *petite* negative yeasts carries a gene or genes necessary for viability. The mitochondrial DNA of these strains may well undergo excision–insertion events to produce defective genomes, but in these cases the cell containing the smaller genomes would be deficient for one or a number of mitochondrial genes necessary for survival, and would be inviable.

Mitochondrial genomes, as illustrated by *S. cerevisiae*, may encode information necessary for the optimal growth of the yeast cell (Clark-Walker & Linnane, 1967). In this previous study a comparison was made between growth rates of isogenic respiratory competent and respiratory deficient strains in the presence or absence of chloramphenicol, which inhibits mitochondrial translation. It was found that the growth rate of the *petite* was 80% that of the ρ^+ strain, either in the presence or absence of the drug, whereas the initial growth rate of the ρ^+ strain on fermentable substrate in the presence of chloramphenicol was the same as the ρ^+ control. These findings may be interpreted to mean that a transcriptional product of the ρ genome, which is translated by cytoplasmic ribosomes, is necessary for optimal growth rate. Hence it may be possible that *petite* negative yeasts are much more, or even entirely dependent on mitochondrial transcription products for their

growth and survival. In the case of chromosomally inherited lesions leading to the respiratory deficient phenotype in *petite* negative yeasts, the mitochondrial DNA would remain intact.

An alternative explanation for the lack of cytoplasmically inherited respiratory deficient phenotypes could be that ρDNA in *S. cerevisiae* has some peculiar structural properties which favour the excision–insertion processes we have discussed. The fact that ρDNA has some unusual properties is apparent from the high AT content of this DNA (82 %), in comparison to the known upper limit of 75 % AT for bacteria. This suggests that ρDNA has AT-rich sequences not utilized for coding purposes and further evidence supporting this suggestion comes from several studies. Bernardi *et al.* (1968) and Bernardi, Piperno & Fonty (1972), following the initial studies of Mehrota & Mahler (1968), have extensively investigated the melting profile of ρDNA from ρ^+ cells and have found a striking compositional heterogeneity with AT-rich sequences suggested to comprise as much as 50 % of the mitochondrial genome. Buoyant density studies have also shown a compositional heterogeneity in ρDNA. Four peaks have been resolved using sonicated ρDNA in CsCl and unsonicated DNA in $Ag^+ - Cs_2\,SO_4$ gradients (Carnevali & Leoni, 1972; Vedel *et al.* 1972). Additional studies have shown this heterogeneity to be due to AT-rich sequences interspersed in the genome (Piperno, Fonty & Bernardi, 1972). Furthermore, the AT-rich stretches of ρDNA may well be composed of highly reiterated sequences, since this is known to be the structure of Ag^+ satellites from other sources (Yunis & Yasmineh, 1970). These AT-rich sequences may well be what we have termed 'sites of partial homology', facilitating excision–insertion and rearrangement events leading to *petite* formation.

An indication that structural considerations may be involved in *petite* negativity, is illustrated by the *petite* negative yeast *Candida parapsilosis*, where the circular mitochondrial DNA has a buoyant density of 1·698 g/cm^3 and length 11 μm (Clark-Walker, unpublished). Thus *C. parapsilosis* and *S. cervisiae* may have quite similar basic genomes of about 11 μm with regard to informational content and buoyant density, but differ in that the latter carries an additional total of 14 μm non-coding partially reiterated AT-rich sequences. This may imply a difference in the frequency and facility of excision–insertion events which may be of critical significance in the establishment of a bud lacking complete genomes.

Although we have suggested that the peculiar structural properties of ρDNA may provide an alternative explanation for the presence of the *petite* mutation in *S. cerevisiae* and its absence from *petite* negative yeasts, some *petite* negative yeasts do have AT-rich mitochondrial DNA similar in buoyant density and abnormal melting behaviour to *S. cerevisiae* (Bak, Christiansen & Stenderup, 1969). Thus a simple explanation for *petite* negativity in terms of the structural peculiarities of mitochondrial DNA may be insufficient to account for the *petite* negative phenomenon. There may be two components to *petite* negativity, one being the existence of essential genes located on mitochondrial DNA, the loss of which leads to cellular inviability, and the second being the restrictions imposed by the actual structural features of mitochondrial DNA.

7. EFFECT OF MUTAGENIC AGENTS

Our discussion of the mechanism of the *petite* mutation has involved a consideration of the spontaneously arising *petites*. However, a large variety of agents can induce the respiratory deficient phenotype, the most studied of these being ultraviolet irradiation and dyes such as ethidium bromide and acriflavin which intercalate into DNA (Nagai *et al.* 1961). Hollenberg & Borst (1971) have pointed out the complexities involved in interpreting the action of ethidium bromide in inducing *petites* and suggest that 'the characterization of the mechanism of *petite* induction requires a clear distinction between primary and secondary effects of ethidium bromide'. Furthermore, they conclude that in addition to inhibiting ρDNA synthesis, ethidium bromide induced mutagenesis may involve repair or 'recombination' of ρDNA.

We think that a fruitful avenue of approach may be to view these agents as exacerbating an already frequently occurring spontaneous process involving excision, insertion and structural rearrangements in ρDNA.

8. CONCLUDING REMARKS

The events and structural considerations invoked for the generation of *petites* involving excision–insertion between circular ρDNA molecules can also explain the diverse phenomena found in the mitochondrial genetic system in yeast. Furthermore, our hypothesis can account for the high spontaneous frequency of *petite* formation because it does not involve normal gene mutation. Rather, the frequency will be governed by many factors such as the structure of the ρDNA, the nuclear genes coding for ρDNA replication, repair and recombination enzymes and the number of molecules of ρDNA per cell. Whilst the events we have discussed for yeast may be influenced by the structural properties of ρDNA itself, the behaviour of plasmids and episomes in bacterial systems lends considerable support to our hypothesis. For instance, recombination between bacterial plasmids such as Colicin factors, Resistance factors and Sex factors has been extensively documented (Novick, 1969). Furthermore, hybrid structures, which have been suggested to form by a single insertion event between circular DNA plasmids, are known to break down asymmetrically producing different gene combinations (Richmond, 1967; Fredericq, 1969). In some bacterial cells containing plasmids, the data favour continually changing gene rearrangements due to fusion and fragmentation cycles and to recurring chromosomal integration and excision events.

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