(perhaps a monolayer) of water separates the solute from the ice. This layer might reflect the shape of the macromolecule. Such water could be held by hydrogen bonds, or it might be in a region where the normal ice structure was disrupted through "hydrophobic" interactions (*13*). Another source of unfrozen water could exist if the macromolecule was able to actually trap water in sufficiently small channels or pores (*12*). One might anticipate a relatively sharper resonance line for water of this type because, other things being equal, water in a cavity would have lower activation energy than water on a surface (*14*). In a molecule with both types of water, considerable averaging would be expected (*15*).

Approximate combinations of "adsorbed" and "trapped" water can be used to explain the protein results on an ad hoc basis. Assume the native protein contained some of both types of water. Denaturation of the proteins could leave the amount of surface binding approximately unaltered, or even augmented, as interior sections of the molecule were exposed. However, the water held inside the native structure would become accessible as the molecule opened, losing any special character or mobility. If the "internal" water is associated with a narrow signal, the line broadening on denaturation can be understood. The simplest description of the water detected by the NMR technique would seem to be water that is constrained but has no definite "long-range" organization.

Our method is not limited to either water as a solvent or biomacromolecules as solutes. Any system in which the solute has a moderate-to-high molecular weight and the solvent has a moderately high freezing point might well yield similar results.

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## Cytoplasmic DNA from Petite Colonies of Saccharomyces cerevisiae: A Hypothesis on the Nature of the Mutation

Abstract. *The density of the cytoplasmic DNA of two strains of "petite" mutants of yeast, obtained by treatment with acriflavin and with ultraviolet light, was examined in cesium chloride density-gradient centrifugation and in all cases appeared to be less than that of the wild type. A cytoplasmic respiratory-deficient strain, treated with additional acriflavin, can show a further shift of the position of the satellite band, always in the direction of reduction of density. Also, from the $\rho^+ \times \rho^-$ cross, $\rho^-$ strains can be recovered in which the density of the satellite DNA is different from the density of the parent $\rho^-$ strain. This finding suggests the existence of recombination in cytoplasmic DNA molecules.*

In yeast the enzymes linked to the respiratory processes, with the single exception of cytochrome c, are under double genetic control [for a concise review of the problem, see Mounolou *et al.* (*1*)]. The cytoplasmic mutation, which leads to the formation of petite colonies, in addition to having an exceptionally high spontaneous frequency of mutation (*2*), can also be induced with a frequency near 100 percent by acriflavin (*3*) and other agents, such as fluorouracil (*4*). Therefore, the mutation has been thought to consist of the elimination of cytoplasmic genetic determinants from the cells (*5*). Discovery of the phenomenon of "suppressiveness" (*6*), that is, that the mutant can be dominant with respect to the wild type, has lead to the belief that the cytoplasmic determinant was still present, although altered, in the mutated cells. Further, Carnevali and Tecce (*7*), Carnevali *et al.* (*8*), and Mounolou *et al.* (*9*) noted that the $\rho^-$ strains contain a band of satellite DNA, corresponding to mitochondrial DNA (*9*), having a density different from that of the DNA of the wild type $\rho^+$ strain. In particular, Carnevali *et al.* (*8*) found that the density of the satellite band was 1.671 g/cm$^3$ as compared to 1.686 g/cm$^3$ for the DNA from the $\rho^+$ strain (*10*). The density of 1.671 g/cm$^3$ corresponds to the density of a polymer that contains mainly deoxyadenylate and deoxythymidylate. In a later work (*11*) it has been confirmed that the DNA from the mutant strain is composed almost exclusively of adenine and thymine. We now report data that, together with previous data, permit the formulation of a hypothesis on the mechanism of the mutation.

We used strain 42 $\rho^+$ diploids that were heterozygous for some nutritional markers and strain 34, derived from the former by means of spontaneous somatic recombination and homozygous for arginine and methionine requirements. We also used strain 16 $\rho^+$.

Cytoplasmic mutations were obtained by treatment with acriflavin and with ultraviolet light. The treatment with acriflavin was carried out with 0.005 percent (weight to volume) acriflavin,

with yeast growing in a complete liquid medium, for 48 hours.

The petite strains were grown under aerobic conditions in enriched Czapek Dox medium. DNA was isolated by a modification of the method of Marmur (*12*); because of the large quantity of RNA in yeast cells, the samples of DNA were treated with pancreatic ribonuclease and ribonuclease $T_1$.

The DNA samples, at a concentration of 10 $\mu$g/ml in citrate-saline standard, were analyzed by cesium chloride density-gradient centrifugation. *Streptomyces fradiae* DNA, having a density of 1.731 g/cm$^3$, was used as a marker.

The density of samples was calculated by the method of Schildkraut (*13*). Table 1 shows the density of cytoplasmic and nuclear DNA from all the strains that were examined. Strains 32 and 33 were obtained by treatment with acriflavin, while strain 34/2 derives from treatment with ultraviolet light. Strains 33/2, 33/3, 33/16-A, 33/16-7, 33/16-11, 69, and 70 derive from complex experiments to be described later. In all cases, the density of the satellite DNA of the mutants was different from that of the wild type, showing a decrease in density which probably reflects a relative increase in adenine and thymine. This was also true for the mutants obtained by treatment with ultraviolet light.

Strain 33, derived from strain 34 by treatment with acriflavin, was given additional treatment with acriflavin. The methods were those previously used, which normally produce 100 percent mutant colonies. Two colonies (33/2 and 33/3) obtained from two identical treatments were selected at random, and the DNA was examined. The density of the satellite DNA appeared to be different from that of the parent colony (Table 1). In this case, also, there was a further reduction of density. The mutagenic action of acriflavin expresses itself on the DNA even when it is not possible to characterize it from a phenotypic point of view.

Diploid strain 33 was crossed with haploid strain 16. From this cross, which shows a moderate suppressiveness (*14*), there were colonies that were normal from the respiratory aspect, petite colonies, and other genetically unstable colonies that continuously produced petite colonies with a frequency of nearly 50 percent. Three petite strains (33/16-A, 33/16-7, and 33/16-11), isolated from these colonies, were examined for density of the DNA (see Table 1). In two cases out of three, the density of the satellite band was decreased with respect to that of the petite parent. Moreover, strain 34/2 $\rho^-$, with mitochondrial DNA of density 1.672 g/cm$^3$, has been crossed with strain 16 $\rho^+$. Again two petite strains (69 and 70) were isolated from genetically unstable colonies and their DNA analyzed in cesium chloride density gradient. The satellite DNA of both strains turned out to have a density between the densities of the parent strains (see Table 1).

Table 1. Buoyant density of nuclear and mitochondrial DNA from some strains of *Saccharomyces cerevisiae*. Strains 33/2 and 33/3 were obtained from strain 33 by an additional treatment with acriflavin; strains 33/16-AG, 33/16-A, 33/16-7, and 33/16-11 derived from the cross between strains 33 and 16, and strains 69 and 70 derived from the cross between strains 34/2 and 16. Mit, mitochondrial.

| Strains | Buoyant density (g/cm$^3$) | |
|---|---|---|
| | Nuclear DNA | Mit-DNA |
| DM (wild type) | 1.700 | 1.686 |
| DM$_1$ (petite) | 1.700 | 1.671 |
| 32 (petite) | 1.700 | 1.678 |
| 33 (petite) | 1.700 | 1.677 |
| 33/3 (petite) | 1.700 | 1.676 |
| 33/2 (petite) | 1.700 | 1.672 |
| 34/2 (petite) | 1.700 | 1.672 |
| 16 (wild type) | 1.700 | 1.684 |
| 33/16-AG (wild type) | 1.700 | 1.683 |
| 33/16-A (petite) | 1.700 | 1.674 |
| 33/16-7 (petite) | 1.700 | 1.678 |
| 33/16-11 (petite) | 1.700 | 1.671 |
| 69 (petite) | 1.700 | 1.675 |
| 70 (petite) | 1.700 | 1.675 |

This research was carried out for the purpose of finding the mechanism by which the $\rho^-$ strains are formed, with altered density, in the DNA satellite band. In order to do this, we must first consider which data have already been obtained. They are listed below.

1) Extremely varied chemical or physical agents can cause the mutation from $\rho^+$ to $\rho^-$ with very high efficiency, up to 100 percent. Among the best-known are acriflavin, fluorouracil, ultraviolet light, and heat (*3, 4*). These agents, which have no apparent relation among themselves, have the capacity, however, to interfere with the synthesis of DNA. Even in the absence of any treatment, the frequency of spontaneous mutation is exceptionally high—up to 1 percent per cellular generation (*2*).

2) In all cases analyzed so far, both by us and in the laboratory of Slonimski, the mutation leads to a shifting in the density of the DNA satellite band. In all but one case (*9*), this shifting is in the direction of a decrease in density, and presumably causes a relative increase in adenine and thymine. It has been demonstrated, particularly in the strain analyzed by Bernardi *et al.* (*11*), that the DNA of the colony examined contained about 96 percent of adenine and thymine in equimolar amounts. Shiftings in DNA density require a change of a high percentage of bases, and therefore cannot be a point mutation.

3) The satellite band DNA in the wild-type colony is extraordinarily rich in adenine and thymine, approximately 80 percent of the DNA being composed of these two compounds (*15*).

4) The strains show the suppressiveness phenomenon, that is, the genetic determinants succeed in excluding the normal determinant, when the two are found together in competition in the same cell (*6*).

5) Treatment with acriflavin causes a new change in density of the satellite DNA, although this is not evident at the phenotypic level. In this case, also, there was a decrease in density.

6) An identical result was obtained by examining colonies derived from a cross between $\rho^+ \times \rho^-$. In two cases out of three, a decrease was obtained in the density of the satellite DNA. Since the cells were not submitted to any treatment, it seems logical to conclude that this last change is due to nonhomologous crossing-over between molecules of cytoplasmic DNA (*16*).

A hypothesis that can be proposed to explain all the reported data is that DNA polymerase normally has a definite and nonnegligible probability of detachment from its template. This event is evidently lethal when it happens in the nucleus, but is compatible with the life of the cell when it happens on the mitochondrial DNA of the yeast. If one admits, and it is very probable, that the DNA polymerase always begins at the same point, the detachment of the enzyme before the end of the duplication would lead to the formation of fragments of the original molecule; these would all have in common the initial segment, that is, the point of attachment. They would, therefore, possess a greater affinity for the polymerase. Naturally these fragments, no longer possessing the complete genetic information, would be unable to function, thereby determining the character of respiratory deficiency. Agents as different as acriflavin and fluorouracil would operate simply by facilitating the detachment of the DNA polymerase from its template. In order to explain the fact that the mutation almost al-

ways leads to a reduction in density of the satellite DNA and probably to a relative enrichment of adenine and thymine, it may be conceived that the initial part of the molecule is particularly rich in these two compounds. Alternatively, one might think that the DNA polymerase, once detached from the template, continues to function in the synthesis of a polymer that contains mainly deoxyadenylate and deoxythymidylate (*17*).

If the first explanations were true, there should be, in theory, a decrease of the molecular weight of the cytoplasmic DNA. Nevertheless, this is not necessarily true because molecules especially rich in adenine and thymine can once again extend themselves by crossing-over; this is facilitated by the ample homologies that probably exist among the polymers very rich in adenine and thymine. The existence of crossing-over between different molecules of cytoplasmic DNA is, moreover, suggested by the work of Hudson and Vinograd (*18*). This existence is also indicated by the fact that (see Table 1) the cross between a $\rho^+$ and a $\rho^-$ strain produces, in the absence of any treatment, new strains that have a satellite DNA density different from that of either of the parents.

The theory that we have set forth has the advantage of explaining the suppressiveness phenomenon. Mills *et al.* (*19*) have demonstrated that molecules from an RNA virus, replicating in vitro can be subjected to a selective pressure. If this selective force is simply the necessity to replicate quickly, it evolves, after a certain number of generations, into a new type of viral RNA that multiplies much more quickly than the initial one. The greater speed of replication is due to the fact that the new molecule is shorter and has a greater affinity for viral replicases. We think that exactly the same process operates on the DNA molecule mutated on the inside of the yeast cell. The incomplete DNA molecules produced by premature detachment of the DNA polymerase can multiply more rapidly than normal molecules, thereby leading to the phenomenon of pseudodominance, otherwise known as suppressiveness.

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## Two Unusual Unionid Hermaphrodites

Abstract. *In a survey of the gonads of 97 species of North American freshwater mussels representing 59 genera, only four species were found to be hermaphroditic (monoecious). Among several other "occasional" hermaphrodites, two were unique in that the same follicles in the gonads produced eggs and sperm simultaneously. Evidently the control mechanism failed to function normally in these species* [Actinonaias ellipsiformis *and* Villosa (*formerly* Micromya) iris (*Lea*)]. *This simultaneous production of eggs and sperm is apparently quite unusual among mollusks.*

In view of the rapid depletion of the mussel fauna, considerable effort has been made to collect and preserve properly relaxed and fixed specimens. Paraffin sections of 97 species, representing 59 genera, have been examined to determine to what extent these animals are monoecious or dioecious (*1*). The mussel fauna group is clearly dioecious (gonochoristic) with only four species (about 4 percent) found to be usually hermaphroditic (monoecious or ambisexual). Among the 1871 specimens sectioned, only one specimen each of two species, representing two genera, appeared abnormal, sperm and eggs being produced simultaneously in the same follicles.

While hermaphroditism is widespread among animals, the reasons for its development are poorly known. Several authors (*2*) have suggested that it may function in the survival of species living in habitats where the success of the reproductive process becomes difficult. Since most of the specimens collected in the same habitats and under similar conditions as the two featured here were normal, dioecious specimens, the theory that for these two specimens conditions were unfavorable seems unlikely. Although Purchon (*3*) concluded that "in the majority of cases, hermaphroditism is an adaptive feature of evolutionary advantage to the species," he also indicated that there are perhaps stimuli other than environmental causes that account for this monoecious development. In addition to the strictly genetic factors, he indicated that the change may be initiated by the gonad itself since in the male phase there is a heavy consumption of nucleoproteins in the production of spermatozoa. The ratio between nucleoproteins and cytoplasm may be upset at a certain point so that sex-reversals are automatically affected. Whether genetic, hormonal, or cytogenetic, the reasons for the sex changes observed in freshwater mussels are, as yet, unknown.

Paraffin sections of 238 specimens of *Actinonaias ellipsiformis* (Conrad) have been studied. Males and females were clearly separable (108 females to 130 males). However, one specimen was an unusual hermaphrodite. Instead of having a predominance of male or female tissue and a small focus of tissue of the opposite sex (occasional hermaphrodites) as found in a number of other groups (*1*), tissues were extensively mixed so that it appeared that the mechanism of sex control was most