The petite mutation in yeast

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The cytoplasmic 'petite' mutation in yeast, which is characterized by the loss of respiratory functions, is caused by large deletions in the mitochondrial genome. The 'petite' mutants arise spontaneously when a segment of the wild-type mitochondrial genome, excised by an illegitimate, site-specific recombination process, is amplified to form a defective mitochondrial genome.

In 1948, the Centre National de la Recherche Scientifique, organized a colloquium entitled 'Unités Biologiques Douées de Continuité Génétique' (Biological Units Endowed with Genetic Continuity). Thirty years later, the proceedings still make most interesting reading. Among the contributions of several outstanding biologists including Lwoff, Delbrück and Monod, there is a paper which can be considered the starting point of extrachromosomal genetics. In this paper, Boris Ephrussi gave the first account of investigations, started three years earlier, on the 'petite colonie' mutation in *Saccharomyces cerevisiae* [1]. It is difficult to describe the initial observation more clearly than in Ephrussi's own words: 'When a culture of baker's yeast, whether diploid or haploid, is plated, each of the cells gives rise in the course of the next few days to a colony. The great majority of these colonies are of very nearly identical size, but one usually finds also a very small number – say 1 or 2% – of distinctly smaller colonies (Fig. 1). These facts suggest that the population of cells which was plated was heterogeneous and that it may be possible to purify it by taking cells from either the big or the small colonies only. The results of such a selection show, however, that cells from the big colonies again and again produce the two types of colonies, while the cells from the small colonies give rise to small colonies only' [2]. Besides describing the mutation and its irreversibility, this paper also reported a number of fundamental observations: (a) that acriflavine treatment increases the number of 'petite' mutants from 1–2 to 100% (Fig. 1); (b) that the mutants grow slowly because they cannot respire, owing to the loss of their ability to synthesize a whole series of respiratory enzymes; and that in anaerobiosis wild-type cells and petite mutants grow at the same slow rate using fermentative pathways; (c) that crosses of wild-type with petite mutants show a non-Mendelian segregation of the mutation, in that they lead either to wild-type progeny exclusively or to both wild-type cells and petite mutants in different proportions; the petite mutants entering the cross were called neutral in the first case and suppressive in the second one [3]. The conclusion drawn by Ephrussi was that wild-type cells and petite mutants differed by 'the presence in the former and the absence in the latter of cytoplasmic units endowed with genetic continuity and required for the synthesis of certain respiratory enzymes' [2].

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This paper is dedicated to the memory of Boris Ephrussi.

The petite mutation is due to gross alterations of mitochondrial DNA

The cytoplasmic units postulated by Ephrussi in 1948 were only identified as mitochondrial genes 20 years later. Indeed, the first hard facts about the molecular basis of the petite mutation were published in 1968 [4,5], when mitochondrial DNAs from two genetically unrelated, acriflavine-induced, petite mutants were shown to have a grossly altered base composition (GC = 4%) compared to DNA from the parent wild-type strain (GC = 18%). These findings unequivocally established that massive alterations in the nucleotide sequences of the mitochondrial genome may accompany the petite mutation and be responsible for it; conclusions that were quickly confirmed by more detailed investigations, which probed the structure of mitochondrial DNA from both wild-type cells and petite mutants [6,7].

The AT spacers and the deletion hypothesis

Mitochondrial DNA from wild-type yeast cells was found to be extremely heterogeneous in base composition, about half of it melting at a very low temperature and being almost exclusively formed by long stretches of short alternating and non-alternating AT : AT and A:T sequences, and the rest melting over an extremely broad temperature range. Interestingly, the existence of two sorts of AT sequences in the AT-rich stretches (later called AT spacers) had been predicted for the first petite genome investigated [4]. Compared with mitochondrial DNA from wild-type cells, DNAs from three spontaneous suppressive petite mutants [6,7] were shown to have lower amounts of GC, to lack a number of components that melt at high temperatures and to renature very rapidly. At that time, I interpreted these results as indicating that petite mutants had defective mitochondrial genomes, in which large segments of the parental wild-type genomes were deleted; I suggested that such deletions arose by a mechanism of the Campbell type [8], involving illegitimate, site-specific recombination events in the AT spacers which I supposed to contain sequence repetitions because of their com-

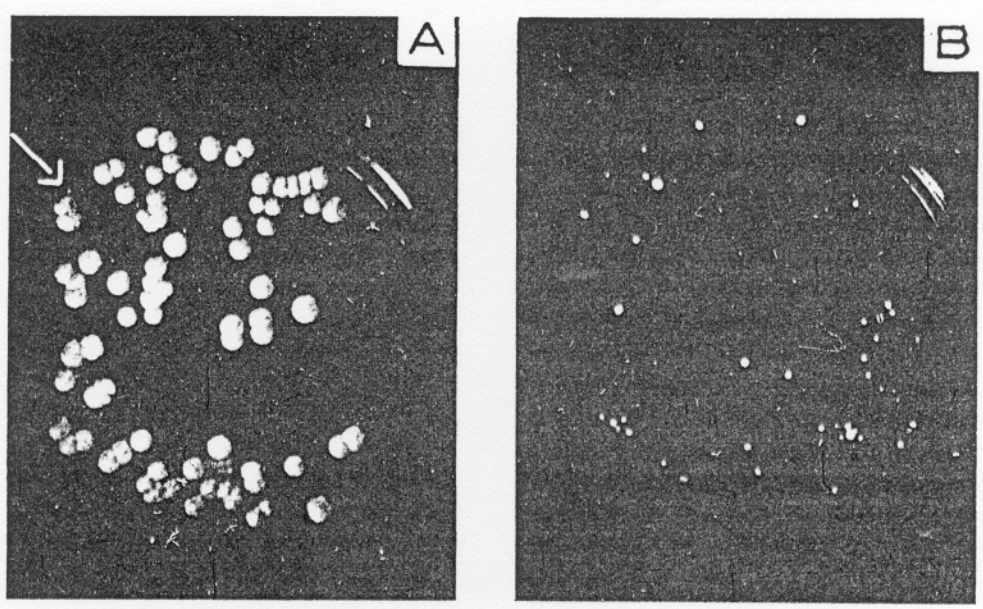

Fig. 1. Colonies formed by baker's yeast on a solid medium. (A) *Colonies of a normal yeast, showing one small colony.* (B) *Colonies formed by the same yeast grown prior to plating in the presence of acriflavine* [2].

position. It was evident that the loss of any known mitochondrial gene products (ribosomal RNAs, tRNAs, the sub-units of enzymes involved in respiration and oxidative phosphorylation) would have a pleiotropic effect and lead to a loss of respiratory functions.

The petite mutation is due to large deletions

Further work [9–12] showed that the AT spacers formed 50% of the wild-type mitochondrial genome, had a GC content lower than 5%, were indeed repetitive in nucleotide sequence and were, therefore, likely to be endowed with sequence homology over stretches long enough to allow illegitimate site-specific recombination. Five years ago, direct evidence was provided for both a deletion mechanism [13] and an accompanying amplification of the excised genome segment [13,14]: only a fraction of the restriction fragments of wild-type mitochondrial DNA were present in petite genomes and these were present in multiple copies per genome unit. These findings disposed of a number of strange *ad hoc* hypotheses put forward to explain the petite mutation [15–17] and led to the scheme shown in Fig. 2 in which the excised segment from the wild-type genome becomes the repeat unit of the petite genome. This may in turn undergo further deletions leading to secondary petite genomes having simpler repeat units. Incidentally, the analysis of restriction patterns of mitochondrial DNA from wild-type cells [13] provided the first unequivocal estimate of the size of the mitochondrial genome unit, about 50×10^6 [13].

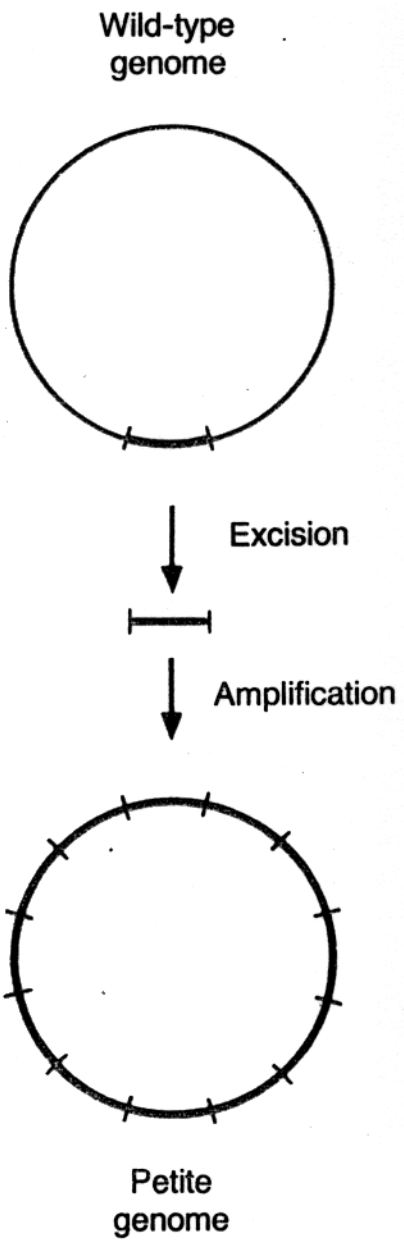

Fig. 2. Scheme of the process leading to the formation of spontaneous petite genomes. A segment of the mitochondrial genome unit from wild-type yeast cells is excised and amplified to yield the mitochondrial genome unit of a petite mutant. The excised segment from the wild-type genome becomes the repeat unit of the petite genome. This may in turn undergo further deletions leading to secondary petite genomes having simpler repeat units [25].

The GC clusters

Another important advance in our knowledge of the organization of the mitochondrial genome was the discovery of short segments of mitochondrial DNA extremely rich in GC, the GC clusters [18–20]. Operationally, two sorts of GC clusters can be distinguished, the (CCGG, GGCC) clusters, present in 60–70 copies per genome unit and recognizable because they are degraded by two particular restriction enzymes, Hpa II and Hae III, and the GC-rich clusters which do not contain CCGG or GGCC sequences, but are often close to (CCGG, GGCC) clusters and to isolated CCGG sequences. A certain number of these clusters were likely to be endowed with sequence symmetry and to be homologous in sequence. Very recent sequence analyses carried out in several laboratories (see below) indicates that the GC clusters are located in the middle of AT spacers and not, as first suggested [20], at one end of them so that an overall scheme of the organization of the mitochondrial genome of yeast is that given in Fig. 3.

The excision sites

The next step was the precise definition of the sequences involved in the excision process. The basic idea of the deletion model mentioned above was that the instability of the mitochondrial genome of yeast was due to the existence in each genome unit of a number of nucleotide sequences having enough homology to allow illegitimate, site-specific recombination to take place. Clearly the newly discovered GC clusters were at least as good candidates as the AT spacers.

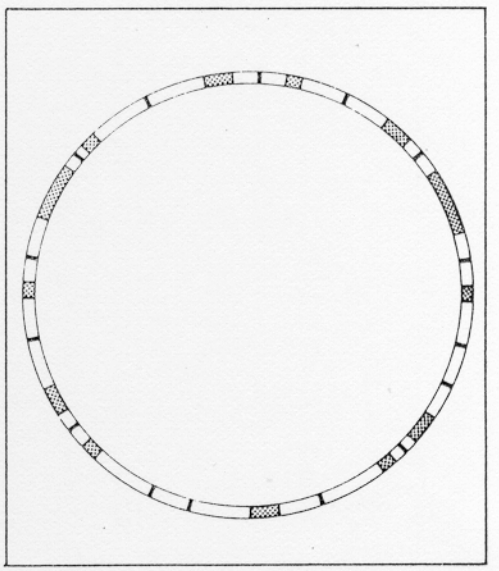

Fig. 3. A schematic representation of the organization of a mitochondrial genome unit of yeast. Grey stretches represent genes, white stretches AT spacers, black bars GC clusters.

Detailed investigations of a number of spontaneous petite mutants [21] showed that most frequently the ends of the repeat units were formed by (CCGG, GGCC) clusters; less frequently, they appeared to correspond to GC-rich clusters and to AT spacer sequences.

For example, in the petite genomes of Fig. 4, excision of the repeat units occurred at, or very near (CCGG, GGCC) clusters in the top four cases; and at GC-rich cluster or AT spacers in the other two. Furthermore, it was shown that repeat units were organized in a perfect tandem (head-to-tail) fashion. Interestingly, secondary excision of simpler repeat units from the petite genomes originally derived from the parental wild-type genome appears to take place at the same kind of sites used in the primary process, the end product being rather simple and stable petite genomes, such as those presented in Fig. 4. The spontaneous petite mutation should, therefore, be visualized as a cascade of excisions, which is slowed down or stopped only by the fact that sequences appropriate for excision are used up in the process.

Such a simple excision mechanism was not found in petites induced by ethidium bromide, practically the only ones studied in most other laboratories. These contained inverted repetitions, multiple deletions, sequence rearrangements and, above all, were caused by much less specific excisions [22]. This is not surprising if one considers that the tremendous increase in petite formation upon mutagenization is

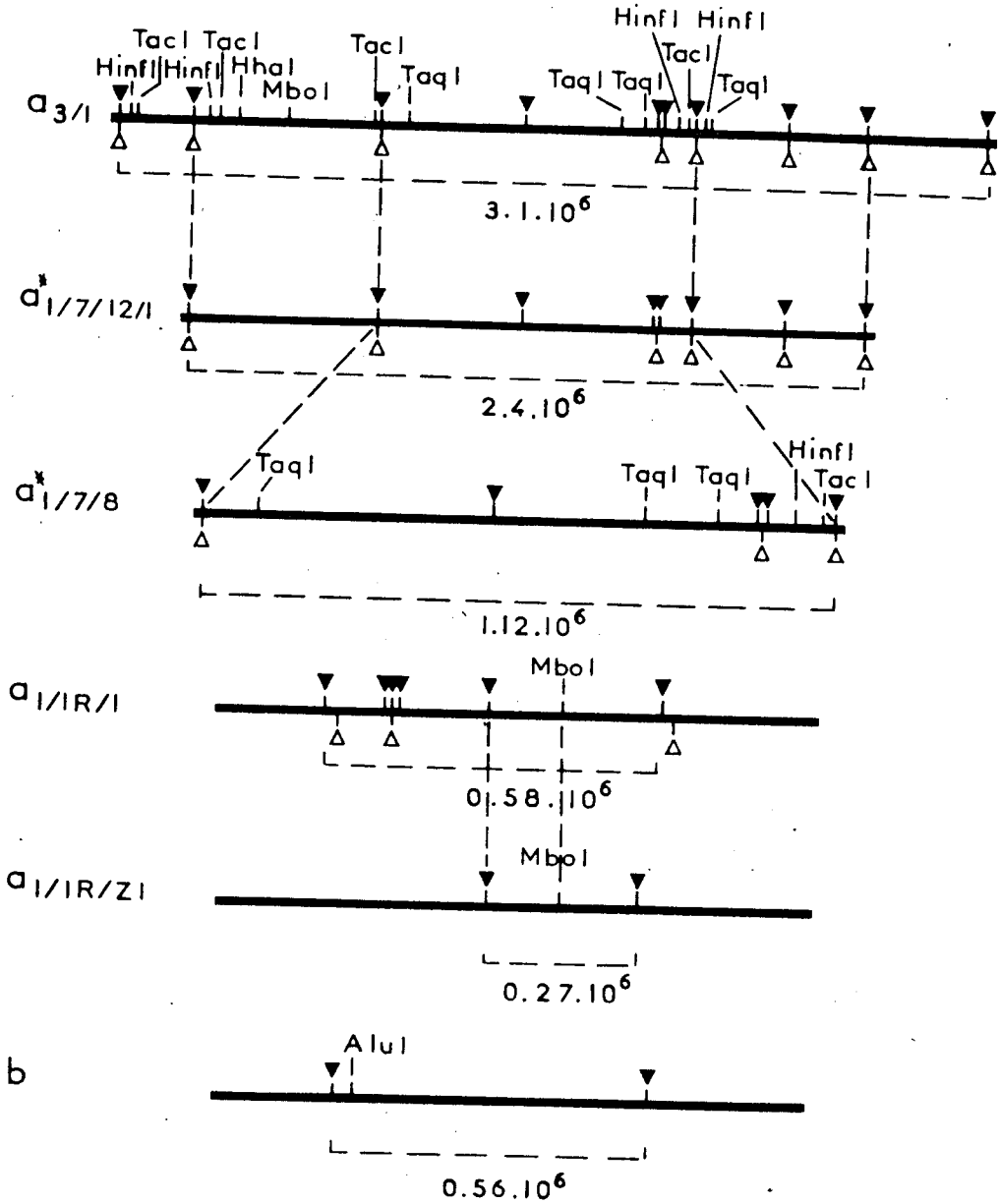

Fig. 4. Restriction enzyme maps of the repeating units of the mitochondrial genomes of several spontaneous petite mutants. The molecular weights of the repeat units are indicated, along with the positions of Hae III (△), Hpa II (▼) and other restriction sites. In the case of $^{a}_{3b/1}$, five isolated Hpa II sites and a Hinc II site are not shown. The broken lines indicate corresponding restriction sites in different repeat units [21].

accompanied by extensive genome fragmentation [23] and that petites lacking mitochondrial DNA altogether are frequently formed [23,24].

The excision mechanism

It is obvious that what is needed now is detailed knowledge, at the nucleotide level, of the sequences involved in the excision of petite genomes. Such work is in progress and hopefully will lead to a more precise understanding of the excision process. The sequence data already available strongly suggests that excision is due to a crossing-over process. If this is so, the primary event in the spontaneous petite mutation is very similar to the excision of the lambda prophage from the *E. coli* chromosome or to the dissociation of a transposon from its host plasmid; in this case, the GC clusters and sequences in the AT spacers play the same role as the insertion sequences delimiting a bacterial transposon, and should also play a role in what can be considered the reverse process, namely the recombination of petite genomes with wild-type genomes or other petite genomes.

The sequence of the repeat unit of a petite mutant

The recent determination of the nucleotide sequence of the repeat unit of the mitochondrial genome of the spontaneous petite mutant $^{a}_{1/1R/Z1}$ ([25]; Fig. 5) and preceding work in Tzagoloff's laboratory [26,27] provided complementary confirmations of several previous results and predictions: that AT spacers are made up of short alternating and non-alternating AT sequences [7,11] and contain repeated sequences and palindromes [12]; that GC-rich clusters are largely contiguous to CCGG sequences and to (CCGG, GGCC) clusters [20]; and that the latter are to some extent endowed with both symmetry and homology [20]. The genome of $^{a}_{1/1R/Z1}$ is of interest in three other respects: (a) it does not contain any gene, and is therefore a clear example of the lethality of petite mutations as far as mitochondria are concerned; (b) it replicates; in fact this is the only function left; since this genome was shown to be made up of a perfect tandem repetition of the basic unit [21], the latter must contain the signal for the initiation of replication; (c) it is excised, in all likelihood, from another petite genome, $^{a}_{1/1R/1}$ (Fig. 4) and not directly from the wild-type genome; the complete sequence of the parental petite genome $^{a}_{1/1R/1}$ should, therefore, provide precise information as to the excision sites involved in the formation of $^{a}_{1/1R/Z1}$.

Some general issues

From the brief account just presented, two general points are clear. First, the organization of the mitochondrial genome of yeast is typically eukaryotic, in that coding sequences are interspersed with non-coding sequences, the AT spacers and the GC clusters; the existence of split genes in this genome (see [28] for a review) points in the same direction. Second, the non-coding sequences of the mitochondrial genome of yeast are similar to the interspersed repeated sequences and the fold-back sequences of the nuclear genome of eukaryotes, in that identical or similar sequences are present in many copies in the genome and that several of these sequences exhibit symmetry.

It is evident that regulatory sequences acting as promotors, operators, sites for the initiation of replication, and sites involved in the processing of transcripts are present in the non-coding sequences of yeast mitochondrial DNA; none of these have so far been identified, although several suggestions concerning the GC clusters have been put forward [20]. Another function of the non-coding sequences is already well documented and has to do with illegitimate, site-specific recombination. The excision of the spontaneous petite genomes just described is an example of these extragenic recombinational events. The same basic mechanism appears, however, to be more general and to account for: (a) the divergence of the mitochondrial genome of wild-type yeast cells; it has been shown [19] that different strains have mitochondrial genomes differing in the

length of AT spacers, apparently the result of unequal crossing-overs in the sequences of allelic spacers; (b) similar changes in the mitochondrial genomes of the progeny arising from crosses of different wild-type strains [29]; an important point here is that the underlying extragenic unequal crossing-overs seem to be much more frequent than intragenic exchanges.

The organization of the mitochondrial genome of yeast, as it has emerged from our work, is of interest in three additional respects: first, it points to the fact that in eukaryotic genetic systems, where so much of the DNA is non-coding, there is a real need for a molecular approach because approaches based on classical genetics or on the study of gene products suffer from serious intrinsic limitations and are unable to provide an overall picture of the genome; secondly, it disposes of a series of ideas centered about a prokaryotic organization of the mitochondrial genome of yeast; these ideas, which were promoted for many years, can be summarized in the following statements: (1) that the mitochondrial genome of yeast has a unique nucleotide sequence, namely a sequence lacking internal repetition; this view had its origin in a misunderstanding of the renaturation kinetics of mitochondrial DNA; (2) that the mitochondrial genome of yeast has an informational content five times larger than that of animal mitochondrial genomes (which have a unit size of only 10^7); this idea should have been considered with suspicion because there are practically no known gene products encoded in the yeast system and not also in the animal system; (3) that the unit size of the mitochondrial genome decreases in the evolution from unicellular organisms to animals; recent results have shown that some protists have indeed a mitochondrial genome as small as animals; (4) that mitochondria are the remnants of prokaryotic endosymbionts; although this view is almost of a philosophical nature, arguments against it abound (see [30]); finally it suggests that the mitochondrial genome may be a useful, simple model for its big brother in the nucleus.

Conclusions and perspectives

It is a matter of satisfaction to see that the problem of the petite mutation in yeast is now essentially solved at the molecular level and that predictions made ten years ago have proven to be correct. Sequence work currently under way should provide additional details within a short time. It is clear that understanding the molecular mechanism of the petite mutation, has come at least as much from a knowledge of the organization of the mitochondrial genome of wild-type yeast cells as from investigations on petite genomes. The scene is now set to understand the other phenomenon discovered by Ephrussi, suppressivity. The petite mutation and suppressivity seem to be the two faces of the same coin, the former basically consisting of the excision of a petite genome segment from the wild-type genome, the latter having to do with the insertion of a petite genome segment into the wild-type genome. In retrospect, it is evident Ephrussi's work not only opened the field of extrachromosomal genetics, but also provided a fantastic stimulus for the investigations which followed up to this day.

Concerning the future of mitochondrial genetics of yeast, there is ample ground for optimism for three main reasons. The first is that the striking advances of the molecular approach have been accompanied by a rapid progress in the genetic approach following the discovery of mitochondrial recombination of antibiotic resistance markers by Thomas and Wilkie [31] and the discovery of mutants of mitochondrial genes that code for polypeptides by Tzagoloff *et al.* [32]. The second reason for

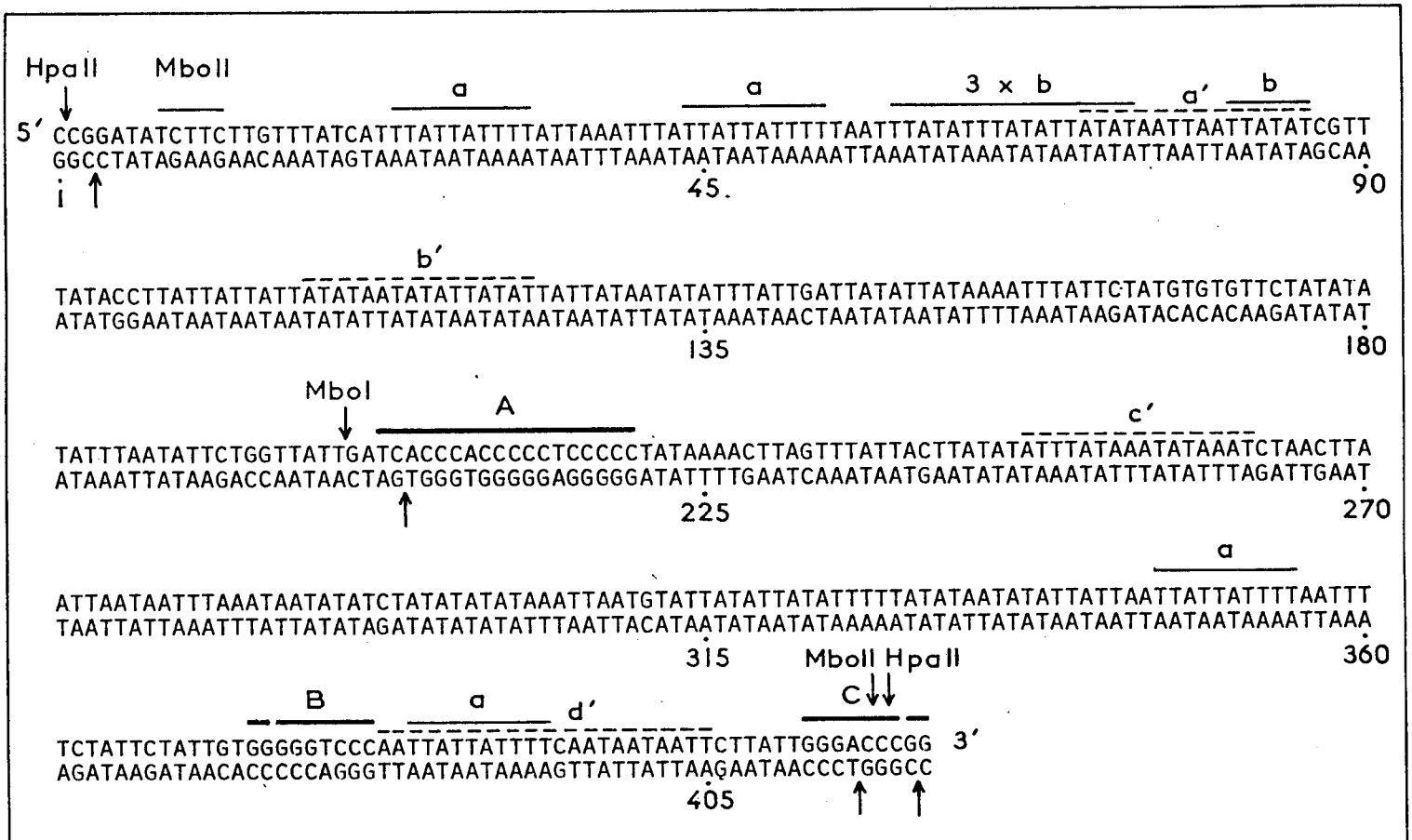

Fig. 5. Nucleotide sequence of the repeat unit of the mitochondrial genome of spontaneous petite mutant ^{a}1/1R/Z1 *(see Fig. 4).* A, B *and* C *indicate GC-rich clusters;* a *and* b, *a repeated decanucleotide and a repeated hexanucleotide, respectively;* a', b', c' *and* d', *palindromic sequences in the AT stretches. The restriction sites of Hpa II, Mbo I and Mbo II are indicated by arrows; the recognition site of the latter enzyme is also indicated* [25].

optimism is that after much tedious but necessary work, detailed physical and genetical maps of the mitochondrial genome have been produced. The third is that our knowledge of the transcription products has progressed very considerably. Under these circumstances, it is not impossible that the mitochondrial genome of yeast will be the first eukaryotic genome to be satisfactorily understood in terms of both structure and function, and this should pave the way to understand the evolution of organelle genomes and the relationships between the latter and the nuclear genomes.

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