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WITH AN APPENDIX

Construction of the Physical Maps of Petite Genomes

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The Mitochondrial Genome of Wild-type Yeast Cells VIII.† The Spontaneous Cytoplasmic "Petite" Mutation‡

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The mitochondrial genomes of a number of spontaneous "petite" mutants of *Saccharomyces cerevisiae* were investigated by restriction enzyme analysis and by hybridization with restriction fragments from parental wild-type genomes. The nucleotide sequences forming the ends of the repeat units of the petite genomes were shown to be formed by GC clusters and, possibly, by AT spacers. These non-coding elements are characterized by the fact that they consist of, or contain, sequences which are repeated a number of times in the parental, wild-type genome and which are often symmetrical. The excision process leading to the formation of the spontaneous petite genomes appears to involve site-specific, illegitimate recombination events which take advantage of localized sequence homology, in agreement with a deletion model previously proposed. The same kind of excision process appears to be operative in the further deletions undergone by the mitochondrial genomes of spontaneous petite mutants. The genome organization and the excision mechanism appear to be largely different in spontaneous and ethidium-induced petite mutants.

1. Introduction

Several years ago it was shown (Bernardi *et al.*, 1968; Mehrotra & Mahler, 1968) that the mitochondrial DNAs from two genetically unrelated, acriflavine-induced, cytoplasmic "petite" mutants of *Saccharomyces cerevisiae* had a grossly altered base composition (G + C = 4%)‖, compared to the DNAs from the parent wild-type¶ cells (G + C = 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.

Subsequent investigations (Bernardi *et al.*, 1970; Bernardi & Timasheff, 1970) showed that the mitochondrial DNA from wild-type yeast cells was extremely heterogeneous in base composition, about half of it melting at a very low temperature and

† Paper VII in this series is Fonty *et al.* (1978).

‡ This paper is dedicated to the memory of Professor Boris Ephrussi who founded extrachromosomal genetics with his pioneering studies on the petite mutation (Ephrussi, 1949).

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‖ A, T, G and C indicate deoxyadenosine, thymidine, deoxyguanosine and deoxycytidine, respectively. G + C is used to indicate the molar fraction of deoxyguanosine and deoxycytidine in DNA.

¶ The term wild-type refers here, as in previous publications from this laboratory, to the mitochondrial genome.

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being almost exclusively formed by long alternating and non-alternating AT stretches (which were later called AT spacers by Prunell & Bernardi, 1974), and the rest melting over an extremely broad temperature range. Compared to mitochondrial DNA from wild-type cells, DNAs from three different spontaneous suppressive† petite mutants were shown (Bernardi *et al.*, 1970) (1) to have lower G + C levels, (2) to lack a number of high-melting components, and (3) to renature very rapidly. These results were interpreted‡ as indicating that petite mutants had defective mitochondrial genomes, in which large segments of the parental wild-type genomes were deleted; it was suggested that such deletions arose by a mechanism of the Campbell (1962) type, involving illegitimate, site-specific recombination events in the AT spacers which were supposed to be internally repetitive in sequence.

Further work showed that these spacers formed 50% of the mitochondrial genome, had a G + C content lower than 5%, were internally repetitive in nucleotide sequences, and likely to be endowed with sequence homology over stretches long enough to allow site-specific recombination (Bernardi *et al.*, 1972; Piperno *et al.*, 1972; Ehrlich *et al.*, 1972; Prunell & Bernardi, 1974). Five years ago, direct evidence was provided for both the deletion mechanism (Bernardi *et al.*, 1975), and the accompanying amplification of the excised genome segment (Locker *et al.*, 1974; Bernardi *et al.*, 1975). Indeed, only a fraction of the restriction fragments of wild-type cell mitochondrial DNA were still found in the petites and these were present in multiple copies per genome unit. These findings disposed of a number of hypotheses put forward to explain the petite mutation (Slonimski, 1968; Carnevali *et al.*, 1969; Borst & Kroon, 1969).

In 1976 short mitochondrial DNA segments extremely rich in G + C were discovered in the mitochondrial genome of wild-type cells, the GC clusters (Bernardi, 1976*a*,*b*; Prunell, *et al.*, 1977*a*; Prunell & Bernardi, 1977). Operationally, two sorts of GC clusters were distinguished, the (C-C-G-G, G-G-C-C) clusters, present in 60 to 70 copies per genome unit and recognizable because they were degraded by two particular restriction enzymes (*Hpa*II§ and *Hae*III) and the G + C-rich clusters, that do not contain C-C-G-G or G-G-C-C sequences, but are often close to (C-C-G-G, G-G-C-C) clusters and to isolated C-C-G-G sequences. A certain number of these clusters were likely to be endowed with sequence symmetry and to be homologous in sequence; this raised the possibility that the excision events leading to the petite genomes could take place not only in the AT spacers but also in GC clusters. Obviously these two localizations of excision sites were not mutually exclusive. In any case, a basic idea of the deletion model was that the instability of the mitochondrial genome of yeast (the spontaneous petite mutation has a rate of 1 to 5% per generation in most laboratory strains) 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.

† Suppressive and neutral petite mutants are distinguished on the basis of the outcome of their crosses with wild-type cells; in the first case, the progeny contains a certain percentage of petite colonies, in the second case it is only formed by wild-type cells (Ephrussi, 1953). Later work showed that neutral petite mutants may lack mitochondrial DNA altogether (Goldring *et al.*, 1970; Nagley & Linnane, 1970).

‡ This model was first presented in a lecture at the Karolinska Institute, Stockholm, in October, 1969. It was subsequently presented at a number of meetings and mentioned by Piperno *et al.* (1972), and by Prunell & Bernardi (1974).

§ Restriction enzymes are indicated according to Smith & Nathans (1973); *Hpa*II and *Hae*III will be indicated henceforth as *Hpa* and *Hae*, respectively.

In the present work, we have investigated the molecular mechanism of the spontaneous petite mutation, and have verified the correctness of the deletion model mentioned above, mainly by studying the nucleotide sequences delimiting the repeat units of such genomes. The spontaneous petite mutants used were either heterogeneous or homogeneous in terms of mitochondrial genome. The latter contained stable, low-complexity genomes and were derived from the former by subcloning. The experimental approach and the results obtained can be briefly outlined as follows.

(1) Since preliminary work (Bernardi *et al.*, 1975,1976) had suggested that the sequences involved in the excision process might frequently correspond to C-C-G-G, G-G-C-C sequences and/or to clusters of these sequences, this point was studied by investigating the restriction patterns of mitochondrial DNAs from heterogeneous petite mutants as produced (a) by *Hpa*II or *Hae*III, and (b) by other restriction enzymes. As shown in Figure 1, if excision takes place at, or very close to, C-C-G-G, G-G-C-C sequences, and/or clusters of these sequences, *Hae* and/or *Hpa* digests of petite mitochondrial DNA will only show bands also present in the patterns of parental, wild-type genomes; if excision takes place elsewhere in the genome, petite mitochondrial DNA will originate new *Hae* or *Hpa* bands, namely bands that are not present in the restriction pattern of the parental wild-type genome and which correspond to junction fragments bridging subsequent repeat units. In either case, restriction patterns produced by enzymes other than *Hae* or *Hpa* should show, as a rule, new bands (Fig. 1). The results obtained confirmed our preliminary work, in that new

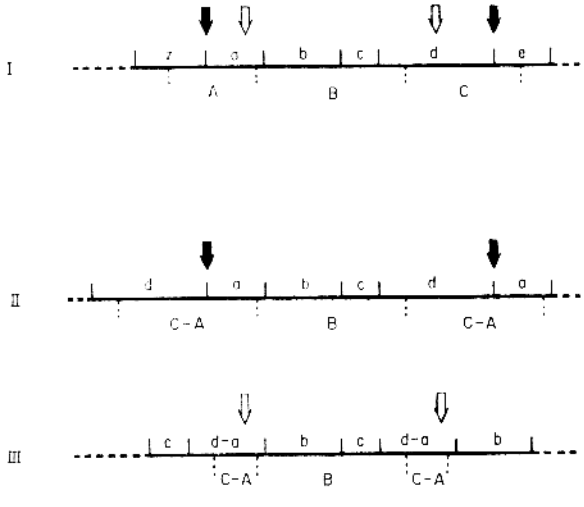


FIG. 1. Restriction fragments of mitochondrial DNAs from spontaneous petite mutants, as expected on the basis of the localization of the original excision event. The upper line (I) represents a segment of the mitochondrial genome unit of the parental wild-type yeast cells; lower case letters indicate fragments delimited by *Hae* sites (G-G-C-C), *Hpa* sites (C-C-G-G) or *Hae–Hpa* clusters; capital letters indicate fragments delimited by restriction sites other than *Hae* or *Hpa*. If excision takes place (solid arrows) at, or very near to, the first kind of sites, the *Hae* and/or *Hpa* fragments from the petite DNA will only be of parental type (II). In contrast, the patterns produced by a different restriction enzyme will show a new band, not present in the corresponding parental pattern, and representing the junction fragment connecting neighboring sites of subsequent repeat units (II; C–A). If excision takes place (open arrows) outside the first kind of sites, all restriction patterns, including those produced by *Hae* and *Hpa*, will show a new band containing the excision sites (III; d–a, C–A). Arrows delimit the repeat units of the petite genomes.

bands were found in the latter digests, but were either absent or strikingly rare in *Hae* or *Hpa* digests.

(2) Several homogeneous petite mutants were isolated from the heterogeneous petite mutants just mentioned. The repeat units of the corresponding mitochondrial DNAs were formed by one or more *Hpa* and/or *Hae* fragments; no new fragments were present; partial digests showed oligomeric fragments corresponding to exact multiples of the repeat units; hybridization of most of these DNAs on restriction fragments from the parental, wild-type genomes revealed that this mainly took place on *Hae* and/or *Hpa* fragments identical in size to those found in the petite DNAs, thus indicating that the nucleotide sequences present at, or very close to, the ends of the DNA segment originally excised corresponded to C-C-G-G and/or G-G-C-C sequences. More rarely, hybridization did not take place on *Hae* and/or *Hpa* fragments identical in size to those found in the petite DNA, and the ends of the repeat unit corresponded to other sequences possibly located in G + C-rich clusters or in AT spacers.

(3) A comparison of restriction patterns of mitochondrial genomes from recently arisen and old petite mutants revealed differences of interest for understanding the evolution of petite mutants and pointed to the fact that the secondary deletions occurring in petite genomes involved excision sites of the same kind as those used in the original excisions from the wild-type genomes.

(4) Finally, this work indicated that the genome organization and the excision mechanism are much simpler for the spontaneous petites investigated here than for the ethidium-induced petites, that are practically the only ones studied so far in other laboratories.

Preliminary reports on the present work were presented at several meetings during the past four years (Bernardi, 1975,1976*a*,*b*; Bernardi *et al.*, 1975,1976,1978).

2. Materials and Methods

(a) *Yeast strains*

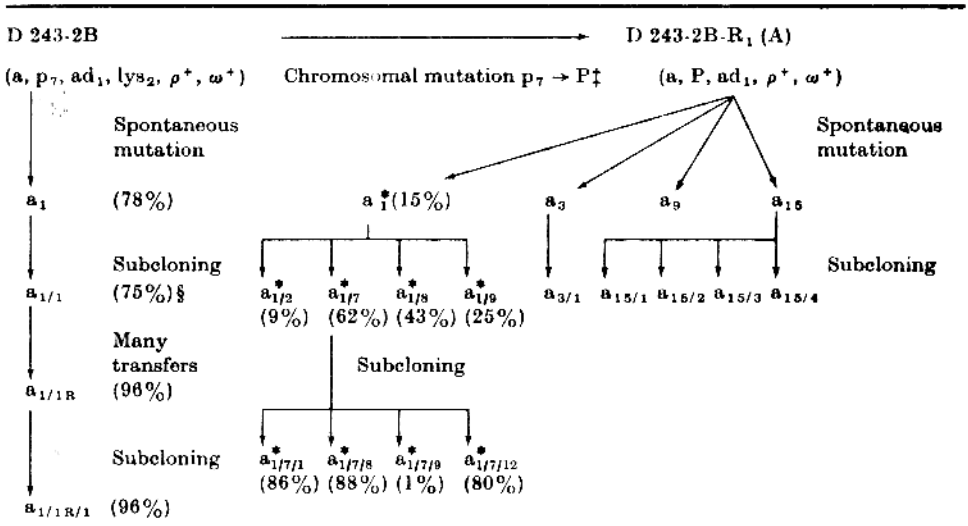
The *S. cerevisiae* strains used in this work were respiratory-deficient, cytoplasmic petite mutants. The genetic relationships among the yeast strains used and their suppressivities are indicated in Table 1. One such strain, a_1 (D 243-2B-106) was a spontaneous mutant of the wild-type strain D 243-2B (Bernardi *et al.*, 1970). Four other strains, a_1^* (D 243-2B-R_1 (A1)), a_3, a_9 and a_{15}, were spontaneous mutants of a chromosomal mutant of strain D 243-2B indicated as D 243-2B-R_1, or A (Bernardi *et al.*, 1970). Several subclones of these petite strains were also used. Two other petite mutants used here were strains b (C 982-19dp 6/6-M15-1B-2/16) and d (DM_1). Strain b (Bernardi *et al.*, 1970) was a spontaneous mutant of wild-type strain C 982-19d (also called B; Bernardi *et al.*, 1970; Prunell *et al.*, 1977*a*). Strain d (Bernardi *et al.*, 1968) was obtained by acriflavine treatment of wild-type strain D (DM; Bernardi *et al.*, 1968; Prunell *et al.*, 1977*a*). A *S. carlsbergensis* wild-type strain, (NCY 74 S, also called C; Prunell *et al.*, 1977*a*), and another *S. cerevisiae* strain (KL 14-4A; Wolf *et al.*, 1973) were used in some experiments. Strains A, B and KL-14-4A were haploid; strains C and D were diploid.

Strains a_3, a_9, a_{15} and their subclones were used for DNA preparation just after they arose, whereas strains a_1, a_1^* (but not their subclones) and b had been stored and replated for several years prior to DNA preparation (at least 15 years for a_1 and b).

Culture conditions for the petite strains derived from D 243-2B, A and B were those described by Bernardi *et al.* (1970); those for strain d were given by Bernardi *et al.* (1968). In all cases, cells were harvested in the late exponential phase.

TABLE 1

Genetic relationships among the yeast strains used†



† Values in parentheses indicate the suppressivity.

‡ The $p_7 \rightarrow P$ reversion may be not a back mutation *sensu stricto*, but a suppressor mutation (Mounolou, 1967).

§ The suppressivity of this strain was determined by Faurès-Renot *et al.* (1974).

‖ $a_{1/1R/Z1}$ was the result of a cross of this strain with B.

(b) *Preparation of mitochondrial DNA*

Four methods were used to prepare DNA from petite strains. Method A involved extraction and purification according to Bernardi *et al.* (1972), except that further purification of the mitochondrial DNA fractions that were first eluted from hydroxyapatite, and were therefore contaminated with nuclear DNA, was done by centrifugation in preparative Cs_2SO_4/Ag^+ density gradients, at pH 9·2 and a Ag^+/nucleotide molar ratio of 0·3 (Bernardi *et al.*, 1972; Filipski *et al.*, 1973). Method A was used for the preparation of mitochondrial DNAs from strains a_1, b and d; DNAs were obtained in quantitative yield, represented 7%, 3% and 13% of total DNAs, respectively, and had *s* values comprised between 13 S and 14 S. In the corresponding wild-type strains A, B and D, mitochondrial DNA represented 12%, 12% and 13% of total DNA, respectively. Method B (Prunell *et al.*, 1977*a*) was used to prepare DNAs from protoplasts of strain a^*_1, $a_{1/1}$ and its subclones, and strain b; in this case, DNAs were obtained in lower yields (60 to 70%) but had *s* values between 18 S and 22 S. Method C (Fonty *et al.*, 1978) was used to prepare DNAs from strain a^*_1 and its subclones; this method yielded DNAs having *s* values close to 28 S, but some nuclear DNA contamination was present, and yields were much lower than in method A (10 to 20%). Method D (modified from Lang *et al.*, 1977) was used to prepare DNAs from the subclones of $a^*_{1/7}$, from strains a_3, a_9, a_{15} and the subclones of a_{15}; yields were around 30%, *s* values were higher than 30 S; nuclear DNA contamination was less than 5%.

DNA preparations from wild-type strains were those previously described (Prunell *et al.*, 1977*a*; Fonty *et al.*, 1978). Method D was used for preparing the DNA from strain KL 14-4A.

(c) *Restriction enzyme degradations and gel electrophoresis*

Restriction enzymes were purchased from New England Biolabs (Beverly, Mass.) and Bethesda Research Laboratories (Bethesda, Md). Some of them (*Eco*RI, *Hind*II + III, *Hae*III, *Hpa*II), were also prepared as described previously (Prunell *et al.*, 1977*a*). Restriction endonuclease digestions, gel electrophoresis and analysis of restriction patterns were performed as described by Prunell *et al.* (1977*a*). As in previous work, all polyacrylamide gels, except the 6% ones, also contained 0·5% agarose. Attempts were made at quantitating the amount of DNA per band in the gel electrophoresis experiments, as previously done by Prunell *et al.* (1977*a*), for DNA digests from wild-type cells. The wide range of band intensities however, made impossible the general use of the photographic method of Prunell *et al.* (1977*b*). For this reason, electrophoretic bands from petite DNAs were simply classified as faint, weak or strong, according to their intensities as compared to those of reference bands from wild-type cell DNAs. Such reference bands were chosen among single bands having molecular weights close to those of petite DNA bands, the loads of wild-type and petite DNAs being the same.

(d) *Hybridization of petite and wild-type genomes*

Restriction enzyme digests of mitochondrial DNA from wild-type yeast strains A, B, C and D (Prunell *et al.*, 1977*a*) were transferred from agarose gels onto nitrocellulose strips (Schleicher and Schull, Dassel, W. Germany; Millipore, Molsheim, France), according to Southern (1975), except that the denaturing transfer solvent was 3 M-NaCl, pH 11·4 (Melli *et al.*, 1975).

Mitochondrial DNAs from petite strains were labeled by nick-translation using ^{32}P-labeled deoxyribonucleoside triphosphates from the Radiochemical Centre (Amersham, U.K.) or New England Nuclear (Dreieich, W. Germany) and DNA polymerase from Boehringer (Mannheim, W. Germany), according to Jeffreys & Flavell (1977). The labeled DNA preparations so obtained had specific activities around 10^7 cts/min per μg.

Hybridization of labeled petite DNA with unlabeled restriction fragments of wild-type DNA was done in 6 × SSC (standard saline citrate, 0·15 M-NaCl, 0·015 M-sodium citrate, pH 7·2), according to the method of Jeffreys & Flavell (1977). Washing of the nitrocellulose filters was done with the following series of solvents: 6 × SSC, 1 × SSC and 0·5 × SSC. Autoradiography was done at room temperature, for 24 h using Neo-Standard ND54 films (Kodak, Rochester).

3. Results

(a) *Restriction enzyme degradation*

In this section, results on the DNAs from heterogeneous petite strains will be presented before those on the DNAs from the homogeneous petite strains derived from them. The reader is referred to Table 1 for the genetic relationships among the strains used and to Figure 1 and its legend for the significance of new restriction bands.

(i) *Mitochondrial DNAs from strains a_3, a_9, a_{15} and their subclones*

(1) The *Hpa* and *Hae* patterns were characterized (Figs 2 and 3; Table 2) by the following features: (a) a number of parental bands were missing; this corresponded to the loss of 28% to 94% of the wild-type genome in various petites; (b) all the *Hpa* bands of petite DNAs were of parental type, and only three *Hae* bands were new: a_{15} and its subclone $a_{15/3}$ showed two such bands†, $5{\cdot}12 \times 10^5$ and $4{\cdot}95 \times 10^5$; subclones $a_{15/1}$ and $a_{15/2}$ (which had identical restriction patterns) one, $4{\cdot}95 \times 10^5$; subclone

† Fragments will be designated henceforth by their molecular weights.

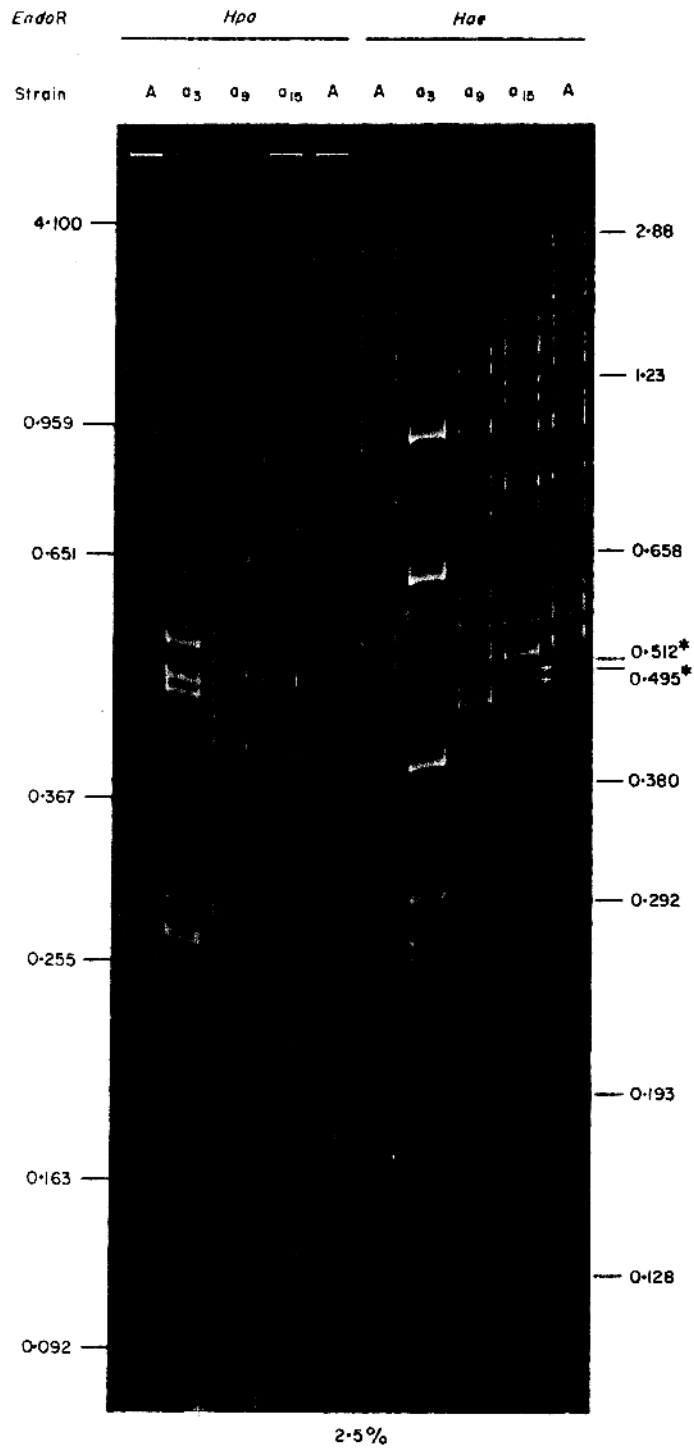


FIG. 2. Electrophoretic patterns of *Hpa* and *Hae* digests on 2·5% polyacrylamide gel of mitochrondrial DNAs from petite mutants a_3, a_9, a_{15} and their parental wild-type strain A. Molecular weights ($\times 10^{-6}$) are indicated for some restriction fragments. Asterisks indicate new bands. The faint bands of the DNA from a_3 and the low molecular weight bands of the DNA from strain A are not visible in this picture.

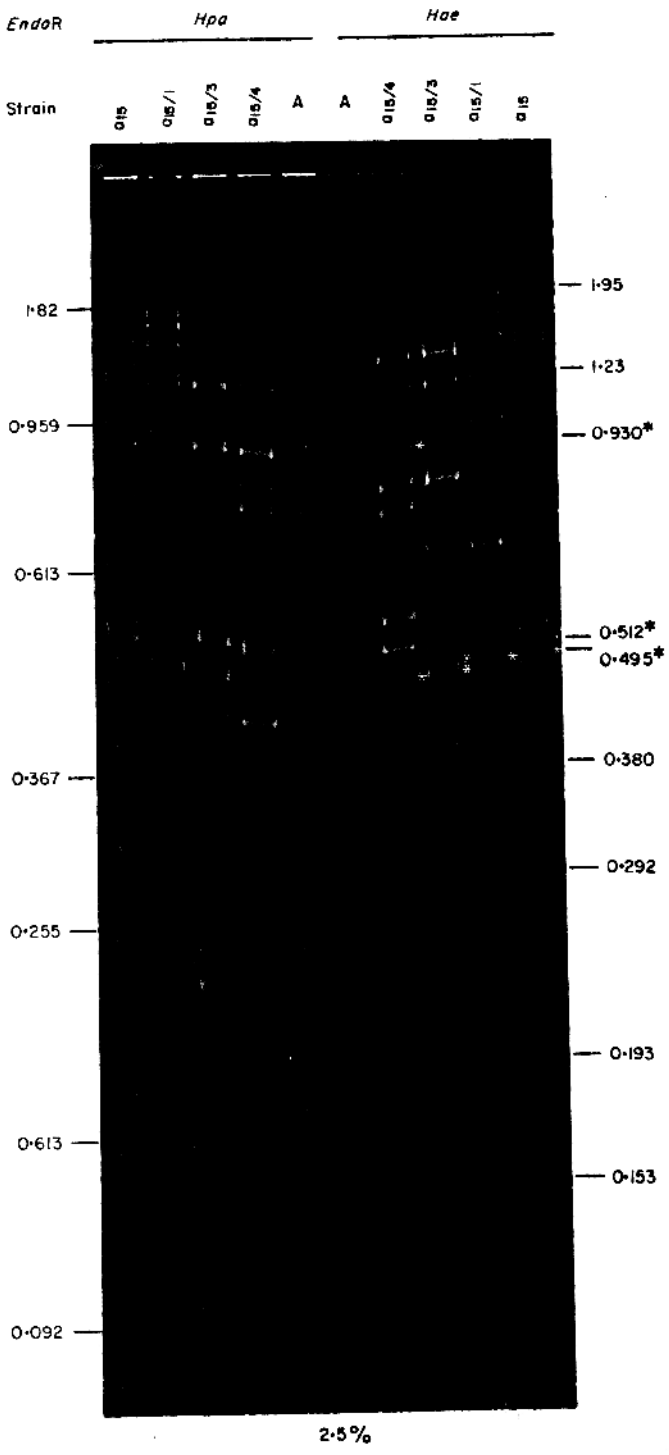


FIG. 3. Electrophoretic patterns of *Hpa* and *Hae* digests on 2·5% polyacrylamide gel of mitochondrial DNAs from petite mutant a_{15}, its subclones $a_{15/1}$, $a_{15/3}$, $a_{15/4}$ and the parental wild-type strain A. Indications as for Fig. 2.

TABLE 2

Results from the restriction patterns obtained with the mitochondrial DNAs of strains $a_{3/1}$, a_9, a_{15}, $a_{15/1}$, $a_{15/3}$ and $a_{15/4}$†

Strain	A		$a_{3/1}$		a_9		a_{15}		$a_{15/1}$		$a_{15/3}$		$a_{15/4}$	
Restriction enzyme	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*
No. of bands‡	75	85	7	13	60	72	45	56	31	39	20	26	16	24
No. of deleted bands	—	—	68	72	15	13	32	29	45	46	57	59	61	61
No. of deleted fragments	—	—	77	93	18	19	36	40	52	62	64	76	69	79
DNA in deleted fragments§ ‖	—	—	49·9	50·3	15	16·0	24·5	24·7	34·1	32·4	40·8	39·7	44·8	42·8
(as % of wild-type genome)	—	—	94·1	93·4	28·2	30·0	46·2	46·3	64·3	60·8	77·0	74·5	84·5	80·3
No. of new bands	—	—	—	—	—	—	2	—	1	—	2	—	2	—
DNA in new bands§	—	—	—	—	—	—	1·0	—	0·5	—	1·0	—	1·4	—
Genome complexity §¶	53·0	53·3	3·1	3·0	38·0	37·3	28·5	28·6	18·9	20·9	12·2	13·6	8·2	10·5

† Patterns obtained on 2·5% and 6% polyacrylamide gels were used. Subclone $a_{15/2}$ showed restriction patterns identical to those of subclone $a_{15/1}$. See the text for strain $a_{3/1}$.

‡ The DNA from strain A showed 84 *Hae* fragments and 108 *Hpa* fragments.

§ Molecular weight $\times 10^{-6}$.

‖ These values correspond to the sum of molecular weights of fragments present in the DNA digest from strain A, which were missing in the DNA from the petite mutants. In the case of homogeneous petite genomes, account was taken of the disappearance of fragments in multiple bands of strain A.

¶ See footnote to p. 502.

$a_{15/4}$ two, $4{\cdot}95\times10^5$ and $9{\cdot}30\times10^5$; except for the latter, the new bands shown by the a_{15} subclones were those already present in a_{15}; (c) the bands were non-stoichiometric.

(2) The *Alu*I, *Mbo*I, *Hha*I and *Hinc*II digests (Figs 4 to 6; Table 3), were characterized by: (a) a smaller number of bands, compared to the DNA from the parental strain; in the case of *Alu*I, only the DNAs from strains $a_{15/1}$ and $a_{15/2}$ were completely digested; (b) the presence of at least one new band per digest, with the only apparent exception of the *Hha*I digest of $a_{15/1}$; it should be noted that the material resistant to *Alu*I corresponds to genomes lacking these sites and was, therefore, equivalent to new bands; (c) genome complexity† estimates (Table 3) close to, or higher than, those obtained from *Hae* and *Hpa* digests, except in the case of *Alu*I, where they were lower because only a fraction of the genome was digested; (d) the non-stoichiometry of the bands.

(3) In the case of a_3, a largely predominant, apparently homogeneous genome was accompanied by a small number of others, the former giving rise to a series of stoichiometric bands, the latter originating faint bands. As expected, several subclones from a_3 showed only the stoichiometric *Hae* and *Hpa* bands corresponding to the majority genome. One of these, $a_{3/1}$, was used for further work. In this case, genome complexity estimates were identical for all digests (Table 3) and equal to about 3×10^6. *Mbo*I (Fig. 4), *Hinc*II and *Hha*I (Fig. 6) had a single site each on this genome, and released a single new band; *Alu*I showed no site. Figure 7 shows the restriction patterns of the DNA from $a_{3/1}$ as obtained with several enzymes. Single, double, triple and partial digests allowed a restriction map to be constructed (Fig. 16). The buoyant density in CsCl of this DNA was found to be equal to 1·683 g/cm^3, which also is the density of mitochondrial DNA from wild-type cells.

(ii) *Mitochondrial DNA from strain a_1^* and its subclones*

(1) The *Hpa* and *Hae* patterns (Figs 8 and 9; Table 4) of the mitochondrial DNA of strain a_1^* were characterized by the features just described for a_3, a_9, a_{15}, namely: (a) the absence of a number of parental, wild-type fragments, (b) the decrease in genome complexity, (c) the striking scarcity of new bands (only one in both *Hpa* and *Hae* digests) compared to the *Hind*III + III digest (see below), and (d) the non-stoichiometry of the bands.

Out of four subclones (Fig. 9, Table 4), three exhibited new bands: $a_{1/2}^*$ showed the same new bands exhibited by a_1^*; $a_{1/8}^*$ showed one new *Hpa* band, and three new *Hae* bands; and $a_{1/7}^*$ showed one new *Hpa* band, and one new *Hae* band. Other (faint) bands of $a_{1/8}^*$ and $a_{1/9}^*$ were new relative to a_1^*, but not to A.

† The complexity of the mitochondrial genome of petite mutants was estimated by subtracting the sum of the molecular weight of the wild-type DNA fragments that were absent in a given petite DNA from the genome unit size, namely, the sum of the molecular weight of the wild-type DNA fragments. It should be pointed out that the genome complexity, estimated as just indicated: (1) assumes that the mitochondrial genome of the parental wild-type strain is made up of a unique DNA sequence; forgetting about internal repetitions in the spacers (Ehrlich *et al.*, 1972), in the clusters of *Hae* and *Hpa* restriction sites (Prunell & Bernardi, 1977), and in the G+C-rich clusters (present work) this is likely to be the real situation (Prunell *et al.*, 1977*a*; see Discussion); (2) ignores the fact that a petite genome may be heterogeneous; if such is the case, the complexity concerns all genomes present in the genome population, at least in so far as each one of them is abundant enough as to show up its own specific bands in the gel pattern; (3) neglects the sequence amplifications characteristic of the petite genomes; (4) takes into account the DNA present in new bands, in the case of homogeneous genomes; in the case of heterogeneous genome populations, new bands should theoretically be taken into account only as far as the corresponding sequences are not already represented in other bands (see Discussion).

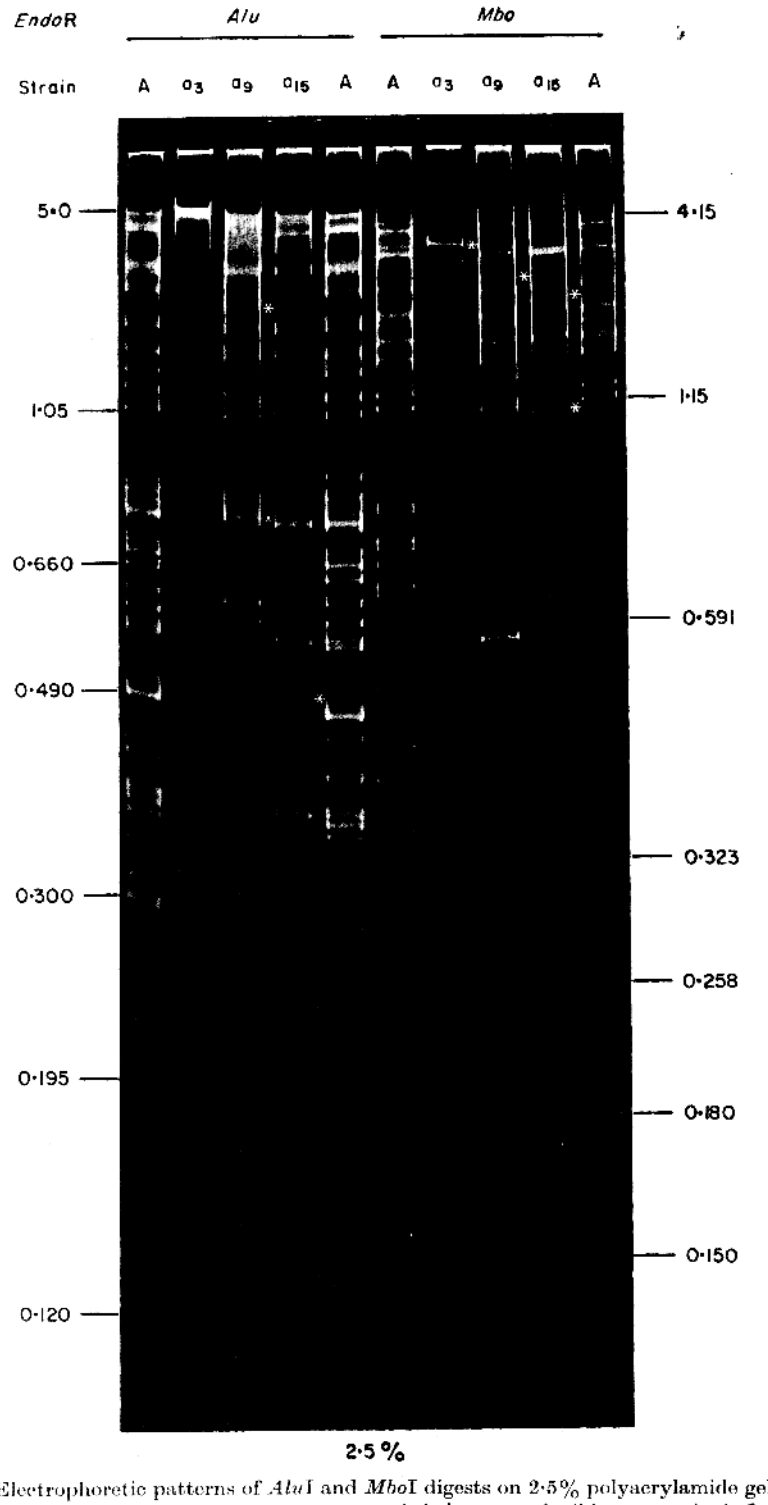


FIG. 4. Electrophoretic patterns of *Alu*I and *Mbo*I digests on 2·5% polyacrylamide gel of mitochondrial DNAs from petite mutants a_3, a_9, a_{15} and their parental wild-type strain A. Indications as for Fig. 2. High molecular weight new bands were better detected on 2% gel (results not shown).

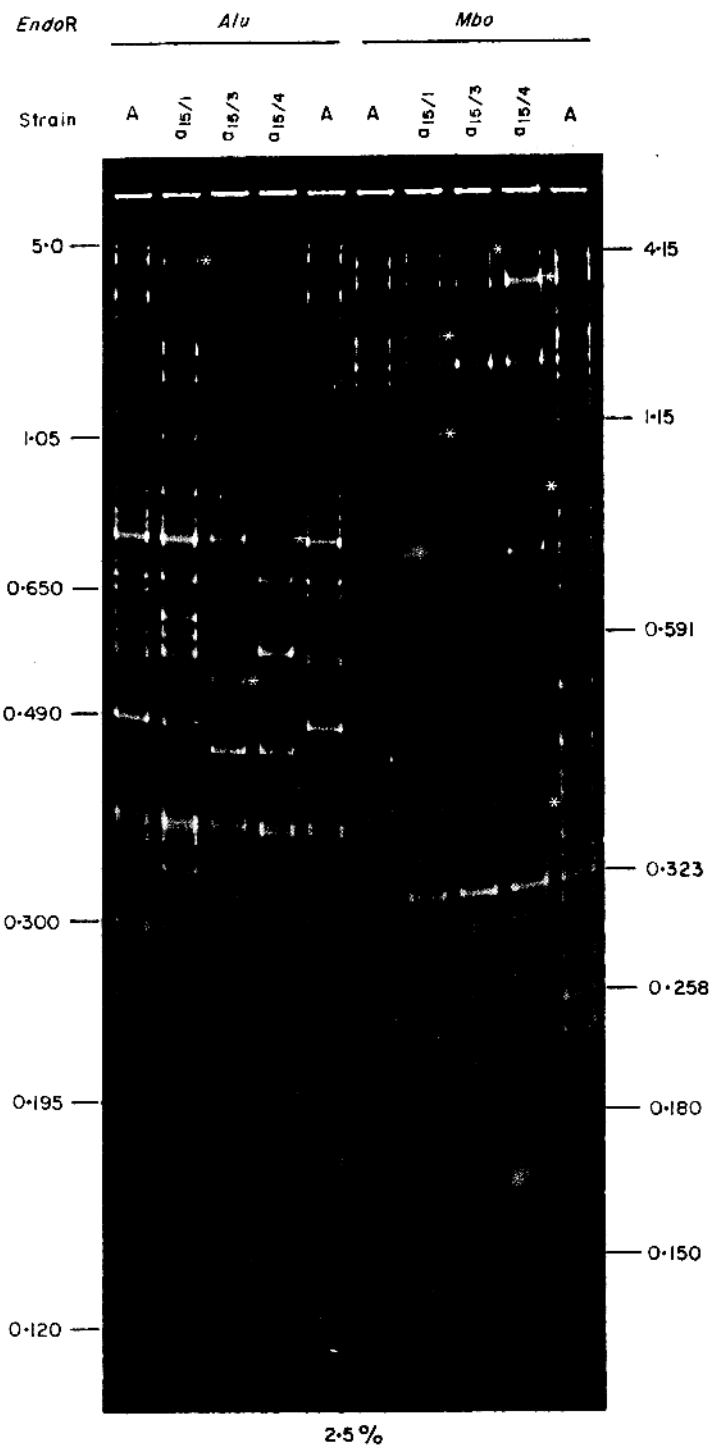


FIG. 5. Electrophoretic patterns of *Alu*I and *Mbo*I digests on 2·5% polyacrylamide gel of mitochondrial DNAs from the petite mutant a_{15}, its subclones $a_{15/1}$, $a_{15/3}$, $a_{15/4}$ and its parental wild-type strain A. Indications as for Fig. 2.

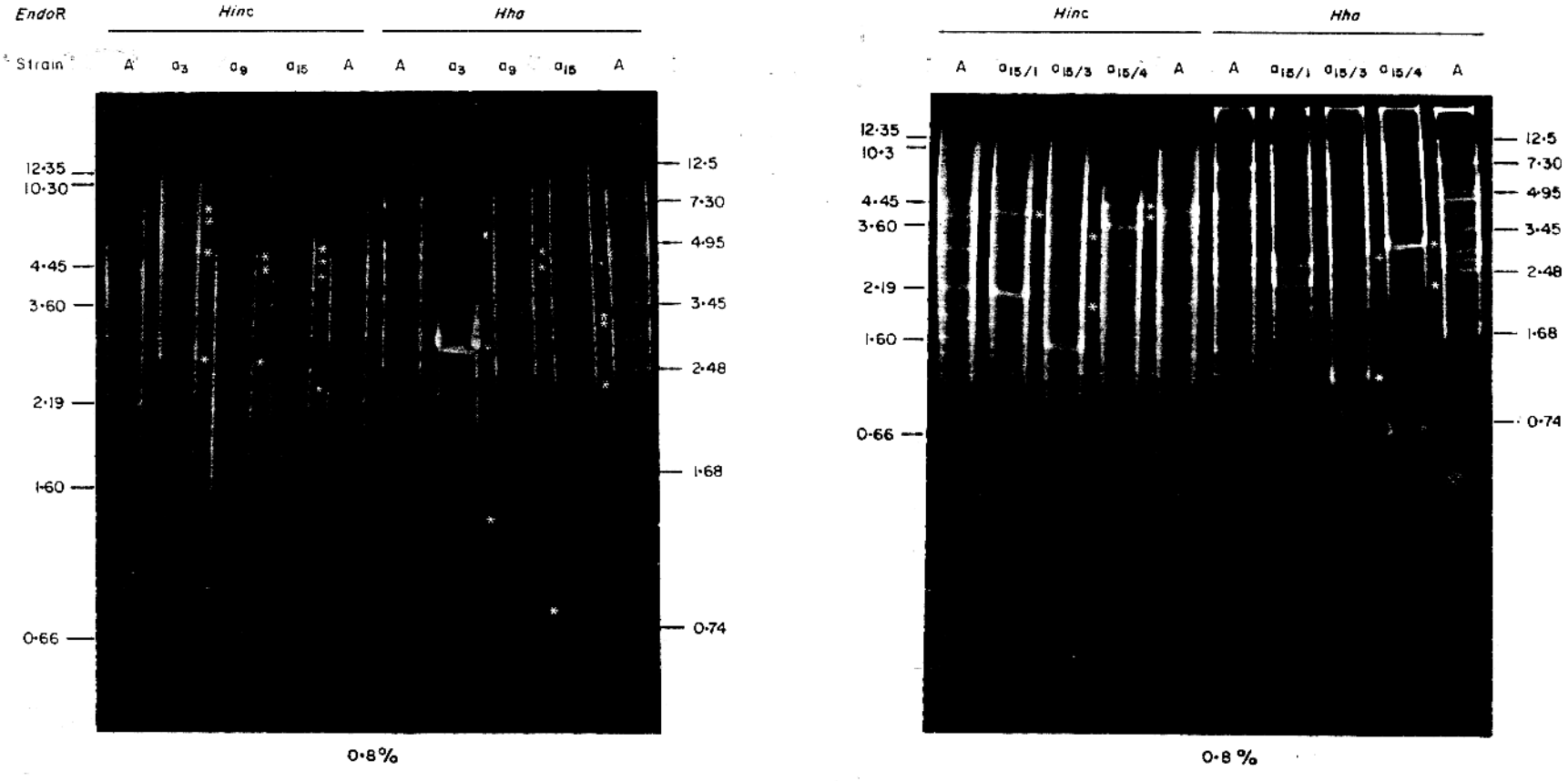


FIG. 6. Electrophoretic patterns of *Hinc*II and *Hha*I digests on 0·8% agarose gels of mitochondrial DNAs from petite mutants a_3, a_9, a_{15}, its subclones $a_{15/1}$, $a_{15/3}$, $a_{15/4}$, and from the parental wild-type strain A. Indications as for Fig. 2.

TABLE 3

Complexities of the mitochondrial genomes of strains a_3, $a_{3/1}$, a_9, a_{15}, $a_{15/1}$, $a_{15/3}$ *and* $a_{15/4}$†

Strains	A	a_3	$a_{3/1}$	a_9	a_{15}	$a_{15/1}$‡	$a_{15/3}$	$a_{15/4}$
Hae	53·0	3·1	3·1	38·0	29·5	19·4	13·2	9·6
Hpa	53·3	3·0	3·0	37·3	28·6	20·9	13·5	10·5
*Alu*I§	51·1	7·85	—	21·1	19·9	16·9	7·2	3·4
*Mbo*I	51·3	4·2	2·85	37·9	28·0	18·5	15·2	14·9
*Hinc*II	52·2	23·0	2·8	35·5	39·3	17·3	15·0	16·3
*Hha*I	51·6	9·8	2·8	37·0	42·2	17·8	15·7	27·0

† Complexities are defined as in footnote ¶ to Table 2 except that account was taken of new bands. Values are $\times 10^{-6}$.

‡ Subclone $a_{15/2}$ showed restriction patterns identical to those of subclone $a_{15/1}$.

§ Except for $a_{15/1}$ and $a_{15/2}$, digests of all petite DNAs showed variable amounts of undigested material (up to 100% in the case of $a_{3/1}$).

(2) The *Hin*dII + III digests of petite a_1^* (Fig. 8) revealed (a) the absence of four of the 13 parental bands corresponding to 19×10^6 of DNA; (b) the presence of eight new bands, representing 37×10^6 of DNA; (c) a genome complexity estimated as 74×10^6, if the new bands were taken into account; (d) a non-stoichiometry of the bands.

(3) Subclone $a^*_{1/7}$, showing the least complex mitochondrial genome and the highest suppressivity (Fig. 9 and Table 4), was subcloned further. Out of 17 subclones, four were examined (Table 1) in terms of mitochondrial DNA; their selection was made on the basis of their suppressivity, one of the four subclones being the least suppressive and three the most suppressive. The first one, $a^*_{1/7/9}$, with a suppressivity of 1%, contained no mitochondrial DNA.

Subclone $a^*_{1/7/12}$ (Fig. 9 and Table 4), with a suppressivity of 80%, contained a DNA differing from $a^*_{1/7}$ in that it lacked one *Hae* fragment, $9{\cdot}6\times10^5$, and one new *Hpa* fragment, $2{\cdot}3\times10^5$; like $a^*_{1/7}$, subclone $a^*_{1/7/12}$ contained a new *Hae* band, $5{\cdot}6\times10^5$. Interestingly, all *Hpa* and *Hae* fragments of $a^*_{1/1/12}$ were stoichiometric with each other and present in the mitochondrial genome of the totally independent petite $a_{3/1}$, except for the new fragment of $5{\cdot}6\times10^5$. Since no *Hpa* fragments corresponding to this *Hae* fragment were present in the DNA from $a^*_{1/7/12}$, it is clear that $a^*_{1/7/12}$ contains two homogeneous genomes, one of which comprises the $5{\cdot}6\times10^5$ *Hae* fragment and lacks *Hpa* sites, and the other one has a repeat unit formed by all other *Hae* fragments. In spite of the fact that this genome has not yet been actually isolated, we will call it $a^*_{1/7/12/1}$. A map of its repeat unit taking into account the data obtained on $a_{3/1}$ and $a^*_{1/7/8}$, is shown in Figure 16.

Subclones $a^*_{1/7/8}$ and $a^*_{1/7/1}$ had the same suppressivity, 88% and contained DNAs showing identical and very simple *Hae* and *Hpa* restriction patterns with no new bands (Fig. 9 and Table 4). These homogeneous genomes were formed by *Hpa* and *Hae* fragments also present in $a^*_{1/7/12}$. Figures 10 and 11 show the electrophoretic patterns obtained by degrading the DNA from subclone $a^*_{1/7/8}$ with several restriction enzymes. Single, double and partial digests allowed us to construct a physical map of this DNA (Fig. 16). The buoyant density in CsCl of this DNA was 1·681 g/cm^3.

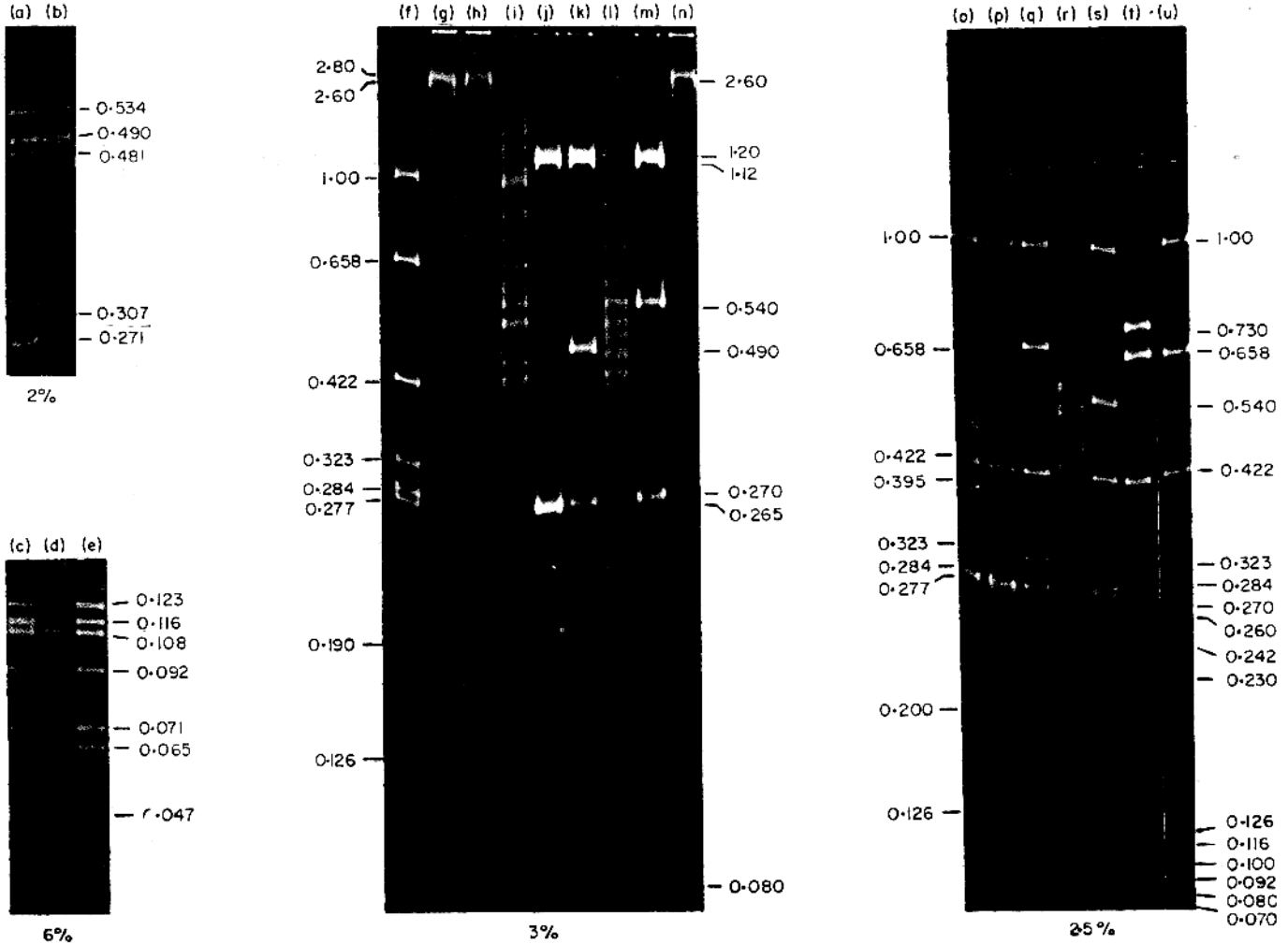


FIG. 7. Electrophoretic patterns on polyacrylamide gels of restriction fragments of mitochondrial DNAs from petite mutant $a_{3/1}$ and its parental wild-type strain A. The patterns concern *Hpa* (a and c), *Hpa* + *Hae* (b and e) digests of petite DNA and *Hpa* (d) digest of wild-type DNA; *Hae* (f and q) *Mbo*I + *Hha*I (g), *Mbo*I (h), *Mbo*I + *Tac*I (j), *Tac*I + *Hha*I (k), *Tac*I (m), *Hha*I (n), *Mbo*I + *Hae* (o), *Mbo*I + *Hae* + *Hha*I (p), *Hae* + *Tac*I (s), *Hae* + *Taq*I (t), *Hae* + *Hin*fI (u) digests of petite DNA; *Hae* (i and r) and *Hpa* (l) digests of wild-type DNA. Gel concentrations and molecular weights ($\times 10^{-6}$) of restriction fragments are indicated. The $0{\cdot}047 \times 10^6$ *Hpa* fragment of A is not visible in pattern d. Partial *Hae* and *Hpa* digests were also investigated both on undegraded and on *Mbo*I degraded DNA from petite $a_{3/1}$. Gel concentrations and molecular weights ($\times 10^{-6}$) of some restriction fragments are indicated.

TABLE 4

Results from the restriction patterns obtained with the mitochondrial DNAs of strains a and its sub-clones*†

Suppressivity (%)	a_1^* 15		$a_{1/2}^*$ 9		$a_{1/8}^*$ 24·5		$a_{1/8}^*$ 42·5		$a_{1/7}^*$ 62		$a_{1/7/12}^*$ 80		$a_{1/7/8}^*$ 88	
Restriction enzyme	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*
No. of bands‡	42	53	35	46	23	30	19	20	7	9	6	8	2	3
No. of deleted bands	34	33	41	40	52	55	59	66	69	77	70	77	73	82
No. of deleted fragments	38	42	45	49	56	68	65	82	77	99	78	99	82	105
DNA in deleted fragments§ ‖	27·0	26·6	31·5	31·1	37·0	36·5	42·8	44·0	48·8	49·9	49·8	49·9	51·9	52·1
(as % of wild-type genome)	51	50	59	58·5	69·5	68·5	81	82·5	92·0	93·7	94·0	93·7	97·9	97·7
No. of new bands	1	1	1	1	—	—	3	1	1	1	1	—	—	—
DNA amount in new bands§	2·15	2·13	2·15	2·13	—	—	0·31	0·25	0·56	0·23	0·56	—	—	—
Genome complexity§ ¶	26	26·7	21·5	22·2	16	16·8	10·2	9·3	4·21	3·32	3·26	3·32	1·13	1·16

† to ¶ See corresponding footnotes to Table 2.

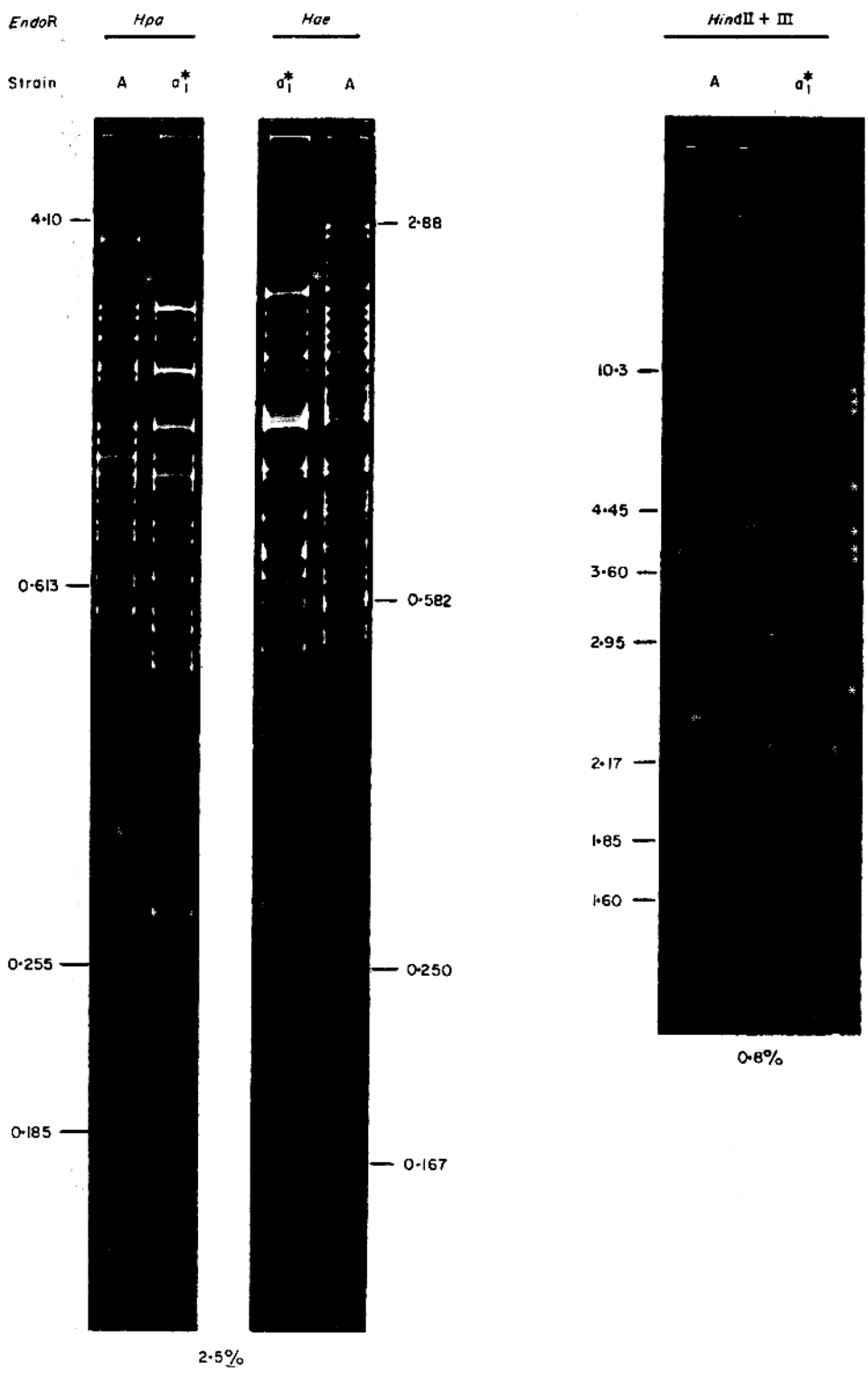


FIG. 8. Electrophoretic patterns of *Hpa*, *Hae* and *Hin*dII + III digests on 2·5% polyacrylamide and 0·8% agarose gels of mitochondrial DNAs from the petite mutant a_1^* and its parental wild-type strain A; a 3% polyacrylamide gel (not shown) revealed, in both DNAs, 3 additional *Hin*dII + III bands $7{\cdot}2 \times 10^6$, $3{\cdot}7 \times 10^5$ and $2{\cdot}7 \times 10^5$, respectively. Indications as for Fig. 2.

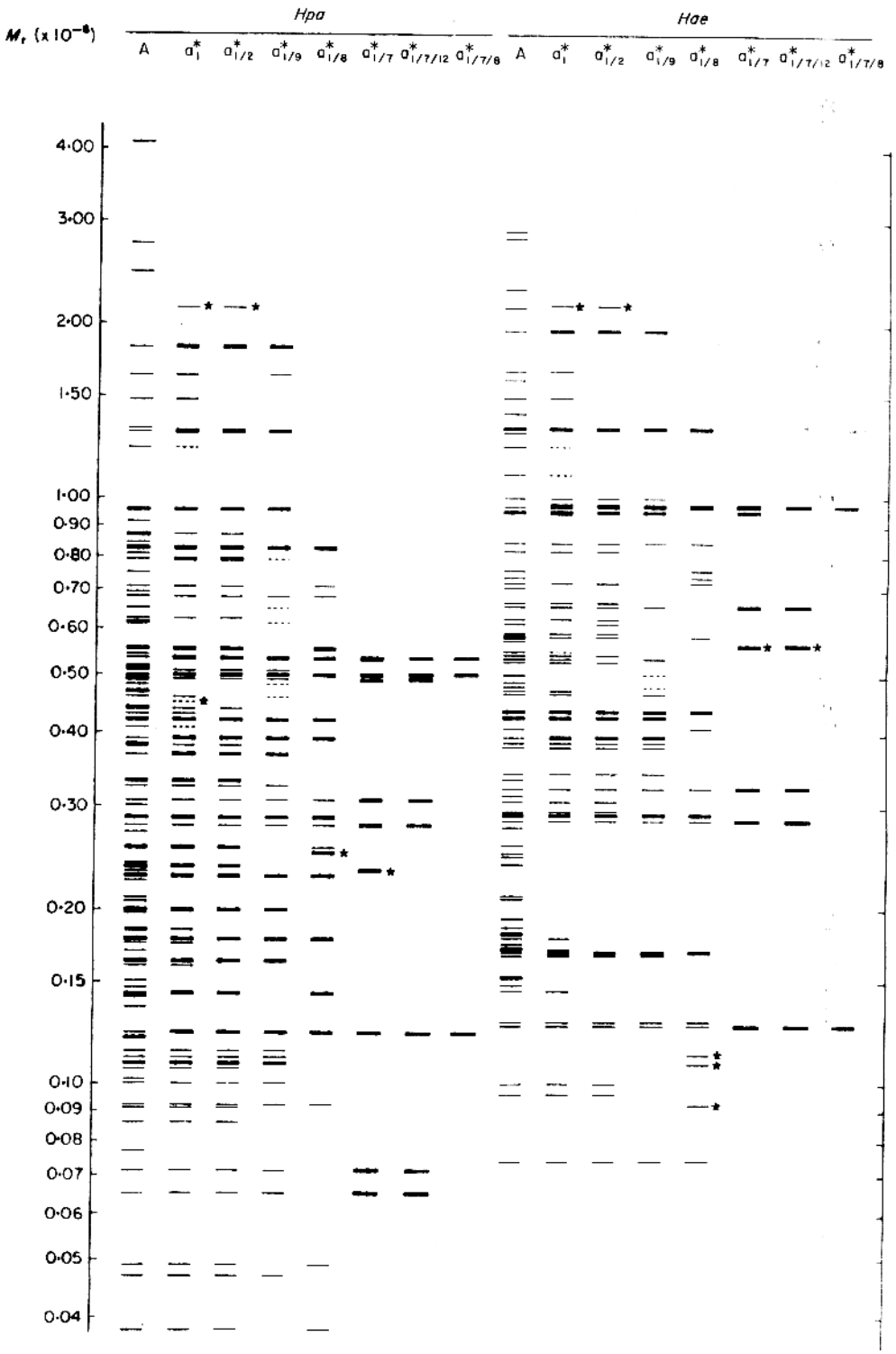


FIG. 9. Scheme of band patterns found in *Hpa* and *Hae* digests of the mitochondrial DNAs from strains A, a_1^* and its subclones $a_{1/2}^*$, $a_{1/9}^*$, $a_{1/8}^*$, $a_{1/7}^*$, $a_{1/7/12}^*$, $a_{1/7/8}^*$, as obtained from 2·5% and 6% gels. Different line thicknesses indicate different band intensities; broken lines indicate faint bands. Asterisks indicate new bands.

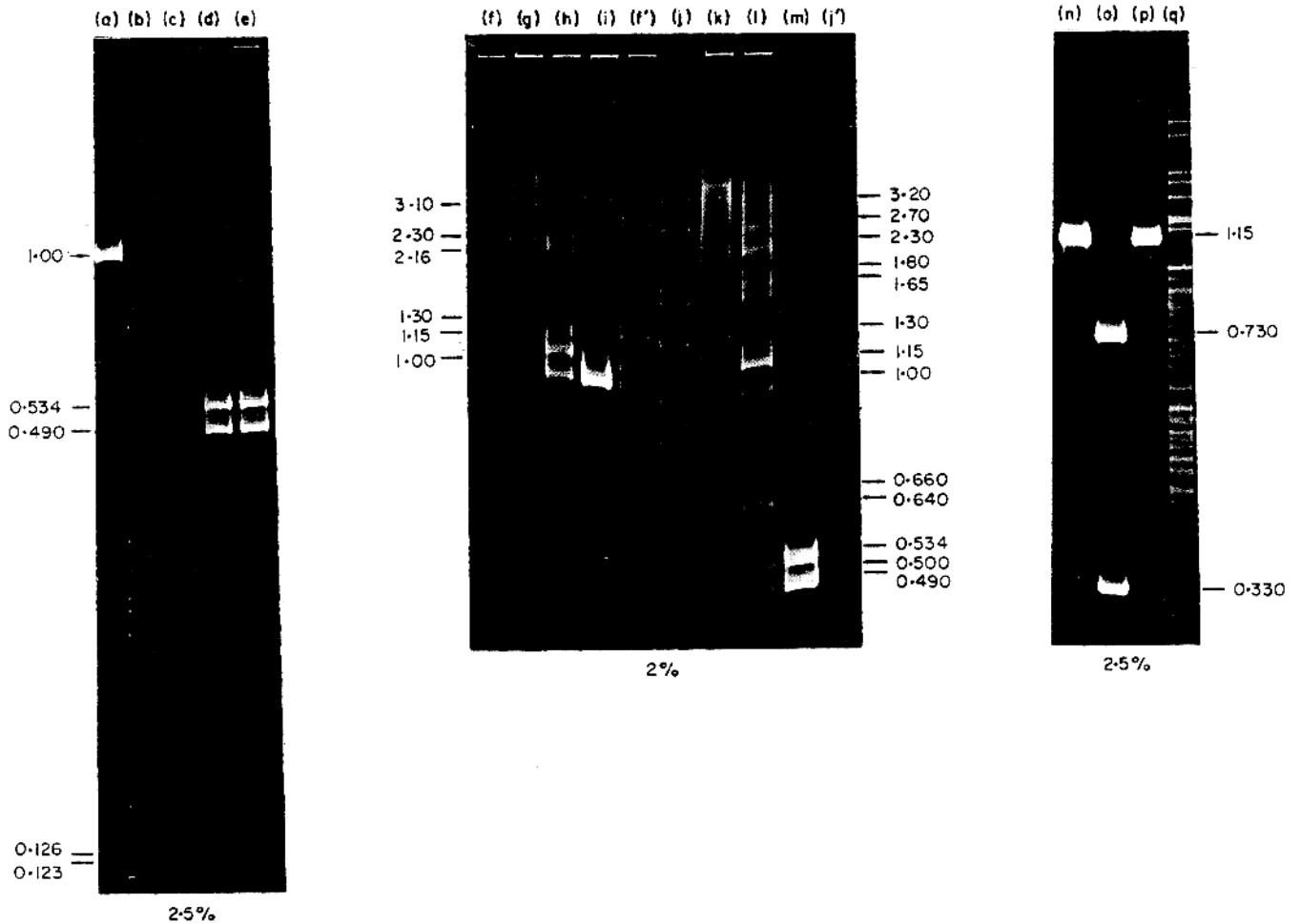


FIG. 10. Electrophoretic patterns on polyacrylamide gels of restriction fragments of mitochondrial DNAs from petite mutant a^*_{1718} and its parental wild-type strain A. The patterns concern *Hae* (b, f, f') and *Hpa* (c, j, j', q) digests of mitochondrial DNA from strain A; *Hae* (a, i) and *Hpa* (d, m), double *Hpa* + *Hae* (e), partial *Hae* (g, h), and partial *Hpa* (k, l), *Hin*f1 (n), *Taq*1 (o), and *Tac*I (p, digests of mitochondrial DNA from strain a^*_{1718}. Gel concentrations and molecular weights ($\times 10^{-6}$) of restriction fragments are indicated. Fragments $1{\cdot}26 \times 10^5$, $1{\cdot}23 \times 10^5$ and $0{\cdot}80 \times 10^5$ could not be seen on lanes (g, h, i), (k, l, m) and (o), respectively, because of their too low molecular weights for the gel concentration used; they were, however, observed on 6% gels.

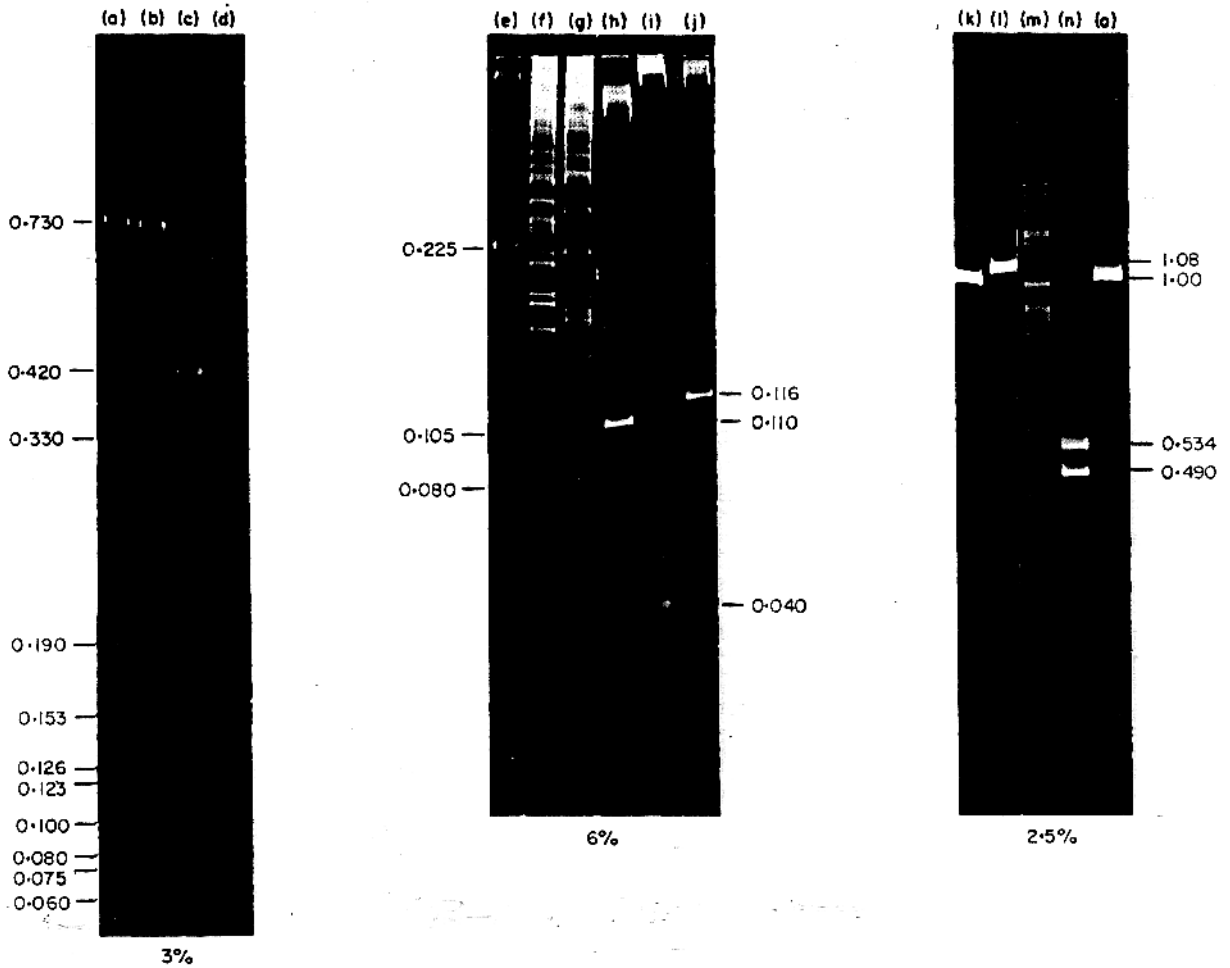


FIG. 11. Electrophoretic patterns on polyacrylamide gels of restriction fragments of mitochondrial DNA from petite mutant a^{*}_{1171s} and its parental wild-type strain A as obtained by different restriction enzymes. The patterns concern *Hpa* (d, f, m) and *Hae* (g) digests of mitochondrial DNA from strain A; double *Hin*fI + *Taq*I (a), *Taq*I + *Hae* (b), *Taq*I + *Hpa* (c), *Tac*I + *Taq*I (e), *Tac*I + *Hpa* (h), *Tac*I + *Hin*fI (i, l), *Tac*I + *Hae* (j, k), *Hin*fI + *Hpa* (n), *Hinf* + *Hae* (o) digests of mitochondrial DNA from strain a^{*}_{1171s}. Gel concentrations and molecular weights ($\times 10^{-6}$) of restriction fragments are indicated.

(iii) *Mitochondrial DNA from strain a_1 and its subclones (Figs 12 and 13, Table 5)*

(1) Strain a_1 showed only 23 *Hae* bands and 28 *Hpa* bands, three of which were new compared to the parental pattern; two *Hae* bands, $4{\cdot}88 \times 10^5$ and $0{\cdot}98 \times 10^5$, and three *Hpa* bands, $3{\cdot}01 \times 10^5$, $1{\cdot}78 \times 10^5$ and $1{\cdot}06 \times 10^5$, displayed a strong intensity and were apparently stoichiometric with each other.

(2) Subclone $a_{1/1}$ only showed the two prominent *Hae* bands exhibited by a_1; seven *Hpa* bands were present, which included the three strong *Hpa* bands of a_1; none of the *Hpa* bands was new compared to a_1, but two were new compared to A; most of the seven *Hpa* bands had different relative intensities compared to a_1; the genome complexity of $a_{1/1}$ was estimated as $1{\cdot}3 \times 10^6$ from *Hpa* digest but as $5{\cdot}9 \times 10^5$ from *Hae* digest, a result demonstrating the heterogeneity of $a_{1/1}$ and the presence in it of genomes deprived of *Hae* sites and only containing *Hpa* sites.

(3) Subclone $a_{1/1R}$ was the result of many replatings of $a_{1/1}$, over a period of two to three years; its *Hae* pattern was identical to that of $a_{1/1}$; its *Hpa* pattern was very similar to that of $a_{1/1}$, but the relative intensity of the bands was different and one $a_{1/1}$ band could not be detected.

(4) Subclone $a_{1/1R/1}$ only showed the prominent *Hae* and *Hpa* bands exhibited by a_1, $a_{1/1}$ and $a_{1/1R}$; the genome complexity was found to be equal to $5{\cdot}9 \times 10^5$ from both *Hpa* and *Hae* digests. Several lines of evidence suggested that this was an homogeneous genome; (a) the *Hae* and *Hpa* bands appeared to be stoichiometric with each other; interestingly, this stoichiometry was already apparent in a_1, $a_{1/1}$ and $a_{1/1R}$, three heterogeneous genomes in which the $a_{1/1R/1}$ genome is differently represented; (b) the genome complexities estimated from both digests were identical, in contrast with the previous cases (a_1, $a_{1/1}$, $a_{1/1R}$; see Table 5); (c) the *Hae* and *Hpa* patterns of the DNAs from two clones issued from individual buds, of a single $a_{1/1R/1}$ cell, and from diploids obtained by crossing the latter, or its buds, with wild-type strain B or with petite strain b (unpublished data) were identical to that of $a_{1/1R/1}$. Restriction patterns obtained with several enzymes (Fig. 14) allowed us to draw a physical map of the repeat unit of the mitochondrial genome of $a_{1/1R/1}$ (Fig. 16). The buoyant density in CsCl of this DNA was 1·687 g/cm^3.

(5) When petite strain $a_{1/1R}$ was crossed with wild-type strain B, a clone, $a_{1/1R/Z1}$, of the residual zygote (namely the zygote resulting after elimination of the early buds) was obtained which contained a DNA showing no *Hae* sites and a single *Hpa* band, corresponding to a molecular weight of $2{\cdot}7 \times 10^5$; this corresponds in molecular weight to a band already present in the DNA of the petite strain used in the cross and also to a band from the parental wild-type strain DNA. Figure 15 (a) to (f′) shows the results obtained with single, double and partial restriction enzyme digestions. A physical map of this DNA is shown in Figure 16. An interesting feature of it is the localization of a *Mbo*I site, which is identical (relative to the isolated *Hpa* site) to that of the same site on the rightmost *Hpa* fragment of $a_{1/1R/1}$. The buoyant density in CsCl of this DNA was 1·683 g/cm^3.

(iv) *Mitochondrial DNA from strain b*

Only two out of ten restriction enzymes tested (*Eco*RI, *Hin*dIII + III, *Hpa*, *Hae*, *Hha*I, *Taq*I, *Mbo*I, *Hin*fI, *Alu*I, *Tac*I), *Hpa* and *Alu*I, were able to split the mitochondrial DNA from strain b releasing a fragment of molecular weight $5{\cdot}6 \times 10^5$.

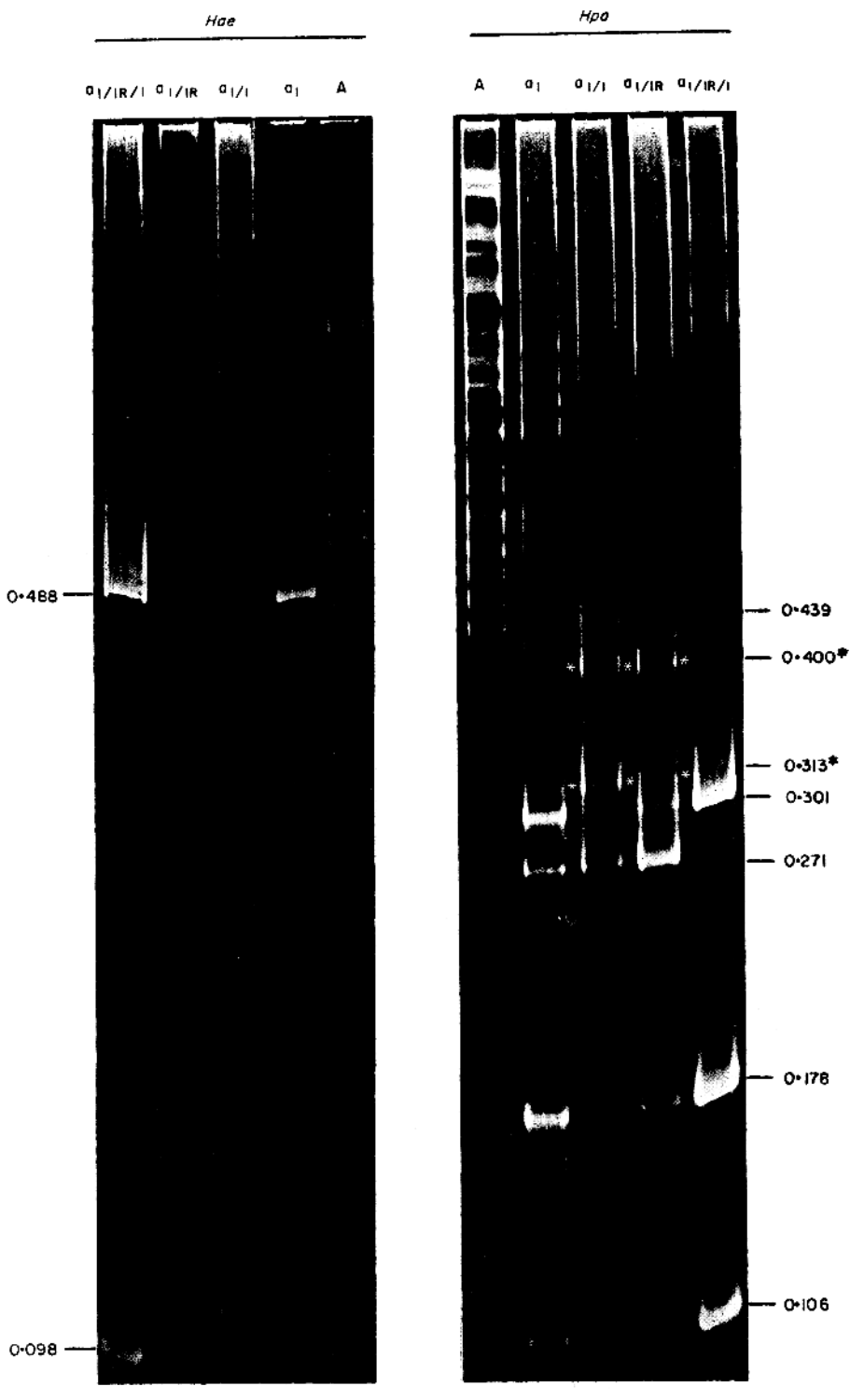


FIG. 12. Electrophoretic patterns of *Hae* and *Hpa* digests on 3% polyacrylamide gel of mitochondrial DNAs from petite mutant a_1, its subclones $a_{1/1}$, $a_{1/1R}$, $a_{1/1R/1}$, and the wild-type strain A. Indications as for Fig. 2.

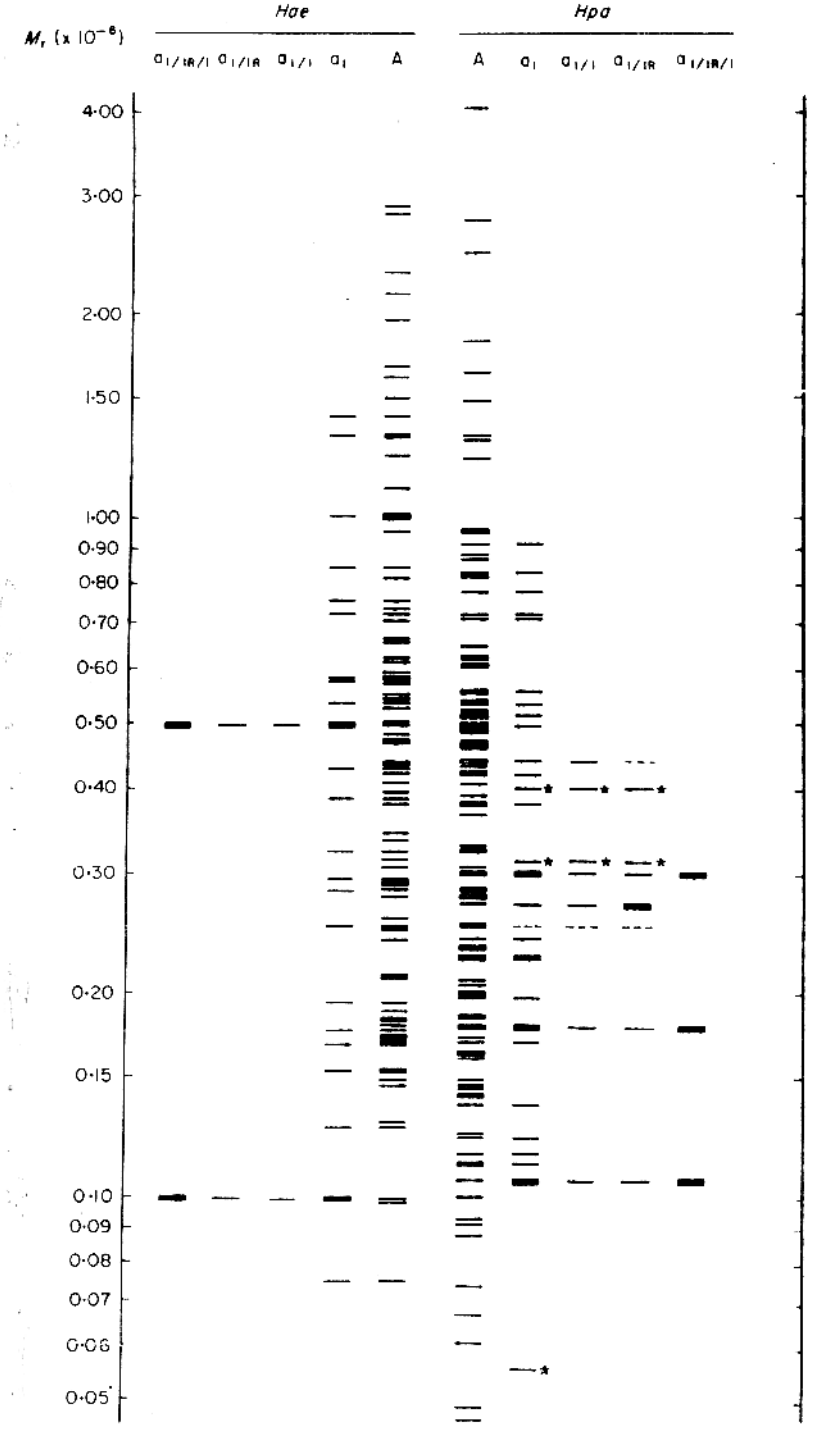


FIG. 13. Scheme of band patterns found in *Hae* and *Hpa* digests of the mitochondrial DNAs from strains A, a_1 and its subclones $a_{1/1}$, $a_{1/1R}$ and $a_{1/1R/1}$, as obtained from 2·5%, 3% and 6% polyacrylamide gels. All other indications as for Fig. 9.

TABLE 5

Results from restriction patterns obtained with the mitochondrial DNAs of strain a_1 and its subclones†

Strain	a_1		$a_{1/1}$		$a_{1/1R}$		$a_{1/1R/1}$		$a_{1/1R/Z1}$	
Restriction enzyme	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*	*Hae*	*Hpa*
No. of bands‡	23	28	2	7	2	6	2	3		1
No. of deleted bands	52	60	73	80	73	81	73	82	—	84
No. of deleted fragments	61	81	82	103	82	104	82	105	—	107
DNA in deleted fragments§	41·8	43·1	52·4	52·0	52·4	52·4	52·4	52·7	—	53·0
(as % of wild-type genome)	78·9	80·9	98·9	97·6	98·9	98·4	98·9	98·9	—	99·5
No. of new bands	—	3		2		2	—	—	—	—
DNA amount in new bands§	—	0·77		0·71		0·71	—	—	—	—
Genome complexity§¶	11·2	10·2	0·59	1·3	0·59	0·86	0·59	0·59	—	0·27

† to ¶ See footnotes to Table 2. For $a_{1/1R/Z1}$ see text.

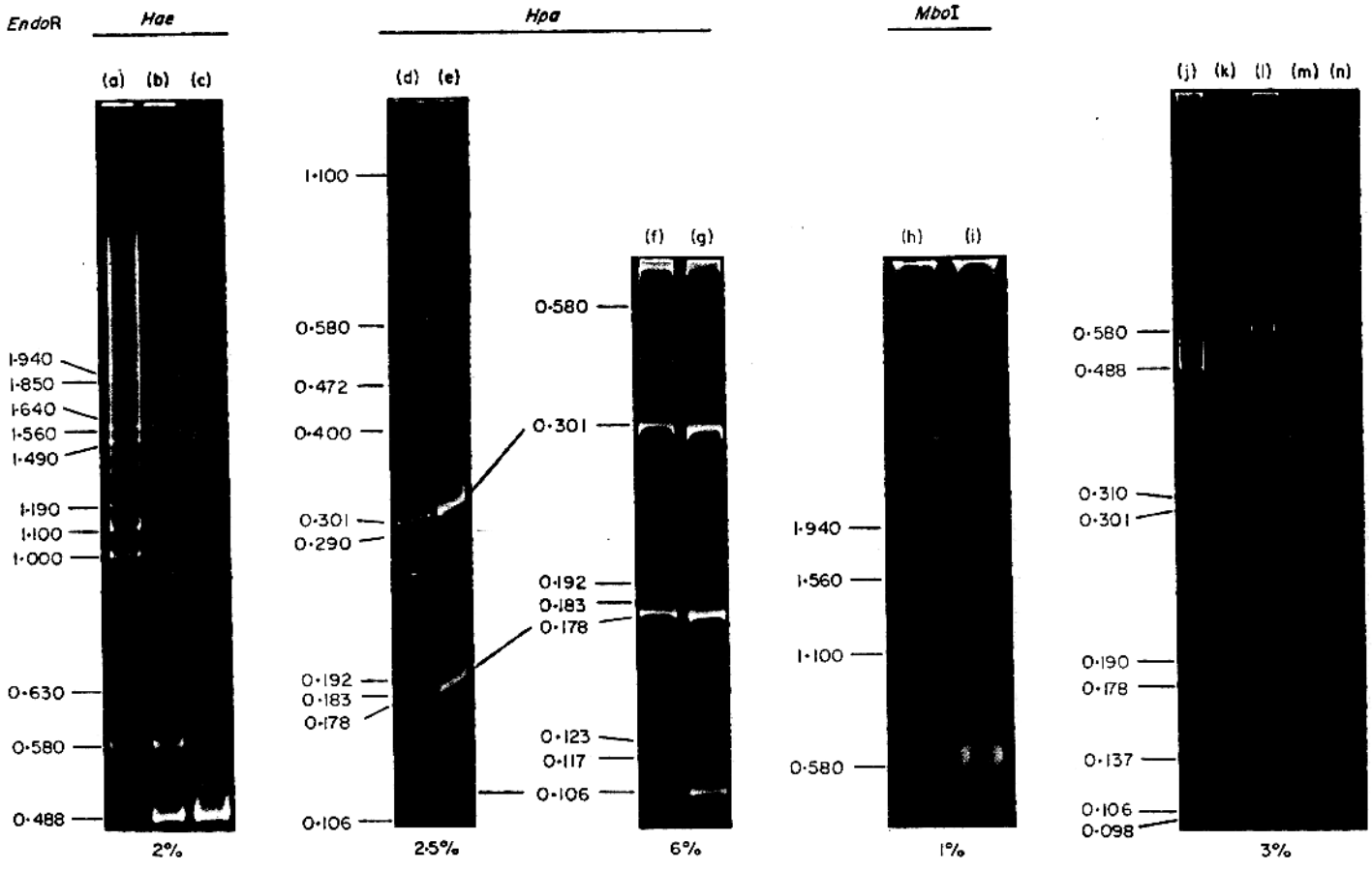
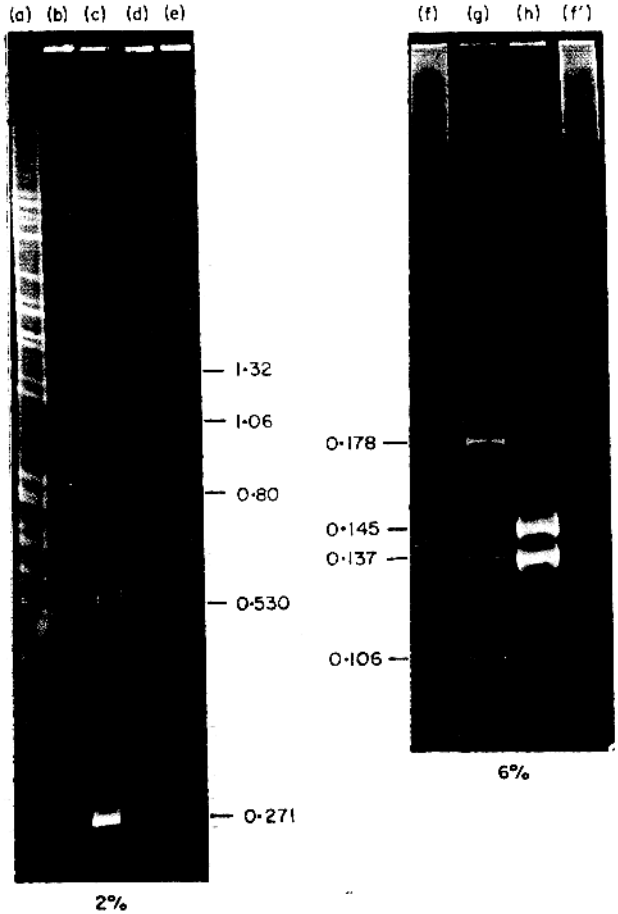


FIG. 14. Electrophoretic patterns of partial and final *Hae*, *Hpa* and *Mbo*I digests of mitochondrial DNA from petite mutant $a_{I/1\,R/1}$ as obtained from polyacrylamide gels (a to g and j to n) and agarose gels (h to i). The patterns concern partial (a, b) and final (c) *Hae* digests; partial (d, f) and final (e, g) *Hpa* digests; partial (h) and final (i) *Mbo*I digests; final *Hae* (j); *Mbo*I (l), and *Hpa* (m) digests; final double *Hae* + *Mbo*I (k) and *Hpa* + *Mbo*I (m) digests. The double *Hpa* + *Mbo*I digest (m) shows only 3 restriction bands, one of these ($1{\cdot}78 \times 10^5$) being a double one containing 2 restriction fragments. Gel concentrations and molecular weights ($\times 10^{-5}$) of restriction fragments are indicated.

a I/IR/II


(a) (b) (c) (d) (e)
1·32
1·06
0·80
0·530
0·271
2%
(f) (g) (h) (f')
0·178
0·145
0·137
0·106
6%

b

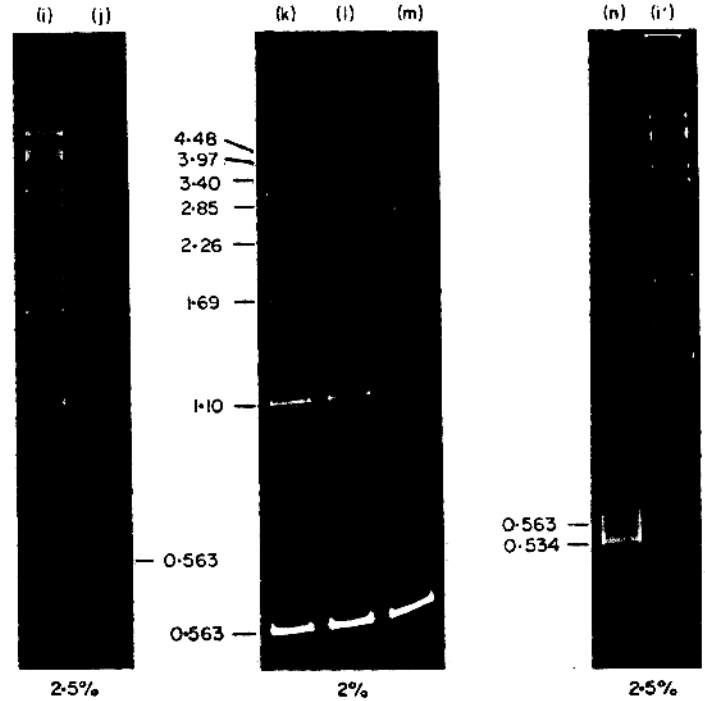

(i) (j)
0·563
2·5%
(k) (l) (m)
4·48
3·97
3·40
2·85
2·26
1·69
1·10
0·563
2%
(n) (i')
0·563
0·534
2·5%

FIG. 15. (a to f)' Electrophoretic patterns of partial, final and double *Hpa* and *Mbo*I digests on polyacrylamide gels of mitochondrial DNA from petite mutants $a_{1/1R/Z1}$, $a_{1/1R/1}$, and the wild-type strain A. The patterns concern final *Mbo*I (a) and *Hpa* (f, f') digests of mitochondrial DNA from strain A, partial (b) and (nearly) final (c) *Hpa*, partial (e) and final (d) *Mbo*I digests of mitochondrial DNA from strain $a_{1/1R/Z1}$, double final *Hpa* + *Mbo*I digest of mitochondrial DNAs from strain $a_{1/1R/1}$ (g) and from strain $a_{1/1R/Z1}$ (h). Gel concentrations and molecular weights ($\times 10^{-6}$) of restriction fragments are indicated.

(i to i') Electrophoretic patterns of final, partial and double *Hpa* and *Alu*I digests on polyacrylamide gels of mitochondrial DNAs from petite mutant b and its parental wild-type strain B. The patterns concern final *Hpa* (i, i') digest of mitochondrial DNA from strain B, final (j) and partial (k, l, m) *Hpa*, double *Hpa* + *Alu*I (n) digests of mitochondrial DNA from strain b. The electrophoretic pattern of a *Hpa* or *Alu*I (not shown) digest of the DNA from strain b obtained under the experimental conditions used for the DNA from its parental wild-type strain B is characterized by the presence of a series of oligomeric bands (k). Increasing the enzyme amount and the digestion time led to the disappearance of the multimers and a strengthening of the monomer band (k to m). A double digest *Hpa* + *Alu*I shows the *Hpa* or *Alu*I monomer band plus a band lower in molecular weight by 3×10^4 (n), a result which could be obtained also on the parental wild-type DNA (not shown).

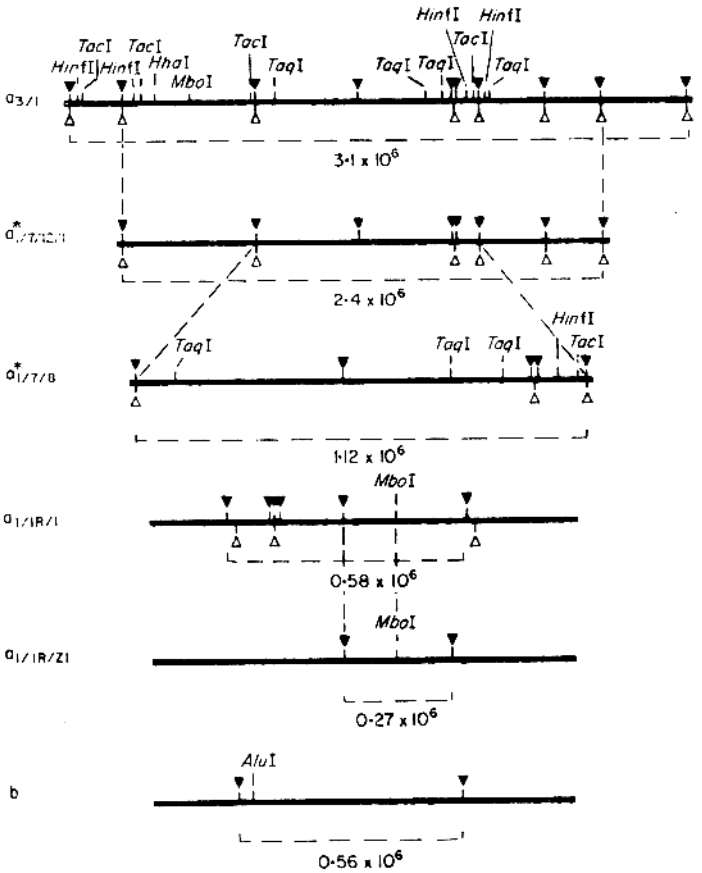


FIG. 16. Restriction enzyme maps of the repeating units of the mitochondrial genomes of petite strains $a_{3/1}$, $a^*_{1/7/12/1}$, $a^*_{1/7/8}$, $a^*_{1/1R/1}a_{1/1R/Z1}$ and b. The molecular weights of the repeat units are indicated. The localizations of *Hae* (△), *Hpa* (▼), and all other restriction sites on the repeat units are shown except for 2 *Hpa* sites on the $6{\cdot}58\times10^5$ fragment of $a^*_{1/7/12/1}$ (this is the leftmost *Hpa* fragment) and $a_{3/1}$, and 3 *Hpa* sites on the $4{\cdot}22\times10^5$ fragment of $a_{3/1}$ (this is the rightmost *Hpa* fragment). Such sites as well the *Hinc*II site on the $a_{3/1}$ genome have not been mapped.

See text for further comments. The molecular weights of the fragments from single, double, triple, and partial restriction enzyme degradations, used to construct the above maps, are given in the Appendix.

Figure 15 (i) to (i') shows the results. A physical map of mitochondrial DNA from petite b is shown in Figure 16. The buoyant density in CsCl of this DNA was 1·678 g/cm^3 (Bernardi *et al.*, 1970).

(v) *Mitochondrial DNA from strain d*

No degradation could be obtained with any of the nine restriction enzymes tested which were those indicated above for b, except for *Tac*I. The buoyant density in CsCl of this DNA was 1·672 g/cm^3 (Bernardi *et al.*, 1968,1970).

(b) *Hybridization of petite DNAs on restriction fragments of wild-type cell DNAs*

(i) *Hybridization on parental wild-type DNAs*

(1) The DNA from petite $a^*_{1/7/8}$ mainly hybridized with two *Hae* fragments and with three *Hpa* fragments of the DNA from the parental wild-type strain A (Fig. 17).

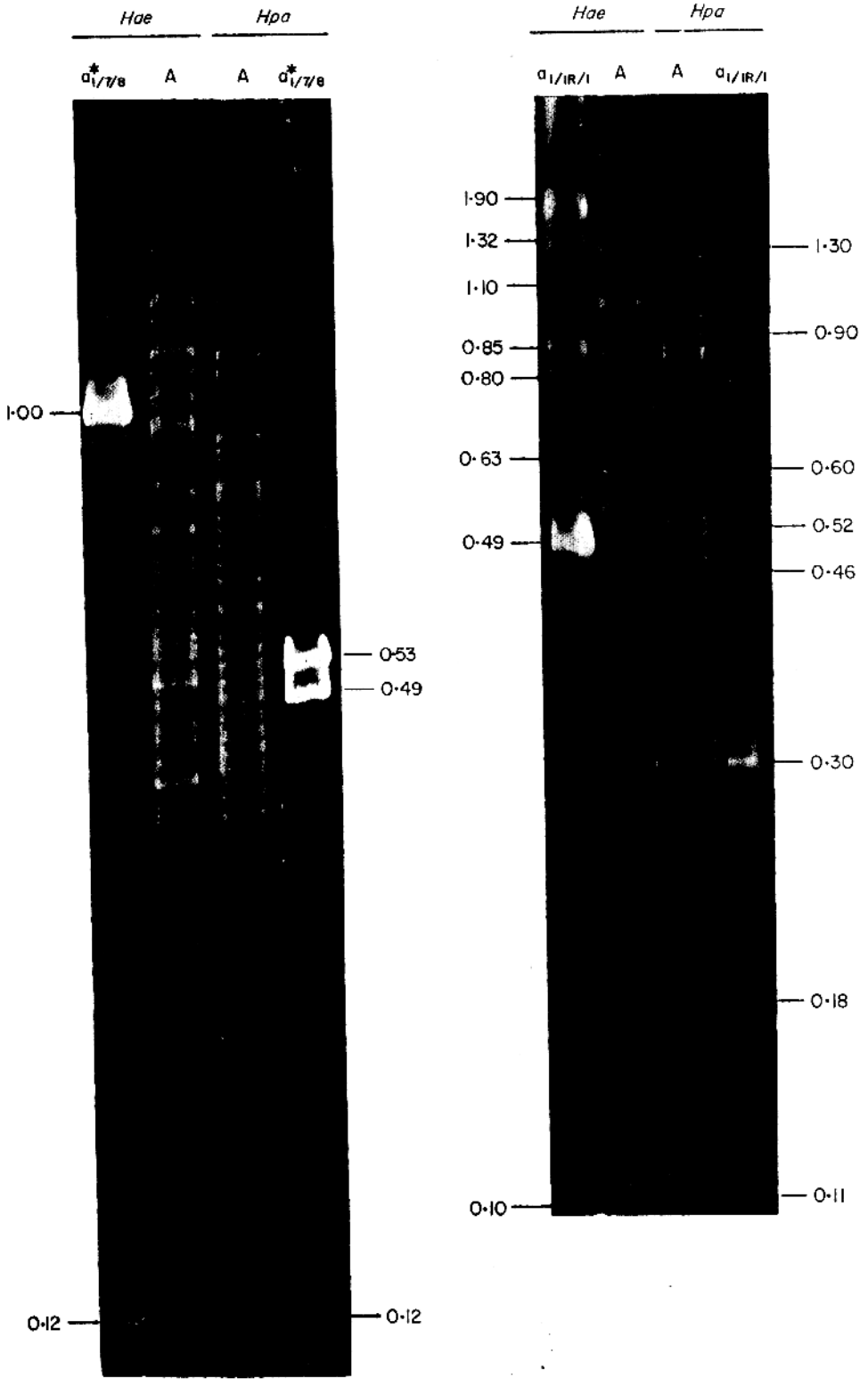
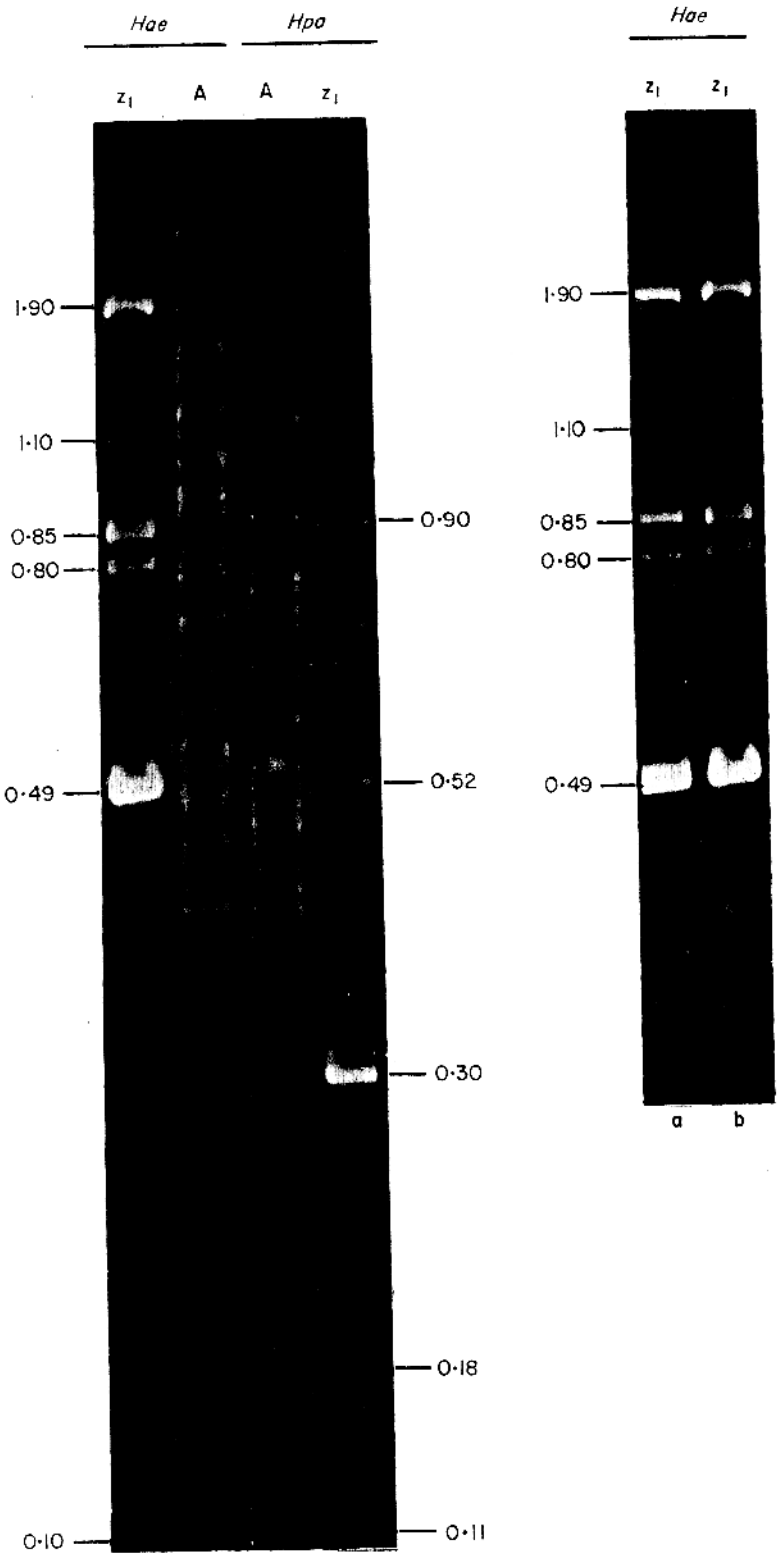


FIG. 17. Hybridization patterns of mitochondrial DNAs from petite mutants $a^{*}_{1/7/8}$, $a_{1/1R/1}$, and $a_{1/1R/z1}$ on *Hae* and *Hpa* fragments of the mitochondrial DNA from the wild-type strain A. Restriction enzymes, strains, and molecular weights ($\times 10^{-6}$) of the restriction fragments are



indicated. z_1 stands for $a_{1/1R/z1}$. a and b correspond to the hybridization of $a_{1/1R/z1}$ DNA digested with micrococcal nuclease up to 33% and 66% degradation, respectively, to *Hpa* fragments of DNA from strain A.

These fragments corresponded in size to the *Hae* and *Hpa* fragments of the petite DNA (Fig. 10). In addition, a number of weaker hybridizations were seen on several *Hae* and *Hpa* bands of the parental genome. This DNA was also found to hybridize on *Hin*cII fragment 7 and *Hha*I fragment 6 of wild-type DNA digests. *Eco*RI digestion of *Hin*cII hydrolysate did not modify the hybridization pattern.

(2) The DNA from petite $a_{1/1R/1}$ mainly hybridized with two *Hae* fragments and three *Hpa* fragments of the DNA from the parental strain, which corresponded in size to *Hae* and *Hpa* fragments of the petite DNA (Fig. 17). A large number of weaker hybridization bands was detected on both *Hae* and *Hpa* fragments from the parental strain as in the case of $a^{*}_{1/7/8}$. Washing the filters with SSC did not change the relative intensity of the hybridization bands compared to the results obtained after washing with 6 × SSC, whereas washing with 0·5 × SSC removed some radioactivity from all bands. The DNA from $a_{1/1R/1}$ also hybridized on *Hin*dII + III fragment 3 and/or 4 of the parental wild-type DNA.

(3) The DNA from petite $a_{1/1R/Z1}$ showed a main hybridization on a $0{\cdot}49\times10^6$ *Hae* fragment and on two *Hpa* fragments, $0{\cdot}3\times10^6$ and $0{\cdot}18\times10^6$, the latter one being relatively weak, as observed with other low molecular weight fragments; all these fragments corresponded to fragments of the repeat unit of $a_{1/1R/1}$ (see Fig. 16), whereas none had the molecular weight of the repeat unit of $a_{1/1R/Z1}$, $0{\cdot}27\times10^6$. Weaker hybridizations of $a_{1/1R/Z1}$ were found on several *Hae* and *Hpa* fragments also showing a weak hybridization with the DNA of $a_{1/1R/1}$. The DNA of $a_{1/1R/Z1}$ was degraded with micrococcal nuclease so as to remove 33% and 66%, respectively, of the segments richest in A + T. Both degraded samples behaved exactly like the undegraded one in the hybridization reaction (Fig. 17 (a) and (b)). When tested on other wild-type DNA digests, this DNA was found to hybridize on *Hin*cII fragment(s) 3 and/or 4 (like that of $a_{1/1R/1}$) and on *Hha*I fragment 5. *Eco*RI digestion of the *Hin*cII hydrolysate did not change the hybridization pattern.

(4) The DNA from petite b mainly hybridized (Fig. 18) to two (or three) *Hpa* bands of $0{\cdot}56\times10^6$ (this seems to correspond to parental band B11), and $0{\cdot}96\times10^6$ (band $b8_1$ and possibly another band), and to three *Hae* bands of $0{\cdot}76\times10^6$ (band $B12_3$) $1{\cdot}60\times10^6$ and $1{\cdot}69\times10^6$ (bands B6 and b5) of the DNA from parental, wild-type strain (Table 6). In both hydrolysates, the lowest molecular weight band showed the strongest hybridization. Much weaker hybridizations with another *Hpa* band of $0{\cdot}74\times10^6$ and another *Hae* band of $1{\cdot}00\times10^6$ were also detected. The effect of subsequent washings of the filters with 6 × SSC, 1 × SSC and 0·5 × SSC was the same as just described for the DNA of strain $a_{1/1R/1}$. The DNA from petite b also hybridized with *Eco*RI band 3 ($7{\cdot}18\times10^6$) of the parental DNA.

(5) The DNA from petite d hybridized to one *Hpa* band of $1{\cdot}14\times10^6$ and one *Hae* band of $1{\cdot}15\times10^6$ of the DNA from the parent strain D (Fig. 18). Hybridization with *Eco*RI and *Hin*dII + III digests did not give any result, possibly because it took place with the largest fragments which were obtained in very low yield.

(ii) *Interspecific hybridizations*

These were performed with the DNA from strain b. This DNA hybridized to *Hpa*, *Hae* and *Eco*RI bands of DNAs from strains A, D and C having either the same or similar molecular weights as the hybridizing bands of the DNA from strain B, the only exceptions concerning the *Hae* bands of strain D and the *Eco*RI band of strain C (Table 6).

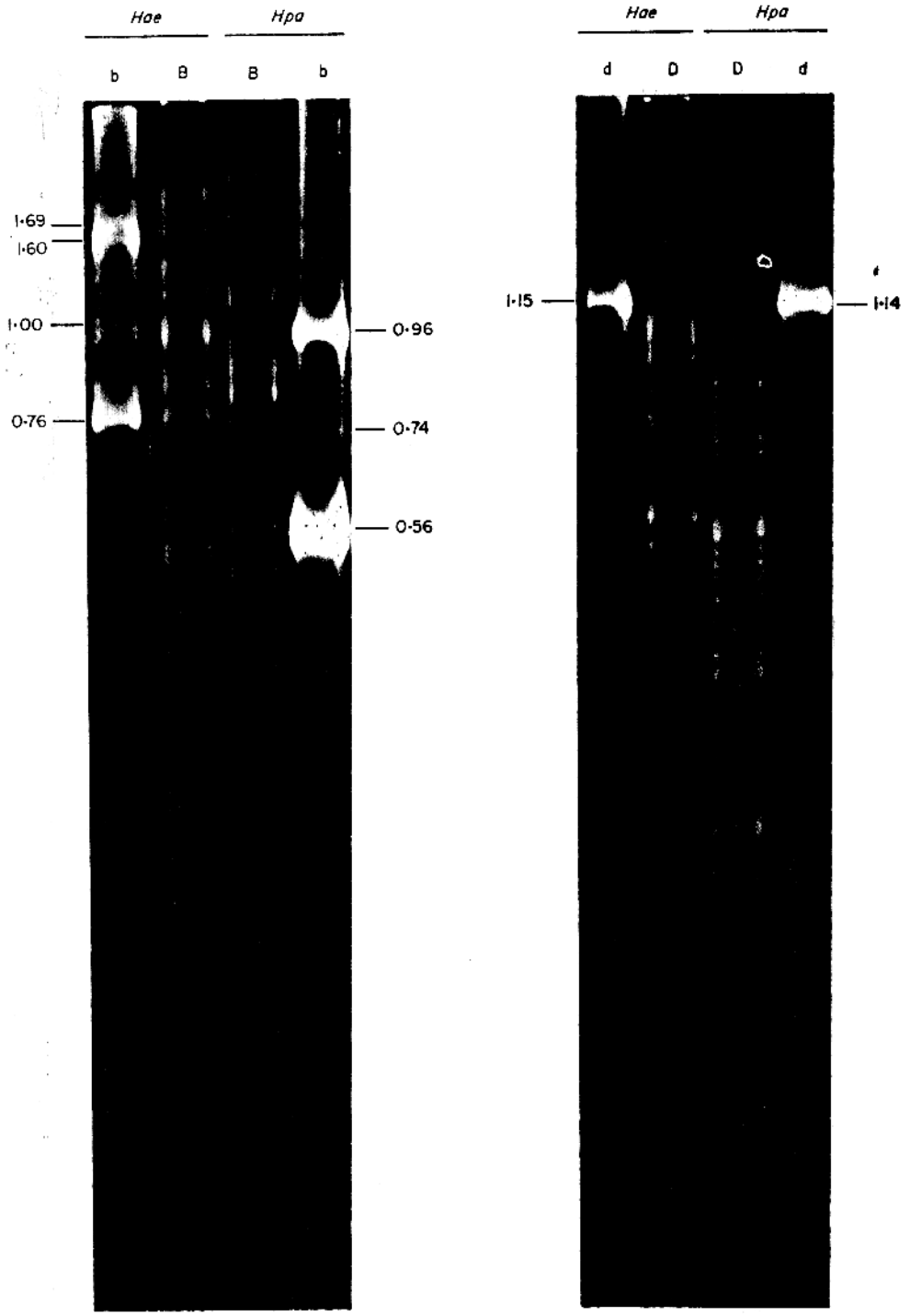


FIG. 18. Hybridization patterns of mitochondrial DNAs from petite mutants b and d on *Hae* and *Hpa* fragments of mitochondrial DNAs from their parental wild-type strains B and D, respectively. All other indications as for Fig. 17.

TABLE 6

Bands of wild-type DNAs hybridizing labeled DNA from petite b

Digest	Strain B		Strain A		Strain D		Strain C	
	M_r† ($\times 10^{-6}$)	Code‡	M_r ($\times 10^{-6}$)	Code	M_r ($\times 10^{-6}$)	Code	M_r ($\times 10^{-6}$)	Code
Hae	1·69	b5	1·66	a5	1·76	$d4_4$	1.63	$c6_1$
	1·60	B6	1·60	A6			1·57	$c6_2$
	0·76	$B12_3$	0·76	$A12_3$	0·95	$d11_4$	0·77	$c12_3$
Hpa	0·96	$b8_1$	0·96	$A8_1$	0·95	$D8_1$	0·90	$c8^5$
	0·56	$B11_1$	0·55	$A11_1$	0·57	§	0·55	c11
*Eco*RI	7·3	R3	7·3	R3	7·3	R3	4·42	R3

† Underlined values indicate main hybridizing bands.

‡ The nomenclature for *Hae* and *Hpa* bands is that of Prunell *et al.* (1977*a*). Capital letters indicate bands considered to be homologous in different strains. R3 is band 3 of *Eco*RI digests.

§ This is a band corresponding in mobility to c_{11}; (see text for the assignment of hybridization to these parental bands).

4.¶ Discussion

(a) *The mitochondrial genomes of spontaneous, heterogeneous petite mutants*

The mitochondrial genomes of a_3, a_9, a_{15} and its subclones, a_1^* and its subclones, a_1 and its subclones $a_{1/1}$ and $a_{1/1R}$, share the following features: (i) absence of a number of parental fragments and decrease in genome complexity; (ii) heterogeneity; (iii) absence or striking scarcity of new *Hae* and *Hpa* bands, and regular presence of new bands in the patterns obtained with enzymes other than *Hae* and *Hpa*.

(i) The restriction patterns of these "petite" genomes are characterized, like those of all petite genomes (whether spontaneous or induced), by the absence of a number of fragments present in the parental, wild-type genome. This feature was the first direct evidence of the deletion phenomenon underlying the petite mutation (Bernardi *et al.*, 1975), and is the cause of a decrease in the genome complexity, a point which will be further commented upon in subsection (ii) (4), below.

(ii) The heterogeneity of these petite genomes, namely the fact that the petite mutants under consideration contain a population of differently deleted and amplified homogeneous petite genomes, is shown by several lines of evidence.

(1) Non-stoichiometry of restriction fragments. Restriction bands having intensities twice as great as expected for single DNA fragments were seen in the patterns of wild-type mitochondrial DNAs and shown to be due to a lack of resolution of fragments having very close molecular weights (Prunell *et al.*, 1977). In the case of heterogeneous petite genomes, however, the lack of stoichiometry covers a much wider range of band intensities. Strong bands are not only due to the amplification phenomena characteristic of petite DNAs (see following sections), but also to the fact that different genomes in the heterogeneous population may share certain stretches of the parental wild-type genome (Fig. 19). Such is the case of the genomes of $a^*_{1/7/8}$ and $a^*_{1/7/12}$, which are both present in $a^*_{1/7}$ (Fig. 9), and of the genomes of $a_{1/1R/1}$ and $a_{1/1R/21}$ which are both present in a_1 and in its subclones $a_{1/1}$ and $a_{1/1R}$. Faint bands are indicative of the presence of minority genomes in the heterogeneous population.

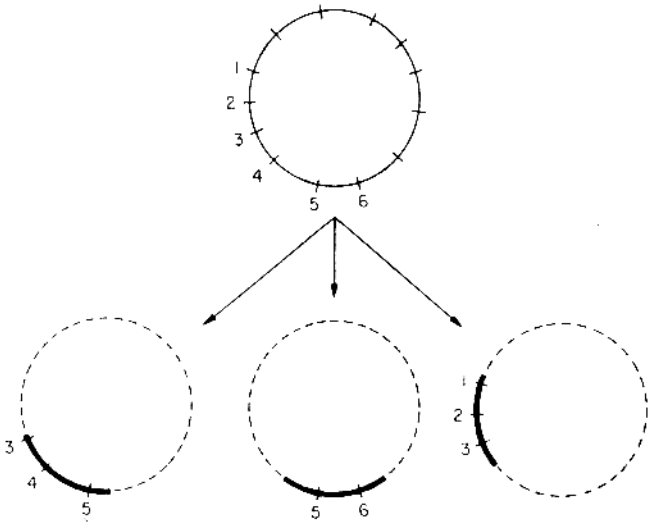


FIG. 19. Scheme of excision of petite genomes from the parental wild-type genome. Radial lines indicate restriction sites other than *Hae* and *Hpa*. The excision process leads to the formation of different petite genomes (thick lines on the bottom circles); these exhibit new bands and overlap in part with each other. The genome complexity calculated on the basis of both parental and new bands produced by the heterogeneous petite genome formed by the 3 homogeneous petite is overestimated relative to the real value.

(2) Differences in the restriction patterns of the DNAs from petite subclones. The DNAs of the subclones of a_{15}, a_1^*, $a_{1/7}^*$, a_1 and $a_{1/1R}$ differ from those of the parental petites in both the restriction patterns and in the relative intensity of parental bands. Three points should be considered here. (a) The presence in some subclones of bands which are new relative to the parental petite genome, but not to the parental, wild-type genome is a very strong indication of the enrichment of minority genomes that had gone undetected in the restriction pattern of the parental petite DNA. (b) Some bands of DNAs from subclones are new relative to both parental petite and wild-type genomes; this emphasizes the possibility that further deletions may affect petite genomes. (c) A case of particular interest is that of $a_{1/1R}$, because it shows that upon repeated replatings a selection took place among the petite genomes present in the heterogeneous population, as shown by the different relative intensity of bands.

(3) Differences in the suppressivity of petite subclones. This has been shown for $a_{1/1}$, a_1^* and $a_{1/7}^*$ (Table 1). If one examines the results of Table 4, an inverse relationship is found between suppressivity and genome complexity. This point will be presented and discussed in detail elsewhere.

(4) Differences in the complexity estimates arrived at using results from different digests. Complexity estimates from *Hae* and *Hpa* sites (Tables 2 to 5), generally were in agreement, a result essentially due to the clustering of these sites and to the absence or striking scarcity of new bands (see following paragraph). Complexity estimates from other digests, taking into account new bands (Table 3) were higher, in most cases, than those estimated from *Hae* and *Hpa* digests and could reach, as in the case of a_1^*, values higher than that of the parental wild-type genome. This

phenomenon is due to the fact that several petite genomes in the population arise from excision events affecting partially overlapping regions of the parental wild-type genome and that the DNA stretches present in new bands from such regions are counted more than once (Fig. 19).

(iii) A very striking property of *Hae* and *Hpa* digests from the spontaneous, heterogeneous petite genomes under consideration is the scarcity or absence of new bands, namely of bands that do not exist in the parental wild-type genome and that correspond to junction fragments. In sharp contrast, all other digests from these petite DNAs show new bands (see Fig. 1).

It should be noted that the number of new bands can be underestimated because (a) they may happen to have the same mobility of parental bands: this case is probably rare, but it has been found for the single *Hpa* band of the $a_{1/1R/Z1}$ genome (see section (b) (ii) (1) (c), below); (b) they may be buried in bands having the mobility of parental bands; this possibility is greater in the case of *Hae* and *Hpa* digests, which usually show more bands than other digests; it should be stressed, however, that in several cases (a_{15}, $a_{15/1}$, $a_{15/3}$, $a_{15/4}$, $a^*_{1/7/12}$) only new *Hae* bands are seen, in spite of the fact that *Hpa* patterns have comparable complexities. It should also be mentioned that new *Hae* and *Hpa* bands could be observed in all 37 ethidium-induced petite mutants examined by Lewin *et al.* (1978); this finding has also other implications which will be further commented upon in the Conclusions.

In spite of some uncertainties in the precise estimation of the number of new *Hae* and *Hpa* bands, it can be concluded that they are rare, compared to the new bands formed by other restriction enzymes on the following grounds: (a) the overall finding of absence or striking scarcity of new bands in a number of heterogeneous petite genomes that had different complexities, and were further analyzed by subcloning. If the excision process had taken place at random locations on the wild-type genome, each homogeneous petite genome present in the heterogeneous population should produce, upon degradation with a restriction enzyme, one new band (corresponding to a junction fragment) per excision event, and yet the number of *Hae* or *Hpa* new bands is extremely rare. In the case of a^*_1, for instance, *Hin*dIII + III released eight new fragments, but only one new *Hae* fragment and one new *Hpa* fragment; (b) five out of six homogeneous genomes, isolated so far from the heterogeneous populations, lack new *Hae* and/or *Hpa* bands. The repeat units of four of them, $a_{3/1}$, $a^*_{1/7/12/1}$, $a^*_{1/7/8}$ and $a_{1/1R}$, are formed by parental *Hae* and *Hpa* fragments. One, b, lacks *Hae* sites and seems to correspond to a parental *Hpa* fragment. One, $a_{1/1R/Z1}$, corresponds to a new *Hpa* fragment, apparently derived from $a_{1/1R/1}$ through an additional deletion.

(b) *The mitochondrial genomes of spontaneous, homogeneous petite mutants*

(i) *The repeat units and their physical maps*

(1) The results of different partial restriction enzyme degradations of all spontaneous homogeneous petite genomes examined here indicate that they are formed by DNA segments, ranging in size from $0{\cdot}27\times10^6$ to $3{\cdot}2\times10^6$ (and corresponding, therefore, to 0·5% to 6% of the wild-type genome), that are tandemly repeated (head-to-tail). No evidence was found in any case for the presence of inverted repeats (head-to-head) or of sequence rearrangements. The fact that in all cases oligomeric series representing precise multiples of the repeat units were found rules out the possibility that sizeable junction fragments were missed. The absence of such hypothetical fragments was

confirmed by electrophoresis on 6% polyacrylamide gels. Therefore, if such fragments exist, they should be small enough to melt under the experimental conditions used and, therefore, to be missed on the gels. In all cases, restriction enzymes were found which had a single site in the repeat unit; in such digests a single monomeric fragment is found, as expected for a tandem repetition, and not two as expected for an inverted repetition or for a mixed repetition.

(2) The restriction maps of four out of six petite genomes (Fig. 16) provide a direct evidence for the *Hae–Hpa* site clusters of Prunell & Bernardi (1977). It should be noted that the data of Figure 16 should be taken as representing a minimal number of *Hae* and *Hpa* sites. Sequence data are required to assess precisely the actual number of these sites in the clusters. All restriction maps of Figure 16 are characterized by regions that do not contain any of the 12 or so restriction sites routinely checked. That part of these regions correspond to AT spacers is strongly suggested by the extremely low G + C content of the spacers ($<5\%$) and the known sequences of the restriction sites.

(3) It should be pointed out (a) that the orientation of the repeat units of Figure 16 on the physical map of the parental wild-type genome is as yet unknown; and (b) that in the map of $a_{3/1}$ the two fragments external to the genome of $a^*_{1/7/12/1}$ might also be both on the left or on the right side of the map in the parental genome. For these reasons, there is uncertainty as to the exact localization of the excision sites of $a_{3/1}$ and $a_{1/1R/1}$; it is certain, however, that these correspond to (C-C-G-G, G-G-C-C) site clusters, since these form both ends of the segments that cannot be positioned on the wild-type genome map.

(4) The identity of restriction fragments present in the repeat units of the genomes of totally independent petites $a_{3/1}$ on one hand and $a^*_{1/7/12/1}$ and $a^*_{1/7/8}$ on the other raises the possibility that spontaneous petite genomes may arise preferentially from certain regions of the wild-type genome, because these have a higher frequency of excision sites, and/or a site for the initiation of DNA replication. The genome overlapping in heterogeneous petite mutants (Fig. 19) may be due, in part at least, to the same reason. Interestingly, these petite genomes arose from a region of the wild-type mitochondrial genome that has been found (Mathews *et al.*, 1977) to originate spontaneous petites with a very high frequency.

(5) The identity of restriction fragments from the repeat units of genomes present in the same heterogeneous petite, like $a^*_{1/7/12/1}$ and $a^*_{1/7/8}$ or $a_{1/1R/1}$ and $a_{1/1R/21}$ may have two explanations: (a) these genomes may have arisen independently from the parental wild-type genome, in which case this would further support the idea of a preferential localization of the excision of spontaneous petite genomes. (b) Alternatively, and more likely, the simpler genome of each pair may have arisen from the more complex one by a secondary excision; the interesting point here is that similar excision sites appear to be used in the secondary excision as in the original one.

(ii) *The hybridization results*

(1) (a) The main hybridization of the mitochondrial DNA from $a^*_{1/7/8}$ on parental *Hae* and *Hpa* digests took place on fragments having the same size as those of the petite genome. Two to three faint secondary hybridization bands were seen in both digests; none of these corresponded to parental segments located on either side of the repeat unit of $a^*_{1/7/8}$ (see Fig. 16), an evidence against the excision sites being relatively far from the (C-C-G-G, G-G-C-C) clusters delimiting the repeat unit.

(b) Likewise, the main hybridization of the DNA from $a_{1/1R/1}$ took place on parental *Hae* and *Hpa* fragments having the same size as those of $a_{1/1R/1}$. The secondary hybridization was, however, much more important than in the previous case; interestingly, it was remarkably weaker on *Hpa* digests compared to *Hae* digests, perhaps owing to the contiguity of *Hpa* sites with G + C-rich clusters (see section (2), below).

(c) In contrast to the preceding cases, the DNA from $a_{1/1R/Z1}$ did not hybridize on a parental *Hpa* fragment having the same size as that corresponding to its repeat unit, $2{\cdot}7\times10^5$. Instead it took place mainly on the $0{\cdot}49\times10^5$ *Hae* fragment and the $0{\cdot}30\times10^5$ and $0{\cdot}18\times10^5$ *Hpa* fragments that also hybridized the DNA from $a_{1/1R/1}$. Thus, the repeat unit of $a_{1/1R/Z1}$ appears to contain sequences from the $0{\cdot}30\times10^5$ *Hpa* fragment (with which it shares the *Mbo*I site, but from which it differs by being shorter by 3×10^4 and by lacking the *Hae–Hpa* cluster) and also from the neighboring $0{\cdot}18\times10^5$ *Hpa* fragment. The simplest explanation is that the excision of the repeat unit of $a_{1/1R/Z1}$ took place at two sites located at the left of the two *Hpa* sites shown in Figure 16; but alternative explanations cannot be ruled out. In addition, the DNA from $a_{1/1R/Z1}$ showed a secondary hybridization on several of the parental bands hybridizing the DNA from $a_{1/1R/1}$. None of these hybridizations was changed upon a very extensive removal of AT spacers from the $a_{1/1R/Z1}$ genome, a strong suggestion that secondary hybridization is due to one or more of the three G + C-rich clusters found in this genome (Gaillard & Bernardi, 1979).

(d) The main hybridization of DNA from petite b took place on a parental *Hpa* fragment apparently having the same size as the repeat unit of b, $5{\cdot}6\times10^5$ and on a *Hae* fragment, $7{\cdot}6\times10^5$. The ends of the repeat unit of b are therefore likely to correspond either to two isolated *Hpa* sites (possibly located next to G + C-rich clusters) or to a *Hae–Hpa* cluster and an isolated *Hpa* site. The secondary hybridization of b was very strong.

(2) The explanation given above for the secondary hybridization of the DNA from $a_{1/1R/Z1}$, namely that G + C-rich clusters are responsible for it, is likely to be valid for the other petite DNAs as well. None of the alternative explanations appears to be correct: (a) no evidence exists in favor of gene duplications; (b) AT spacers were directly ruled out in the case of $a_{1/1R/Z1}$; other indirect evidences are the fact that decreasing the stringency of hybridization did not cause a preferential disappearance of secondary hybridization and the single hybridization of the DNA from d, which is formed by AT spacers to an extent higher than 90%; (c) (C-C-G-G, G-G-C-C) clusters were largely or totally removed from the *Hae* or *Hpa* fragments on which hybridization was carried out.

(3) The hybridization of the petite genomes under consideration on *Eco*RI, *Hin*cII and *Hin*dIII + III fragments from the parental wild-type genomes, showed that these genomes originated from three different regions. The quasi-identity of *Eco*RI, *Hin*cII, *Hin*dIII and *Bam*HI restriction patterns exhibited by the DNAs from our parental strain A and from strain KL14-4A (a genome that has been mapped by Sanders *et al.*, 1977) justifies the assumption that these genomes have very similar physical maps. In this case, our results show that the DNAs from $a_{1/1R/1}$ and $a_{1/1R/Z1}$ originated from a sector comprised between map positions 27 and 46, where the 15 S RNA gene is localized, the DNAs from $a_{3/1}$, $a^*_{1/7/12/1}$ and $a^*_{1/7/3}$ from a sector comprised between map positions 88 and 95, between the var_1 locus and the 21 S RNA gene. Because of the identity of the large *Eco*RI fragments of the DNA from strains A and B, and their

very likely homology in sequence, it can also be concluded that the DNA from b originated from a sector comprised between map positions 64 and 76 in the *cob-oli*-2 region.

(4) The results of the main hybridization of the DNA from strain b on the *Hae* and *Hpa* fragments of the DNAs from three other wild-type yeast strains (Table 4) confirm the suggestion of Prunell *et al.* (1977*a*) that fragments having identical or similar sizes in different strains are homologous in sequence. The data of Table 6 confirm homologies previously established on the basis of other criteria, and indicate homologies not evident before. Very interestingly, the secondary hybridization of the DNA from b also takes place on fragments of similar size in different DNAs, some of which were considered homologous previously. This suggests a sequence conservation in different strains of the G + C-rich clusters (presumed to be the sequences responsible for the hybridization), having the same localization on the mitochondrial genome of wild-type cells.

(c) *The mitochondrial genome of induced petite mutant d*

Information on the organization of this genome, the only one derived from a strain submitted to mutagen action among those studied here, is very scanty because of lack of restriction sites.

DNA/DNA hybridization showed, however (Fig. 18), that this petite DNA hybridizes to a single *Hpa* and a single *Hae* band (these bands being close to each other in molecular weight) from the DNA of the parent strain. This result is interesting because it should indicate the localization for the seryl tRNA gene which has been detected on it by Carnevali *et al.* (1973). In this connection, it should be noted that micrococcal nuclease degradation has shown that the amount of spacer sequences in this DNA was higher than 90%; and that 10% of it was as high as 8% in G + C, *versus* 4% for total DNA (Prunell, 1976).

5. Conclusions

(a) *The mitochondrial genome of spontaneous, heterogeneous petite mutants*

A comparison of recent and old, spontaneous, heterogeneous petite genomes provides several interesting insights. Recently arisen petite genomes, like those of a_9 and a_{15}, were characterized by the following features.

(i) A low degree of heterogeneity. This is indicated by three facts: (1) the deviation from stoichiometry of *Hae* or *Hpa* fragments is small; (2) the number of new bands in other digests is small; (3) the genomes of two random subclones, $a_{15/1}$ and $a_{15/2}$, showed identical restriction patterns.

(ii) The properties of the genomes of their subclones. In the case of a_{15}, two points are striking: (1) the complexity of these genomes is high, ranging from 10×10^6 for $a_{15/4}$, to 13×10^6 for $a_{15/3}$, to 20×10^6 for $a_{15/1}$, that of the parental petite a_{15} being about 30×10^6 (Table 3); (2) almost all of the *Hae* or *Hpa* fragments of the least complex subclone genome, $a_{15/4}$, are present in the more complex one, $a_{15/3}$, and those of the latter in the most complex one, $a_{15/1}$ (Fig. 3).

Old petite genomes, arisen years before analysis, like a_1 and a_1^* share with the recent ones the fact that the least complex of their subclones show *Hae* or *Hpa* fragments that are contained in the more complex ones. They differ from them however, in two other respects: (1) they have a high degree of heterogeneity, as shown by the very poor stoichiometry of their *Hae* or *Hpa* fragments, and by the large number of *Hin*dII + III fragments in the case of a_1; (2) low-complexity genomes are found in their subclones.

These findings may be interpreted as follows. The relationships found in the *Hae* or *Hpa* restriction patterns of the genomes from subclones indicate that these are essentially subsets of a single petite genome (or of a very small number of genomes), originally excised from a given region of the wild-type genome. We have already mentioned in the previous section that some regions of the latter may be preferentially used in the formation of spontaneous petites. While in this respect, recent and old spontaneous petite genomes do not show large differences, they do so as far as their heterogeneity and the complexity of their subclone genomes are concerned.

The low complexity of subclones from old petites indicates that the excision process originally acting on the wild-type genome continues to operate in the petite mutants, using the same preferential sites (as indicated by the absence or scarcity of new *Hae* or *Hpa* bands) and causes a progressive decrease in the complexity of the genomes present in the heterogeneous population. Such a process appears to come to an end when excision sites become scarce, and stable, simple petite genomes are formed like those of $a^*_{1/7/8}$, $a^*_{1/7/12/1}$ and $a_{1/1R/1}$. It is conceivable that there is a spectrum of excision sites, the most favorable ones being used first.

The other difference between recent and old petite genomes, the larger heterogeneity of the latter, seems to be due to the fact that, as the number of petite genomes in the population increases because of the continuing excision process, a selection takes place leading to the situation actually seen in old petite genomes, in which some genomes predominate over other ones. This suggestion is supported by the changes found in $a_{1/1R}$ as the result of many replatings of $a_{1/1}$. Since some genomes in the population tend to be wiped out, probably because their replication rate is lower, one could expect a general trend for spontaneous petite genomes to become lower in complexity as they age. This is borne out by our experimental results with a_1 and also with a_1^*.

If this general picture of the evolution of petite genomes is correct, as we believe, it is possible that a recent petite of low complexity, like a_3 (complexity of 3×10^6), may either correspond to an old petite which was present for a great number of generations in the wild-type cell population, or have arisen recently as the result of an excision process involving highly preferred and relatively close excision sites.

(b) *The mitochondrial genome of spontaneous, homogeneous petite mutants*

The findings of the present work indicate that the mitochondrial genomes of spontaneous petite mutants belong in three different classes according to the nucleotide sequences that form the ends of the repeat units and that have, therefore, presumably been involved in the excision process.

(i) *Mitochondrial genomes formed by the tandem repetition (head-to-tail) of a DNA segment which is delimited by (C-C-G-G, G-G-C-C) clusters*

This is the case of the petite genomes $a_{3/1}$, $a^*_{1/7/12/1}$, $a^*_{1/7/8}$ and $a_{1/1R/1}$. Here excision of the repeating unit of the petite genome did take place at two (C-C-G-G, G-G-C-C) clusters of the parental, wild-type genome, or at a few nucleotides distance from them.

Under these circumstances, the most likely primary mechanism for the excision of this class of petite genomes, is a site-specific, illegitimate recombination, as previously suggested (Prunell & Bernardi, 1977). Such a recombination event may conceivably involve clusters located on the same genome unit or on two different genome units. In the first case, the nucleotide sequences involved in the recombination process should basically correspond to inverted sequence repeats; in the second case, to direct sequence repeats. As a result of the illegitimate recombination, one would expect to have changes in the (C-C-G-G, G-G-C-C) clusters located at the ends of the repeat unit of the petite genome, compared to the original clusters in the wild-type genome.

(ii) *Mitochondrial genomes formed by the tandem repetition (head-to-tail) of a DNA segment delimited by C-C-G-G sequences*

This situation may be due to the same primary event described above, namely to the excision of the repeating unit of the petite genome at two (C-C-G-G, G-G-C-C) clusters on the parental wild-type genome, the only difference being that G-G-C-C sequences, which are known to be generally internal to C-C-G-G sequences in the clusters (Prunell & Bernardi, 1977) have been lost in the illegitimate recombination process. Alternatively, one (C-C-G-G, G-G-C-C) cluster is involved on one side, the other side being represented by the G + C-rich cluster contiguous to a C-C-G-G sequence; the third possibility is that two sites of the latter kind are involved. The case of the genome of petite b apparently belongs to one of the last two subclasses.

(ii) *Mitochondrial genomes formed by the repetition of a DNA segment delimited by neither (C-C-G-G, G-G-C-C) cluster nor C-C-G-G sequences*

In this case, excision sites are possibly located on G + C-rich clusters not contiguous to C-C-G-G sequences (whose existence has been shown by Gaillard & Bernardi, 1979) or on AT spacers. This may be the case of $a_{1/1R/Z1}$.

The relative frequency of the three different excision modes outlined above cannot be assessed at the present time. If one were to judge this issue on the basis of absence of new *Hae* and *Hpa* bands exhibited by independent, heterogeneous petite mutants, and on the basis of the situations found in all homogeneous, petite mutants isolated so far, the frequency should decrease when going from the first to the second and third excision mode. It should be stressed here that the data on the homogeneous petite genomes concern all the genomes isolated so far in our laboratory, and not a selected set of petite genomes.

(c) *The mitochondrial genome of acriflavine-induced petite mutant d*

The repeat unit of the mitochondrial genome of petite d strikingly differs from those considered above in that it does not contain *Hae* nor *Hpa* sites and is essentially formed by spacer sequences. It is quite evident that in this case, either the original excision event was different from those discussed above, or considerable sequence rearrangements have followed an excision of the types already described. In either case, we consider it very significant that this petite was the result of mutagenization with acriflavine. In fact, it is well known that the tremendous increase in petite formation upon mutagenization (from a few per cent to 100% per generation) is accompanied by extensive fragmentation of the mitochondrial genome (Goldring *et al.*,

1970) and that petites lacking mitochondrial DNA altogether are frequently formed (Goldring *et al.*, 1970; Nagley & Linnane, 1970). Investigations carried out in other laboratories indicate that ethidium bromide-induced petites fall into far more complex excision patterns compared to those found here. For instance, all the 37 induced petites of Lewin *et al.* (1978), not only regularly exhibited new *Hae* and *Hpa* bands, but also frequently showed inverted repetitions of monomer units, multiple deletions and sequence rearrangements. It appears therefore that the primary events originating spontaneous and induced petite mutants are significantly different.

(d) *General conclusions*

The general conclusions of the present work are the following. (1) The primary event in the excision of the defective genomes of spontaneous petite mutants seems to be a crossing-over process involving site-specific, illegitimate recombination concerning the same genome unit or two different genome units. (2) It appears that the sequences concerned by this process most frequently are the (G-G-C-C, C-C-G-G) clusters, but G + C-rich clusters and AT spacers may also be involved. (3) If recombination occurs by an internal crossing-over process, the primary event in the spontaneous petite mutation is very similar to the excision of the lambda prophage from the *Escherichia coli* chromosome or to the dissociation of a transposon from its host plasmid; in this case, the various GC clusters 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. (4) In fact the intervention of such sequences in recombination processes appears to be more general and to account for the divergence of the mitochondrial genome of wild-type cells (Prunell *et al.*, 1977*a*), and for the recombination events occurring in crosses of wild-type cells (Fonty *et al.*, 1978); obviously in these cases, unequal crossing-over events never lead to the elimination of any essential coding sequences. (5) Our original proposal that the petite mutation is the result of an excision process promoted by the presence of nucleotide stretches that share some sequence homology and are present in a certain number of copies per genome unit appears to be correct†.

APPENDIX

Construction of the physical maps of petite genomes

The molecular weights ($\times 10^{-5}$) of the fragments obtained by degradation of petite DNAs with restriction enzymes are given in the following Tables. Underlined values correspond to double bands. The partial *Hae* digestions of the DNA from $a_{3/1}$ were done on final *Mbo*I digests

† Since this paper was completed, we have learned from Dr A. Tzagoloff that work done in his laboratory has shown that the nucleotide sequences of the (C-C-G-G, G-G-C-C) clusters studied so far fit with the predictions of Prunell & Bernardi (1977): (1) in being palindromic; (2) in being present in several copies in the genome; (3) in being contiguous to G + C-rich clusters deprived of C-C-G-G or G-G-C-C sites. Interestingly, G + C-rich clusters are also found around isolated *Hpa* sites.

$a_{3/1}$

Hpa	*Hpa* + *Hae*	Partial *Hae*	*Hae* + *Tac*I	Hae — *Hha*I + *Mbo*I
5·34	5·34	28·0	10·0	10·0
4·99	4·99	26·0	5·40	4·22
4·92	4·42	23·5	4·22	3·23
3·07	3·07	21·3	3·23	2·84
2·78	2·78	20·0	2·84	2·77
2·71	2·71	18·0	2·60	2·00
1·23	1·23	17·0	1·16	1·26
1·14	1·14	15·0	0·92	
1·08	1·08	13·7		*Hha*I + *Mbo*I
0·92	0·92	11·2	*Hae* — *Taq*I	26·0
0·71	0·71	10·0	7·30	1·90
0·65	0·65	7·70	6·58	
0·47	0·47	6·58	4·22	*Mbo* I + *Tac*I
Hae	*Hae* + *Mbo*I	4·22	3·23	12·0
10·0	10·0	3·95	2·84	11·3
6·58	4·22	3·23	2·30	2·70
4·22	3·95	2·84	1·26	2·65
3·23	3·23	2·77	1·00	
2·84	2·84	1·26	0·80	*Hha*I + *Tac*I
2·77	2·77		0·70	12·0
1·26	1·26	*Tac*I		11·3
		12·0	*Hae* + *Hin*fI	4·90
*Mbo*I	*Taq*I	11·3	10·0	2·70
28·0	22·0	5·40	6·58	0·80
	7·30	2·70	4·20	
*Hha*I	2·84		2·84	
28·0	0·80	*Hin*fI	2·60	
		17·0	2·42	
		11·0	0·49	
		2·80	0·29	
		1·00		

$a^{*}_{1/7/12/1}$

Hpa
5·34
4·99
4·92
3·07
2·78
1·23
0·71
0·65
Hae
10·0
6·58
3·23
2·84
1·26

Hpa	Partial *Hpa*	*Hpa* + *Hin*fI
5·34	32·0	5·34
4·99	27·0	4·99
1·23	23·0	0·50
	18·0	0·21
Hae	16·5	
10·0	13·0	*Hae* + *Hin*fI
1·26	11·5	1·00
	10·0	0·49
Hpa + *Hae*	6·60	0·24
5·34	6·40	
4·99	5·34	*Hpa* + *Taq*I
1·23	5·00	4·20
	4·90	3·30
*Tac*I	1·23	1·23
11·3		1·00
	Partial *Hae*	0·80
*Hin*fI	31·0	0·60
11·3	23·0	
	21·6	*Hae* + *Taq*I
*Taq*I	13·0	7·30
7·30	11·5	1·26
3·30	10·0	1·00
0·80	12·6	0·80
		0·75
*Tac*I + *Taq*I	*Hpa* + *Tac*I	
7·30	5·34	*Hin*fI + *Taq*I
2·25	4·99	7·30
1·05	1·1	1·90
0·80		1·53
	Hae + *Tac*I	0·80
*Hin*fI + *Tac*I	10·0	
10·8	1·16	
0·40		

Hpa	Partial *Hpa*
3·01	11·0
1·78	5·80
1·06	4·72
	4·00
Hae	3·01
4·88	1·92
0·98	1·83
	1·78
*Mbo*I	1·23
5·80	1·17
	1·06
Hpa + *Mbo*I	
1·78	Partial *Hae*
1·37	19·4
1·06	18·5
	16·4
Hae + *Mbo*I	15·6
3·10	14·9
1·90	11·9
0·98	11·0
	10·0
Partial *Mbo*I	6·30
19·4	5·80
15·6	4·88
11·0	0·98
5·80	

Hpa
2·71
*Mbo*I
2·71
Hpa + *Mbo*I
1·45
1·37
Partial *Hpa*
13·2
10·6
8·00
5·30
2·71
Partial *Mbo*I
13·2
10·6
8·00
5·30
2·71

Hpa
5·63
*Alu*I
5·63
Hpa + *Alu*I
5·34
0·30
Partial *Hpa*
22·6
16·9
11·0
5·63
Partial *Alu*I
22·6
16·9
11·0
5·63

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