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Temperature can reversibly modify the structure and the functional efficiency of *ori* sequences of the yeast mitochondrial genome

(Recombinant DNA; environment; replication; evolution; direct repeats; *Saccharomyces cerevisiae*; suppressivity; petite mutants)

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SUMMARY

We have compared the suppressibility of three isonuclear spontaneous, cytoplasmic petite mutants of *Saccharomyces cerevisiae*, as measured at three temperatures, 23°C, 28°C and 33°C. The three petites have mitochondrial genomes made up of repeat units which are about 400 bp in size, and carry an origin of replication, *ori*1. This *ori* sequence is intact in petite Z1, whereas it lacks GC cluster A in petite 26 and cluster A plus some contiguous nucleotides in petite 14. These deletions lead to the impossibility to form a stem-and-loop structure of the *ori* sequence, the 'A-B fold', which involves two GC clusters, A and B, and the nucleotides in between. Instead, a 'replacement fold', only involving AT base pairs, is feasible. In petites 14 and 26, suppressivity decreases when the temperature is raised from 28°C to 33°C, and increases when the temperature is lowered from 28°C to 23°C. In contrast, no changes are seen in petite Z1. These temperature effects correlate with the stability of the 'A-B fold' and the instability of the 'replacement folds'. Since suppressibility measures the replicative competitiveness of the petite genome relative to the wild-type genome, these results indicate that an environmental parameter, temperature, can reversibly affect the structure and the functional efficiency of *ori* sequences in vivo.

The evolutionary implications of these findings are discussed.

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Abbreviations: bp, base pair(s); Δ, deletion; *ori*, origin of DNA replication; wt, wild type.

INTRODUCTION

In evolution the environment mainly acts as a selection agent; organisms adapt to diverse environments by being selected for. The environment can also cause, in a direct or indirect way, chemical alterations in the genome, as exemplified by the action of mutagens. Here, we describe another way in which the environment can affect the genome, namely by directly inducing changes in its higher-order structures. These genome transconformations are reversible and non-inheritable. Nevertheless, they can dramatically modify a basic genome function, such as replication, and so provide selective advantages or disadvantages in the absence of changes in the primary structure of DNA.

The present work concerns the effect of environmental temperature on the replicative ability of the mitochondrial genomes of three isonuclear, spontaneous, cytoplasmic petite mutants of *S. cerevisiae*, a1/1R/14, a1/1R/1/26 and a1/1R/Z1, which will henceforth be referred to as petites 14, 26 and Z1, respectively. The primary structure of the mitochondrial genomes of these petites had been previously determined in our laboratory (Gaillard et al., 1980; de Zamaroczy et al., 1981; 1983; 1984). Each one of these genomes arose by the excision of a DNA segment from the mitochondrial genome of yet another petite, a1/1R/1 (see Fig. 1). As is the rule for spontaneous, cytoplasmic petites (Gaillard et al., 1980; de Zamaroczy et al., 1983), these excisions (i) took place by illegitimate recombination events involving pairs of direct repeats (shown in Fig. 1) and (ii) were followed by tandem amplifications of the excised segments, which became the repeat units of the newly formed genomes of the secondary petites Z1, 14 and 26. While the repeat unit of the parental petite genome is 884 bp long, the repeat units of petites Z1, 14 and 26 are 416, 392 and 398 bp long, respectively. In the latter three cases, each repeat unit corresponds to about 0.5% of the wt mitochondrial genome and only contains about 100 bp in addition to one *ori* sequence, *ori*1. (This is one of the four active *ori* sequences of the mitochondrial genome of the parental wt strain A; de Zamaroczy et al., 1981; 1984.) The amount of mitochondrial DNA in these petites being roughly the same as in wt cells, these petites contain about 50 times (200/4) more active *ori* sequences than the parental wt cells.

While each repeat unit of the mitochondrial genomes of petites a1/1R/1 and Z1 contains complete *ori* sequence, those of petites 26 and 14 contain *ori* sequences which had lost 11 and 27 bp, respectively, in the excision process. In the case of petite 26, the deletion comprises GC cluster A; in the case of petite 14, it comprises also part of the neighboring sequence *p* (de Zamaroczy et al., 1981; 1984; Figs. 1 and 2).

In the complete *ori* sequences of *ori*$^+$ petites a1/1R/1 and Z1, a secondary structure, the 'A-B fold', is possible (de Zamaroczy et al., 1981; 1984). This is a stem-and-loop structure, comprising 11 AT bp and 6 GC bp, which is formed by GC clusters A and B and the sequence in between (Figs. 1, 2 and 3). In contrast, the formation of this structure is impossible in the *ori*$^-$ genomes of petites 14 and 26, which can form, however, 'replacement folds' made of pure AT (Fig. 3) and endowed with a lesser thermodynamical stability (especially in the case of petite 14) compared to the A-B fold (de Zamaroczy et al., 1984).

EXPERIMENTAL

The *ori*$^-$ mitochondrial genome of petites 14 and 26, which lack a normal feature of the *ori* sequence, the A-B fold (this feature is perfectly conserved in all *ori* sequences; de Zamaroczy et al., 1981; 1984), do exhibit lower replicative abilities compared to the *ori*$^+$ mitochondrial genomes of petites a1/1R/1 and Z1 (as well as of all *ori*$^+$ petite genomes having repeat units shorter than about 1000 bp; Rayko et al., 1988). This can be judged by the suppressivity test (Ephrussi et al., 1955; this was carried out here as described by Rayko et al., 1988; see also footnote to Table I), in which petite mutants are crossed with wt (grande) cells. In this test, the degree of suppressivity, namely the % of diploid petites (which carry in every case the parental petite genome; Goursot et al., 1980; de Zamaroczy et al., 1981; Rayko et al., 1988) in the progeny, is determined by the replicative efficiency of the petite genome relative to the mitochondrial genome of the wt cells used in the cross (Bernardi et al., 1980; Blanc and Dujon, 1980; de Zamaroczy et al., 1981). Indeed, when crossed with wt cells, *ori* petites 14

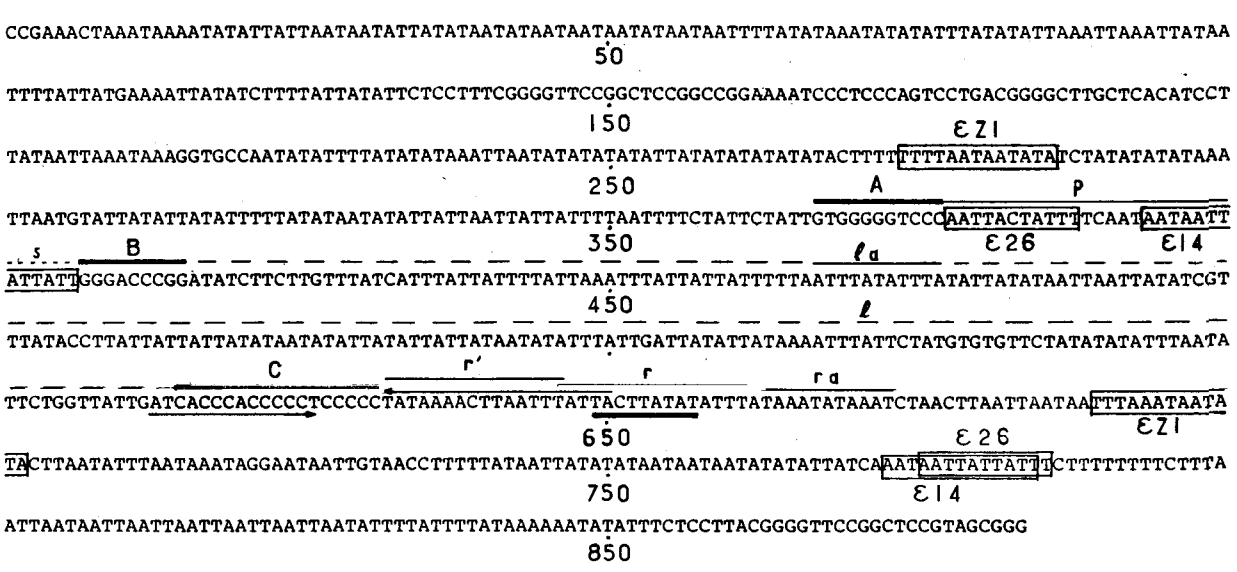

Fig. 1. The repeat unit of the mitochondrial genome of primary petite a1/1R/1 (see Gaillard et al., 1980) is 884 bp in size and contains an *ori* sequence, *ori*1. This comprises three GC clusters, A, B and C (heavy overlines). Cluster B is separated from cluster A by two short AT sequences *p* and *s*, and from cluster C by a long AT sequence *l* (broken overline). Cluster C is followed by sequence *r* (light overline) which comprises, on the strand shown, a nonanucleotide (heavy underline) where transcription of the RNA primer of one nascent DNA chain starts (arrow pointing to the left); transcription of the RNA primer for the other nascent DNA chain starts before cluster C (arrow pointing to the right) (Baldacci et al., 1984). Sequence *r* is followed by a sequence *ra* which might pair with sequence *la* (within the *l* sequence) to form a hypothetical tertiary structure (de Zamaroczy et al., 1984). In the case of *ori*1, sequence *r* is preceded by a duplication, sequence *r'*.

Excision sequences (ε) of the secondary mitochondrial genomes from petites 14, 26 and Z1 (see Gaillard et al., 1980; de Zamaroczy et al., 1984) are indicated by boxes.

and 26 yield progenies comprising only 79% and 85% diploid petites, respectively, instead of 99% produced by crosses involving *ori*$^+$ petites a1/1R/1 and Z1 (Table I). Incidentally, much more dramatic decreases in suppressivity are associated with petite genomes deleted in the crucial C cluster region (de Zamaroczy et al., 1981), or lacking *ori* sequences altogether; these petite genomes replicate by making use of surrogate origins of replication.

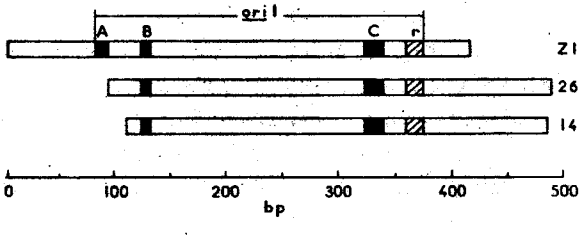

Fig. 2. Scheme of the repeat units of the mitochondrial genomes of petites Z1, 26 and 14. A scheme of the *ori*1 sequence, with its main elements (GC clusters A, B, C [black boxes] and sequence *r* [hatched box]), is also represented.

In view of the presumed lower thermodynamical stability of replacement folds compared to the A-B fold, crosses of petites 14 and 26 with wt cells were also performed at both a higher (33°C) and a lower

TABLE I

Suppressivity of petites 14, 26 and Z1 at different temperatures

Temperature[a] (°C)	Suppressivity (%)[b]		
	Petites: 14	26	Z1
23	90	89	99
28	79	85	99
33	45	58	97

[a] Temperatures indicated were applied to precultures and to cultures used in crossings and in the incubation of diploids. Precultures in stationary phase were diluted ten times upon setting up the cultures used in crossings.

[b] Suppressivity was tested by crossing petite strains (*MAT*a, *ade1*) against the grande strain B (*MAT*α, *trp1*, *his1*). The percentage of petite/total diploid colonies was determined by counting about 1000 colonies on minimal agar.

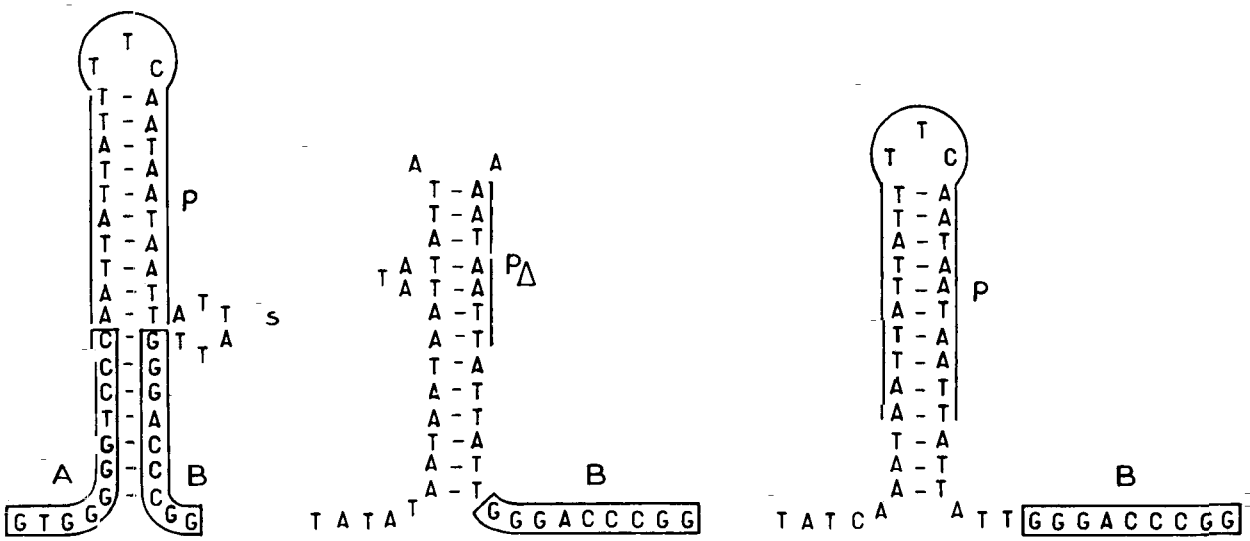

Fig. 3. Potential secondary structure of the 'A-B fold' of the *ori* sequence present in the repeat units of mitochondrial genomes from petites a1/1R/1 and Z1 (*ori*1) and of the 'replacement folds' which can be formed in the *ori*1 sequence present in the mitochondrial genomes of petites 14 and 26. In the case of petite 14, the residual nucleotides from the partially deleted *p* stretch (*pΔ*) can generate a hairpin structure with nucleotides from the preceding repeat unit, but the stem, only formed by 13 AT nucleotides, carries different terminal and side loops compared to the A-B fold. In the case of petite 26, the upper part of the stem and terminal loop are identical to those of the A-B fold, but the lower part is replaced by three A:T pairs (three nucleotides are derived from the preceding repeat unit). The sequences involved in the structure shown (GC clusters A and B, sequences *p* and *s*) are those indicated in Fig. 1.

(23°C) temperature than the standard one, 28°C. The results of Table I indicate that such temperatures, respectively, decrease and increase the replicative ability of the two mutants, whereas that of the control *ori*$^+$ petite Z1 did not show any significant change between these temperatures (Table I). The decrease of the replicative ability was stronger for petite 14 than for petite 26, as expected from the supposed lower thermodynamical stability of the replacement fold of the former. In the first case, the % of wt colonies increased from 10% to 55% between 23°C and 33°C, in the second from 11% to 42%. The changes in replicative ability just described are immediate and reversible (as shown by control experiments).

DISCUSSION

The results presented indicate that an environmental factor, temperature, can reversibly affect the replicative ability of a genome by altering its secondary (and, possibly, its tertiary) structure. Indeed, (i) these changes cannot be ascribed to enzymes involved in DNA replication, as in temperature-sensitive mutants, since the petites discussed here are isonuclear, and lack mitochondrial protein synthesis, like all petites; (ii) the different effects of temperature on the replicative ability of petites Z1, 14 and 26 show an excellent correlation with those expected from the secondary structures of the postulated A-B fold and replacement folds (de Zamaroczy et al., 1981; 1984), an effect on tertiary structures being also possible.

It should be pointed out that the repeat unit of petite Z1 extends 80 bp to the left of cluster A and only 40 bp to the right of sequence *r*, whereas those of petites 26 and 14 extend 115 bp to the right of sequence *r* (Fig. 2); in all cases, however, these extensions to the left of *ori* sequences are just made of AT spacer. Effects of flanking regions of *ori* sequences on the replicative ability of the latter have been demonstrated (Rayko et al., 1988), but they are negligible compared with the different suppressivities exhibited by petites 14 and 26 relative to petite Z1. There is, therefore, no doubt that these differences are due to the deletions in the *ori* sequences of petites 14 and 26.

An alternative explanation for the results reported here would be that an unchanged replication system would interact differently — at different temperatures — with the different primary sequences of the canonical and partially deleted *ori* sequences. This requires, however, a strong temperature coefficient of the protein–DNA interaction accompanied by an invariance of the secondary/tertiary structure of the deleted *ori* sequence, whereas the temperature coefficient in the case of the interaction with the canonical *ori* sequence would be negligible.

Interestingly, the conclusion that DNA secondary structure is required for *ori* activity in vivo has also been very recently reached for bacteriophage G4, where a strong temperature-dependent impairment of replication was found after introducing by site-directed mutagenesis point mutations which destabilize intrastrand base-pairing in the *ori* sequence (Lambert et al., 1987). In the latter case hairpin formation concerns single-stranded and not double-stranded DNA. The possibility should be left open, therefore, that hairpin formation in the petite genomes discussed here may be affected in the single-stranded DNA made during DNA polymerase action. This would not change, however, the basic conclusion that temperature differentially and reversibly affects secondary DNA structures as present during the replication of the mitochondrial genomes of petites 14 and 26.

From a general viewpoint, these results indicate the existence of a novel type of environment–genome interaction, in which reversible changes in higher-order DNA structures are induced with profound consequences on a basic genome function, such as replication. These changes concern genome transconformations which, although non-inheritable, can be maintained for many generations in the presence of the appropriate environmental condition. Genome transconformations can provide, therefore, strong selective advantages or disadvantages and play an important role in evolution, independently of classical mutations, which involve changes in the primary structure of DNA.

It should also be noted that genome transformations of the type just described are likely to be found in organelle genomes from other poikilothermic organisms and also in prokaryotic genomes, which have a similar nucleoprotein organization and can replicate at widely different temperatures. Moreover, similar phenomena might (i) be induced by other environmental factors; (ii) affect other genome functions (e.g., transcription); and also (iii) be operative in other organisms.

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