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THE REPLICATION OF YEAST MITOCHONDRIAL DNA

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Introduction

Investigations from our laboratory have shown the presence of seven ori sequences in the mitochondrial genome of wild-type Saccharomyces cerevisiae (Fig.1). Ori sequences have been mapped and oriented on the mitochondrial genome and their primary structure has been determined (see ref.1 for a brief review). So far, evidence for the involvement of ori sequences in DNA replication, has been strong, yet indirect. Essentially, it rests on the following findings : a) the ori sequences of the mitochondrial genome of yeast show structural similarities with those of animal cells (heavy

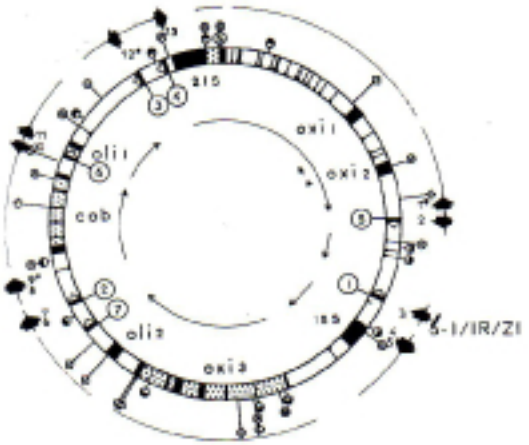

Figure 1. Physical map of the mitochondrial genome of wild-type S. cerevisiae strain A. The inner circle indicates stable transcripts. Circled numbers refer to the ori sequences. The outer circle indicates the sequenced regions of this genome. The location of the repeat unit of the mitochondrial genome of a-1/1R/Z1 is indicated. The location and the orientation of sequences r are indicated by arrowheads pointing in the direction of its TAT end. For further details, see ref. 2.

strands) ; b) at least one ori sequence is present on the repeat units of the mitochondrial genome of the vast majority of spontaneous petites ; it should be stressed, however, that ori 7, was never found alone in a petite genome and ori 4 and ori 6, were only rarely found alone ; c) a correlation exists between the suppressivity of a petite and the intact or altered state of the ori sequence present in its repeat units ; ori^+ petites, containing an intact ori sequence, are highly suppressive (less so, however, in the case of ori 6 ; ori 4 and ori 7 were never tested because not available in haploid petites) ; ori^- petites, deleted in cluster A or cluster C of their ori sequences (see Fig.2) were, respectively, slightly and very much lower in suppressivity than the corresponding ori^+ oetites ; ori^r petites, having repeat units with inverted ori sequences and ori° petites lacking altogether any of the seven canonical ori sequences were low in suppresivity ; d) ori sequences contain on one strand a 16-bp sequence, r, where transcription is initiated (2) ; such sequence is interrupted by the insertion of a GC-cluster, γ, and is not operational in ori 4, 6 and 7.

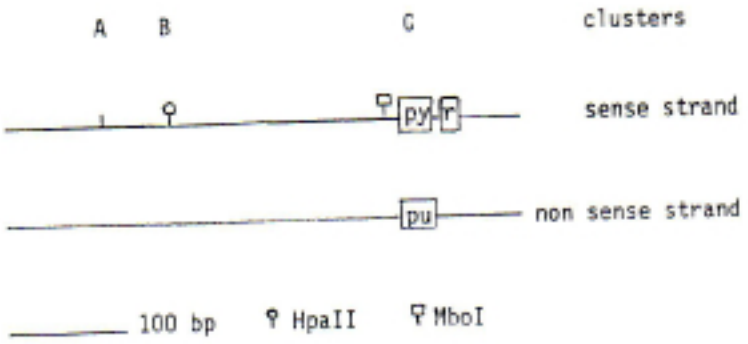

Figure 2. Schematic map of the repeat unit of the mitochondrial genome of petite a-1/1R/Z1. The position of clusters A, B and C of ori 1, and of some restriction sites are indicated. Boxes py, pu refer to the oligopyrimidine and oligopurine tracts of cluster C ; box r to the sequence of transcription initiation.

To sum up, the indirect evidence that ori sequences are involved in DNA replication rests on their similarity with bona fide replication origins, on their expected presence in the repeat units of the vast majority of petites, on the correlation between the intact or altered state of the ori sequence and the level of transmission of the petite mitochondrial genome to the progeny of crosses with wild-type cells, and on the expected association between the sites of initiation of DNA replication and RNA transcription.
Here we report the first direct evidence that, indeed, ori sequences of the mitochondrial genome of yeast are origins of DNA replication, since sites located on both strands in the neighborhood of cluster C are starting points for nascent DNA strands which are preceeded by short primer RNA sequences. The study reported here was done using the mitochondrial genome of petite a-1/1R/Z1. Figure 2 provides a schematic map of the repeat unit, containing ori 1, of this petite.

Materials and Methods

Petite a-1/1R/Z1, culture media and cell growth, preparations of mitochondrial nucleic acids, enzymatic treatment, techniques for DNA labeling and DNA-DNA and DNA-RNA hybridizations were previously described (2-4). Strand separation and DNA sequencing were performed according to Maxam and Gilbert (5).

Results

Mitochondrial DNA from petite a-1/1R/Z1 was degraded with Hpa II, an enzyme cutting every repeat unit at a site located in cluster B of the ori 1 sequence (Fig.2). The fragments so obtained were 5' labelled with P^{32} ; their strands were separated and used in hybridization experiments with total nucleic acids, RNA, or DNA, as present, respectively, in untreated, pancreatic DNas-treated, and pancreatic-treated mitochondrial phenol extracts from petite a-1/1R/Z1. Hybrids were then degraded with S1-nuclease, heat-denatured and submitted to high-voltage electrophoresis in parallel with partial chemical depurinates of the labelled strands.

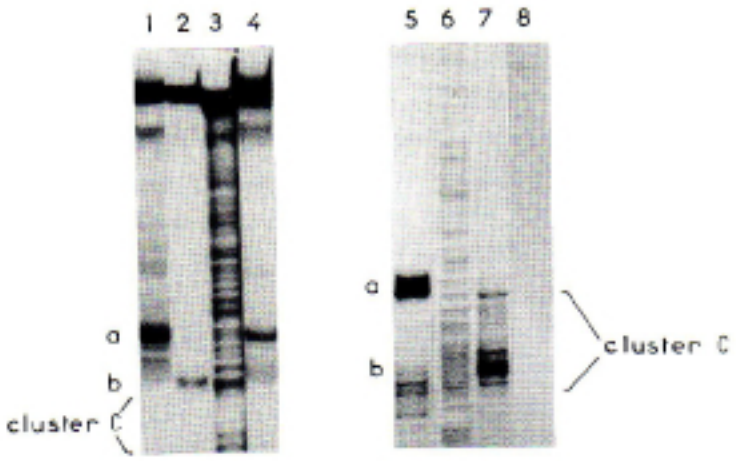

Figure 3. Autoradiograms of S1-nuclease degraded hybrids formed by end-labelled separated strands of Hpa II-degraded DNA from petite a-1/1R/Z1 with total nucleic acids, RNA and DNA from mitochondrial of the same petite. See text for further explanations.

The results (Fig.3) were different with hybrids obtained when the labelled strand was the sense strand used as a template in RNA transcription, namely the strand containing the oligopyrimidine tract of cluster C (2 ; Fig.2), or the other strand.

In the first case, hybrids with RNA yielded a major band (Fig.3 ; lane 4) corresponding to a fragment about 240-bp in size, which ended about 20 nucleotides 3' of cluster C (Fig.4). The same band was found when total nucleic acids were used in hybridization (Fig.3 ; lane 1) , showing that the protection of the labelled strand in this experiment was essentially due to RNA molecules. In contrast, when mitochondrial DNA was used in hybridization, the S1-protected labelled strand ended only very few nucleotides 3' from cluster C. (Fig.3 ; lane 2 and Fig.4).

In the second case, no detectable protected strands were found when DNase-treated mitochondrial nucleic acids were used in hybridization (Fig.3 ; lane 8). This is in agreement with our previous study that the strand carrying the oligopurine tract of cluster C is not detectably transcribed (2). When total nucleic acids were used in hybridization, the protected strand ended just before cluster C (Fig.3 ; lane 5; Fig.4).

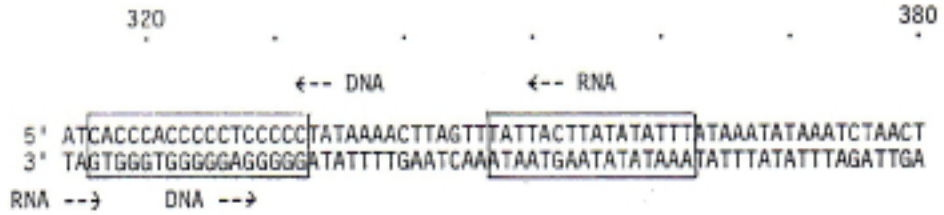

Figure 4. Primary structure of cluster C and sequence r of the ori 1 sequence. The position corresponding to the 5' ends of hybrids resistant to S1-nuclease degradation are indicated by arrows showing the 5' to 3' direction of newly synthesized RNA and DNA molecules.

Finally, when RNase-treated extracts were used in hybridization, the strong band of lane 5 (Fig.3) just described was replaced by another band (Fig.3 ; lane 7) corresponding to a shorter fragment ending inside cluster C (Fig.4).

Several controls were performed. First, self-annealed probes were digested with S1-nuclease to detect any material protected by foldback. Second, the activity of S1-nuclease was tested by showing that the enzyme was able to remove the end label from hybrids reconstituted from the two separated probes. Third, hybrids were reconstituted by using strands derived from an MboI digest and complementary strands derived from an end-labelled Hpa II digest ; after S1-nuclease digestion, the protected hybrids had the expected lengths.

Discussion

The present results show the existence in the mitochondrial of petite a-1/1R/Z1 of a population of DNA molecules having the size of the repeat units and identical ends. The presence of DNA molecules having such a size in DNA preparations from petite mutants is well-known (2). Their identical ends indicate that they are initiated at specific sites. Our experiments map the 5' ends at the level of cluster C of ori. Analogous

experiments aiming at detecting RNA molecules allowed us to find two starting points of transcription, one on each strand, about 20 nucleotides upstream of the sites where 5' ends of DNA molecules map.

The fact that the non-sense strand [i.e. the strand having the oligopurine tract in cluster C does not produce long transcripts, but only short ones which are degraded by RNase, indicates that its replication starts with an RNA primer, which is still present in the majority of monomer DNA molecules. RNase treatment allows the identification of the transition points (RNA → DNA) and the demonstration of intermediate-size nascent DNA molecules in which some ribonucleotides are replaced by deoxyribonucleotides (see the band ladders of Fig.3 ; lane 7).

As far as the sense strand is concerned, the situation is complicated by the presence of transcripts having the size of the repeat units and which start at sequence r. These transcripts are the only ones evident in untreated phenol extracts ; upon RNase treatment, they disappear to be replaced by slightly shorter DNA fragments. The sense strand appears, therefore, to produce both full-length transcripts and RNA primers for DNA replication, the start points being the same.

To sum up, both strands act as templates for a DNA replication which involves short RNA primers having their 5' ends in the neighborhood of cluster C. The critical importance of cluster C and of the neighboring sequences had already been stressed by the dramatic drop in suppressivity and transcription of petites which have lost these segments of their ori sequence (1,2). It is conceivable that the stem-and-loop structure formed by the cluster A-cluster B region acts instead as a recognition sequence. In any case, the loss of cluster A does not affect replication and transcription to any comparable extent (1,2). Interestingly, it is possible that the putative recognition sequence formed by the cluster A-cluster B region is close in space to the initiation of replication and transcription sequences formed by cluster C and sequence r, because of the interaction between two complementary inverted 15-bp sequences located at positions 167-181 and 364-378, respectively (see Fig.4 and ref.1). These sequences were also found (6) in the ori sequence of the mitochondrial DNA of Candida utilis, and (in one copy only) in yeast ars 2 (7).

In turn, the RNA initiation sequence r can be folded in a short loop-and-stem structure, also found in the other r sequence of the mitochondrial genome (2).

Results similar to those discussed in this paper have been obtained in the case of petite mutants containing ori 5 in their mitochondrial repeat units.

REFERENCES

1. Bernardi, G. : Trends in Biochem. Sciences 7, 1-5 (1982)

2. Baldacci, G., Bernardi, G. : EMBO Journal 1, 987-994 (1982)

3. Faugeron-Fonty, G., Culard, F., Baldacci, G., Goursot, R., Prunell, A., Bernardi, G. : J. Mol. Biol. 134, 493-537 (1979)

4. Gaillard, C., Bernardi, G. : Mol. Gen. Genet. 174, 333-337 (1979)

5. Maxam, A.M., Gilbert, W. : Proc. Natl. Acad. Sci. USA 74, 560-564 (1977)

6. Tikhomirova, L.P., Kryukov, V.M., Strizhov, N.I., Bayev, A.A. : Mol. Gen. Genet. 189, 479-484 (1983)

7. Tschumper, G., Carbon, J. : J. Mol. Biol. 156, 293-307 (1982)

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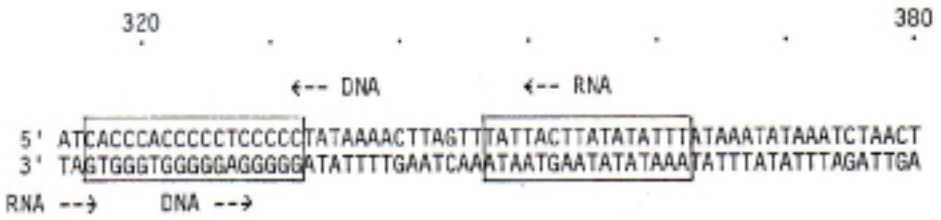

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