PHYSICAL MAP OF CIRCULAR MITOCHONDRIAL DNA FROM *NEUROSPORA CRASSA*

Ulrich BERNARD, Alfred PÜHLER, and Hans KÜNTZEL

Max-Planck-Institut für experimentelle Medizin, Abteilung Chemie, 34 Göttingen, and Institut für Mikrobiologie, Universität Erlangen-Nürnberg, West Germany

Received 13 October 1975

1. Introduction

Mitochondrial DNA (mtDNA) from *Neurospora crassa* has been shown by denaturation mapping to consist of a homogeneous population of circular molecules (mol. wt 41 × 10^6) exhibiting a characteristic distribution of asymmetric AT-rich sequences [1,2].

Although this DNA is almost completely degraded during isolation by cuts at randomly distributed sites, as revealed by denaturation mapping of linear fragments [1,2], it can successfully be analyzed by restriction endonucleases which convert most of the unspecific fragments into specific ones [3]. The sum of the contour lengths of the eleven fragments produced by Eco RI has been shown to be identical to the length of the circular molecules [3]. In this study we have mapped the positions of the four largest Eco RI fragments by comparing denaturation patterns. The positions of two other Eco RI fragments and of the largest fragment obtained by restriction endonuclease Hin III [14] were determined by partial and double digestion experiments.

2. Materials and methods

Growth of *Neurospora crassa* (wild type strain 5256) [1–3], isolation and electron microscopy of mtDNA have been described [1–3]. DNA was digested with Eco RI in a medium containing 100 mM Tris–HCl, 50 mM NaCl, 10 mM $MgCl_2$, pH 7.5, (and with Hin III in the same medium except that Tris–HCl was 10 mM) at 37°C for 2 h. Both enzymes were kindly donated by Dr H. Bujard (Heidelberg) and later purchased from Miles.

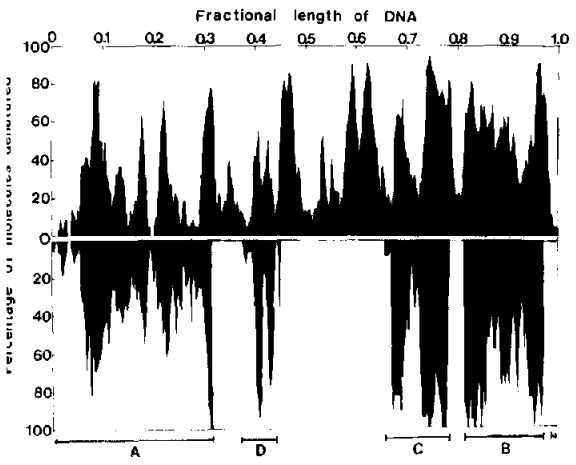
Computer analysis of contour length data of partially denatured DNA molecules was performed as described [1,2] except that the circular molecules were divided into 300 instead of 100 sections. Gel electrophoresis was performed in 0.5% agarose (MCI-SeaKem) containing 36 mM Tris–HCl, 30 mM sodium phosphate, 1 mM EDTA, pH 7.8, for 16 h at 4°C, 30 V, in glass tubes of 1 cm diameter. DNA was reisolated from individual bands as already described [6].

3. Results and discussion

We have recently reported that the length distribution of Eco RI digested mtDNA exhibits four maxima of contour lengths between 1.5 and 7 μm, and that these correspond to the four largest fragments A to D resolved by agarose gel electrophoresis [3]. The same distribution is obtained if the DNA is partially denatured at 49°C after digestion with EcoRI.

All analyzed molecules of the size of fragment A turned out to be almost identical in their denaturation patterns, indicating that these molecules were free of unspecific fragments derived from different regions of the genome [2]. With decreasing fragment lengths the relative content of molecules containing unrelated denaturation patterns increased up to 20% in peak D [2]. From each maximum (A to D) at least 20 molecules having apparently similar patterns were selected and analyzed with the help of a computer program [1,2] in order to construct denaturation maps of the four largest Eco RI fragments.

The fragment maps are shown in the lower part of fig.1. By visual comparison of the patterns of the cir-



'ig.1. Denaturation maps of circular mtDNA and its four ırgest Eco RI fragments A to D. The maps were constructed y quantitative computer analysis as described [1,2]. A .milar program was used to determine the optimal position or the fragments along the linearized circular map by omparing denaturation patterns (manuscript in preparation).

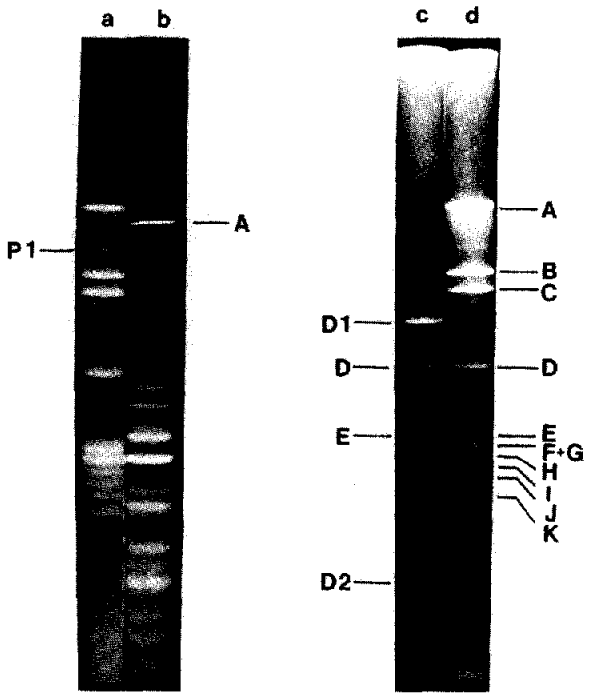


Fig.2. Agarose gel electrophoresis of mtDNA digested by restriction endonucleases. (a) Partial digest by Eco RI. (b) Complete digest by Hin III. (c) Digest of Hin III fragment A by Eco RI. (d) Complete digest by Eco RI. For details see Methods.

ular (linearized) molecules and the fragments it turns ut that there is only one possible location of the ırgest fragment A. The visual identification of the ositions of the three other fragments is more diffiult, but by quantitative computer analysis (details ill be published elsewhere) all four fragments can ptimally be arranged along the circular map as shown ı fig.1.

The positions of the smaller Eco RI fragments (E ɔ K) could not be determined by this method ecause their denaturation patterns were not suffiiently characteristic.

A possible position for one of these fragments is he gap between Eco B and C (fig.1). In order to lentify this fragment we have produced a partial :co RI digest. The agarose gel shown in fig.2a exhibts several fainter bands in addition to the eleven end ıroducts (fig.2d). One of these bands (P1) has a molecılar weight of 9.4×10^6. Although the complete igestion of re-isolated P1 has not yet been performed, : can be assumed that P1 contains both B and H mol. wt 7.7 and 1.7×10^6) [3]. Since the gap ıetween A and B is too small (mol. wt about $).4 \times 10^6$) to account for one of the eleven fragnents, we have placed H between B and C (fig.3).

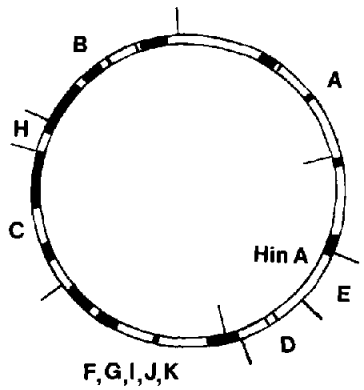


Fig.3. Physical map of circular mtDNA. The black regions represent AT-rich segments which are single-stranded in more than 50% of all analyzed molecules after heating at 49°C. The outside letters represent the eleven Eco RI fragments.

Additional information can be obtained by digestion with another restriction endonuclease, Hin III. This enzyme, which recognizes the sequence A↓AGCTT [4], produces one large fragment of mol. wt 11×10^6 (Hin A) together with about 20 smaller fragments which could not be completely resolved (fig.2b).

The fragment Hin A was re-isolated and digested with Eco RI. Fig.2c shows that it contains the Eco fragments D and E together with two other fragments (D1 and D2). Since the denaturation map of fig.1 exhibits a gap between A and D corresponding to a fragment of the size of E (mol. wt 2.1×10^6) it is safe to map E between A and D. The largest subfragment of Hin A, D1 (mol. wt 5×10^6), can only overlap with Eco A since all possible neighbours at the left side of D, Eco fragments F, G, I, J, K (fig.3), are smaller than D1. Hence the remaining subfragment D2 (mol. wt 0.6×10^6), which is difficult to visualize on gels (fig.2c) because of its small size, can be placed at the left side of D. The position of D1 was confirmed by digesting re-isolated Eco RI fragment A with Hin III.

The data are summarized in the circular map shown in fig.3. The seven mapped fragments cover more than 80% of the genome. The order of the five small Eco fragments between Eco C and D remains to be determined.

The finding that Hin III produces only fragments smaller than Eco D, except for Hin A, suggests that several additional cleavage sites of Hin III map within the regions of Eco fragments A, B and C. Furthermore the map also shows that the Eco fragments A, D and E have a much higher G + C content than fragments B and C.

The identification of restriction fragments carrying genes for mitochondrial ribosomal RNA molecular hybridization has been reported in the yeast system [5]. Similar experiments are in progress to map mitochondrial genes for ribosomal and messenger RNA in *Neurospora crassa.*

Acknowledgement

We thank the Deutsche Forschungsgemeinschaft for support and A. von Gabain for valuable advise.

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