Characterization of Mitochondrial DNA from the Pika
(Ochotona rufescens rufescens)
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DNA, Mitochondria, Pika

Further to anatomical and physiological studies performed on the pika (Ochotona rufescens rufescens), a new laboratory animal, the main characteristics of the mitochondrial DNA from its liver are defined. The buoyant density of this DNA is 1.695 g/cm³, its length 5.30 am, i.e., 3.17 times that of the replicative form of ϕX 174. It could have approximately 16500 base pairs.

The DNA from the pika is very similar to that of the rat or the rabbit, although these animals show great physiological differences.

Introduction

Pikas are Lagomorphs from Central Asia, living at an altitude of 1800—4000 m. They have been brought back to France by one of us and acclimatized in our laboratory to be used as new experimental animals. In experimental toxicology, each animal species is liable to react differently to the same drug, so that the pika could find its original status among other well-known laboratory materials. Anatomical and physiological experiments have been already carried out on the pika. Studies on its histological characters have shown that red cells are small and more numerous than in the Wistar rat or the rabbit bred under the same conditions and at the same altitude. The pikas bred at a low altitude have thus kept the same characters as those living in the highlands from Central Asia. It was therefore highly interesting to check whether life at a high altitude has any influence on cellular respiration and on the development of the mitochondria in this animal.

Electron microscopic observations revealed well-defined differences in the size of the mitochondria, between the pika and the rat. The mitochondria extracted from pika livers and then stained with 4% phosphotungstic acid pH 7 were larger than those of the rat (Fig. 1*). One could infer from this that the same differences could affect the respiration of isolated mitochondria. All our studies have shown that QO₂ and ADP/O values are lower in the pika than in either the rat or the rabbit. However, these are still preliminary works, which do not allow us to draw any definite conclusion. These data could be related to the different evolutions followed by the rat, the rabbit and the pika along parallel phyla. In addition to these studies, it was also of interest to define some characteristics of the mitochondrial DNA from the pika, whose size and topological homogeneity are remarkable, and compare them with those of mitochondrial DNAs from other species. Moreover, such data could be used in studies on the drugs that interact with DNA and more particularly with mitochondrial DNA.

Material and Methods

The animals used were:

Afghan pikas (Ochotona rufescens rufescens) bred in our laboratory,
Wistar albino rats from the C.S.E.A.L. (Centre de Sélection et d’Elevage des Animaux de Laboratoire, 45, Orléans-la-Source, France),
“Petit Russe” rabbits bred in our laboratory.

The mitochondria were prepared by the method of Schneider, Mitochondrial DNA was extracted from mitochondria isolated and suspended in S.S.C. (standard saline citrate: 0.15 M sodium chloride, 0.015 M sodium citrate) by shaking for 2 x 10 min with 0.3, then 0.2 volume of 80% phenol in S.S.C (adjusted at pH 9) at room temperature. The aqueous layer was removed, then dialyzed for 24 h against 4 x 11 S.S.C. Mitochondrial DNA was isolated and purified by ultracentrifugation in a cesium chloride-propidium diiodide gradient. After centrifugation for 60 h at 36000 rpm at 18 °C in the 40 rotor of the Spinco model L centrifuge, the band

* Fig. 1 see Plate on page 146 a.
of circular closed mitochondrial DNA was directly removed by piercing the tube with a needle at the level of the band of mitochondrial DNA. Propidium diiodide was discarded by dialysis against a buffer (1 M NaCl, 0.01 M Tris, 1 mM EDTA), then by application to a column of Dowex 50 W x 8.

Analytical ultracentrifugation

It was carried out in Beckman Model E analytical ultracentrifuge in CsCl, at 44700 rpm for 18 h at 20 °C, with DNA from Micrococcus lysodeikticus (φ: 1.731 g/cm³) added as a marker.

Electron microscopy

The twisted circular molecules of mitochondrial DNA were transformed into circular open molecules under the influence of γ rays (4000 rad).

The technique used for spreading is a modification of the method of Lang and Mitani: drops of 50 μl of 0.1 M ammonium acetate containing 0.3 μg/ml of cytochrome C (Sigma) and in some cases 0 – 50% of denaturing agent (formamide – Fluka), are deposited on a very clean hydrophobic surface. The molecules of DNA embedded in a sheath of cytochrome C come to the surface of the drop and are picked up on carbon grids when the grid and the drop enter into contact. The grid is dried in absolute alcohol, then rotary shadowed with platinum.

A mixture of mitochondrial DNAs from pikas and rats was spread under standard conditions, i.e., in a medium of low ionic strength (0.1 to 0.2 M in ammonium acetate) and without any denaturing agent (formamide or formaldehyde). An O.P.L. 75 K electron microscope was used. The length of the molecules was measured on the micrographs with a map measurer. The magnification of the microscope was determined with Fullman 1002 grids (grating replica, 1135 line/mn) or Polaron M 190-1 grids (grating replica 2160 line/mn). A mixture of mitochondrial DNAs from pika and ΦX 174 bacteriophage replicative form was also spread.

In this procedure pika DNA and standard DNA were included into the same film of basic protein and photographed concomitantly. In each photograph, there must be at least one molecule from each DNA (Fig. 2 *). Lengths were measured and only the length ratio was taken into account. This method enabled therefore to discard the errors due to magnification and to avoid any environmental influence upon the measurement of the length of the unknown DNA.

Results

Analytical centrifugation in CsCl gradient enabled us to determine the buoyant densities of the following mitochondrial DNAs:

- mitochondrial DNA from pika liver: 1.695 g/cm³;
- mitochondrial DNA from rat liver: 1.696 g/cm³;
- mitochondrial DNA from rabbit liver: 1.698 g/cm³.

DNA from Micrococcus lysodeikticus was used as a marker (φ = 1.731 g/cm³) (Fig. 3).

To determine the lengths of rat and pika mitochondrial DNAs exactly, we analyzed the cumulative frequency distribution. We obtained a linear representation of the Gaussian curve and could determine the mean length accurately. These results are

* Fig. 2 see Plate on page 146 b.
Fig. 1.

1. Electron micrograph of mitochondria from pika liver. × 35,000.

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2. Mitochondria extracted from pika liver. Negative staining with 4% phosphotungstic acid pH 7. × 30,000.

3. Mitochondria extracted from rat liver, negative staining with 4% phosphotungstic acid pH 7. × 30,000.
Fig. 2. DNA molecules (pika mitochondrial DNA and DNA from ΦX174 replicative form). After spreading and rotary shadowing with platinum at 5°, with the source 8 cm from the center of the grid.
The mean lengths of rat and pika mitochondrial DNAs are 4.99 μm and 5.30 μm, respectively. The histogram of length distribution is presented in Fig. 5. Both types of molecules (rat and pika) are clearly observed. To discard any error during handling, we used the method of the “intern standard” made of DNA molecules from ϕX 174. The diagrams of (mtDNA length/ϕX 174 length) presented in Figs 6 and 7 reveal the existence of a homogeneous population, which shows that pika DNA is 3.17 longer than that of the replicative form of ϕX 174 (RF I form transformed into RF II by γ ray irradiation at 6000 rad).

**Discussion**

In the present study, mitochondrial DNA from the pika has been characterized by its buoyant density in CsCl gradient as well as by the length of its

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**Fig. 4.** Cumulative distribution analysis of the length of mtDNA molecules extracted from rat and pika livers (the scale is a Gaussian scale).

**Fig. 5.** Length distribution histograms of a mixture of mtDNAs from pikas and rats.

**Fig. 6.** Cumulative distribution of mtDNA length/ϕX 174 length.

**Fig. 7.** Length relationship between mitochondrial DNAs from the pika and from ϕX 174 replicative form.
circular molecules; the obtained values differ but slightly from those found in other mammals. Differences in density could reflect differences in base composition between the three species—rabbit, rat, and pika. The direct measurement of the molecules of the mitochondrial DNAs therefore reveals length differences between the rat and the pika (4.99 and 5.30 \( \mu m \) respectively). However, lengths may vary with several factors as the method of DNA extraction, the spreading method, the protein used to embed DNA (cytochrome c or methylalbumin), the ionic strength, the denaturing agents etc. Thus, Gordon indicates that the replicative form of \( \Phi X \) 174 DNA has lengths from 1.48 to 2.26 \( \mu m \). Furthermore, the magnification cannot be determined precisely with a grid.

DNA from \( \Phi X \) 174 is well known and according to Sharp it has 5200 base pairs and a molecular weight of \( 3.4 \times 10^6 \) daltons. As the standard and the molecule to be measured are submitted to the same conditions, length ratios do not depend upon either the magnification of the microscope or spreading conditions. We can deduct from the mean obtained from these ratios that mitochondrial DNA from the pika possesses approximately 16500 base pairs. The length difference is no artefact, as it is present whatever is the spreading method.

Although the cause of such difference cannot easily be explained, we think it might be found in the fact that the pika separated very early from the phylum which gave birth to other \textit{Lagomorphae}. Actually, Borst says that the less evolved the species, the longer is its mitochondrial DNA; this could apply to the pika, a living fossil, and the length difference, \( i.e., \) 0.3 \( \mu m \) could correspond to the presence of an additional gene maintained during the whole evolution.

In addition, the present work could enable to perform \textit{in vitro} and \textit{in vivo} hybridization of mitochondrial DNAs from rabbits and pikas. \textit{In vitro} experiments have been unsuccessful. \textit{In vivo} studies have been unsuccessful. \textit{In vitro} experiments are in progress.

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