# THE COMPLEXITY OF HUMAN MITOCHONDRIAL DNA

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Received 4 August 1971
Original figures received 1 September 1971

## 1. Introduction

The physicochemical properties of human mitochondrial DNA (mt-DNA) have been studied [1–3], as well as those of mt-DNAs of many species so far examined [4–6]. It has been shown that human mt-DNA molecules have a circular shape and a length of 5 mμ as in other higher organisms like other mammals, birds, amphibians, while in lower organisms, like yeasts, Neurospora, Tetrahymena and in various plants, mt-DNA molecules appear to be mainly linear and of a greater length, up to 20 mμ or more [7] as reviewed recently [5]. Studies on the function of mt-DNA were carried out by investigating protein synthesis in isolated mitochondria and by isolating mitochondrial RNA species and hybridizing them with mt-DNA [8–14].

In the present investigation the genetic complexity of human mt-DNA has been evaluated by determining its renaturation kinetics.

## 2. Methods

The mitochondria were isolated from human placenta, and from human leukemic leucocytes, and the mt-DNA then extracted as previously described [3]. The purity of mt-DNA was checked by centrifuging it at equilibrium in neutral CsCl where it has a characteristic density, and in alkaline CsCl where the complementary strands display different densities [3]. Human mt-DNA was centrifuged in a linear sucrose gradient (5–21%) at 20,000 rpm for 15 hr in the swinging bucket SW 25 rotor of a Spinco L2 preparative ultracentrifuge. Optical reassociation curves of human mt-DNA were determined as reported [15, 16]. Circular mt-DNA molecules were broken and their size reduced by treating with alkali for 18 hr in $Na_2CO_3$-NaOH buffer (pH 12.3). Boundary sedimentation of alkali treated DNA was carried out as previously described [17].

## 3. Results and discussion

Fig. 1 shows the pattern of human mt-DNA centrifuged in a sucrose gradient. There are two major peaks [18] of 39 S and 27 S sedimentation values, as determined by boundary sedimentation in the analytical ultracentrifuge [17], corresponding respectively to the circular form and to the nicked form.

The complexity of mt-DNA has been determined by evaluating the kinetics of renaturation. An inverse correlation between complexity and kinetics of renaturation of DNA had been previously established [15]. In the case of mt-DNA, which is circular, the shape of the molecule can interfere with the determination of the renaturation kinetics. Therefore, the experiment has also been carried out on DNA reduced to linear form by alkali treatment for 18 hr in $Na_2CO_3$-NaOH buffer (pH 12.3). In this condition all circles are surely nicked as proved by the separation of the complementary strands in alkaline CsCl [3], and by the sedimentation value of 7 S of alkali-treated mt-DNA, as determined by boundary sedimentation in the analytical ultracentrifuge [17].

In fig. 2 the '$C_0t$' curves of human mt-DNA are reported. The renaturation of human mt-DNA occurs

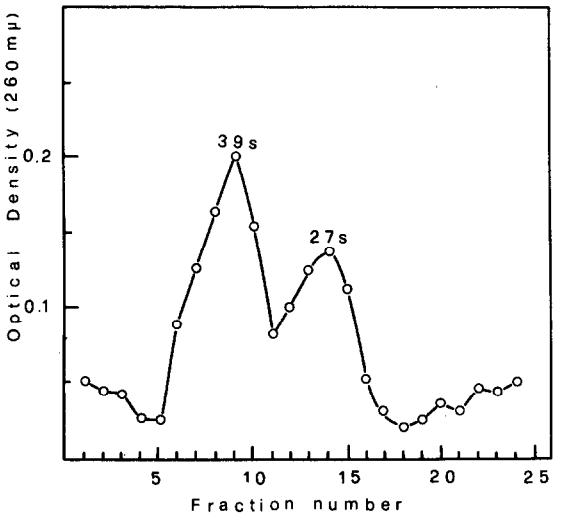


Fig. 1. Human placenta mitochondrial DNA centrifuged in a sucrose linear (5–21%) density gradient at 20,000 rpm for 15 hr in the swinging bucket SW 25 rotor of a Spinco L2 preparative ultracentrifuge. The two peaks have a sedimentation of 39 S and 27 S respectively, as determined by boundary sedimentation in the analytical ultracentrifuge according to Eigner and Doty [17].

more slowly after all the DNA has been reduced to linear form by alkali treatment. This latter renaturation curve can be taken as an indication of the complexity of human mt-DNA. The $C_0t\ \frac{1}{2}$ value in this optical reassociation curve of human mt-DNA is $3 \times 10^{-3}$, which should correspond to a repeated unit length of about 3000 nucleotide pairs [15].

The low complexity of human mt-DNA might not be a generalized feature of all mt-DNAs, as in fact, a higher complexity of mt-DNA in lower organisms compared to higher organisms has been noted [18–21].

The most relevant implication of the low complexity of human mt-DNA is that its capacity to code for RNA species must be rather limited. The molecules of mt-DNA in higher organisms have a very homogeneous size of 10 million dalton molecular weight [5]. Each mitochondrion appears to contain a few mt-DNA molecules, with an average of 3. It has also been suggested on the basis of previous renaturation kinetics experiments that chick mt-DNA molecules are very homogeneous [18]. The present experiments are in favor of the hypothesis that there is a redundancy also within a human mt-DNA molecule. Since the molecular weight of the repeated nucleotide unit would be only 1,800,000 daltons, it is conceivable that human mt-DNA can code only for ribosomal and transfer mitochondrial RNAs, which have been shown to hybridize specifically with mt-DNA in several species [8–12]. It is also known that protein synthesis occurs in the mitochondria. This, however, might be specified by nuclear genes [22–23].

It is likely that a similar situation occurs also in other mammalian mt-DNAs. In fact, preliminary experiments indicate that the '$C_0t$' curves of rat mt-DNA are similar to those of human mt-DNA [24].

There are other facts, besides the renaturation kinetics experiments reported here, in favor of the low complexity of human mt-DNA. Human mt-DNA can be separated into the complementary strands displaying a difference of 0.039 g/ml between their densities in alkaline CsCl [3]. This occurs also in other mammalian mt-DNAs, in which the difference in density in alkaline CsCl between the complementary strands varied according to the species: in fact, it is 0.031 g/ml in rat, 0.027 g/ml in rabbit and sheep, 0.022 g/ml in guinea pig and 0.027 g/ml in mouse [24–26]. If an equimolar mixture of heavy and light strands of these mt-DNAs separated in alkaline CsCl is brought back to neutrality, the two strands stick together reforming a single band in neutral CsCl indicating that a double stranded structure, at least for some length of the sequence, has been reformed. This occurs to our knowledge only in the case of nuclear satellite DNAs, which are among the simplest naturally occurring polynucleotide species [16] A comparison could be made with some viral DNAs, namely some *Bacillus* phages, which separate into two bands in neutral CsCl on denaturation, but do not reassociate unless submitted to conditions favoring renaturation, such as heating at 60° for 5 hr at high salt molarity [27–28]. This suggests that these viral DNAs are more complex than mammalian mt-DNAs.

**Acknowledgement**

This work was supported by grants no. 69.01673.0. 115.253 and no. 70.01207.04.115.253 from the Consiglio Nazionale delle Richerche (Roma).

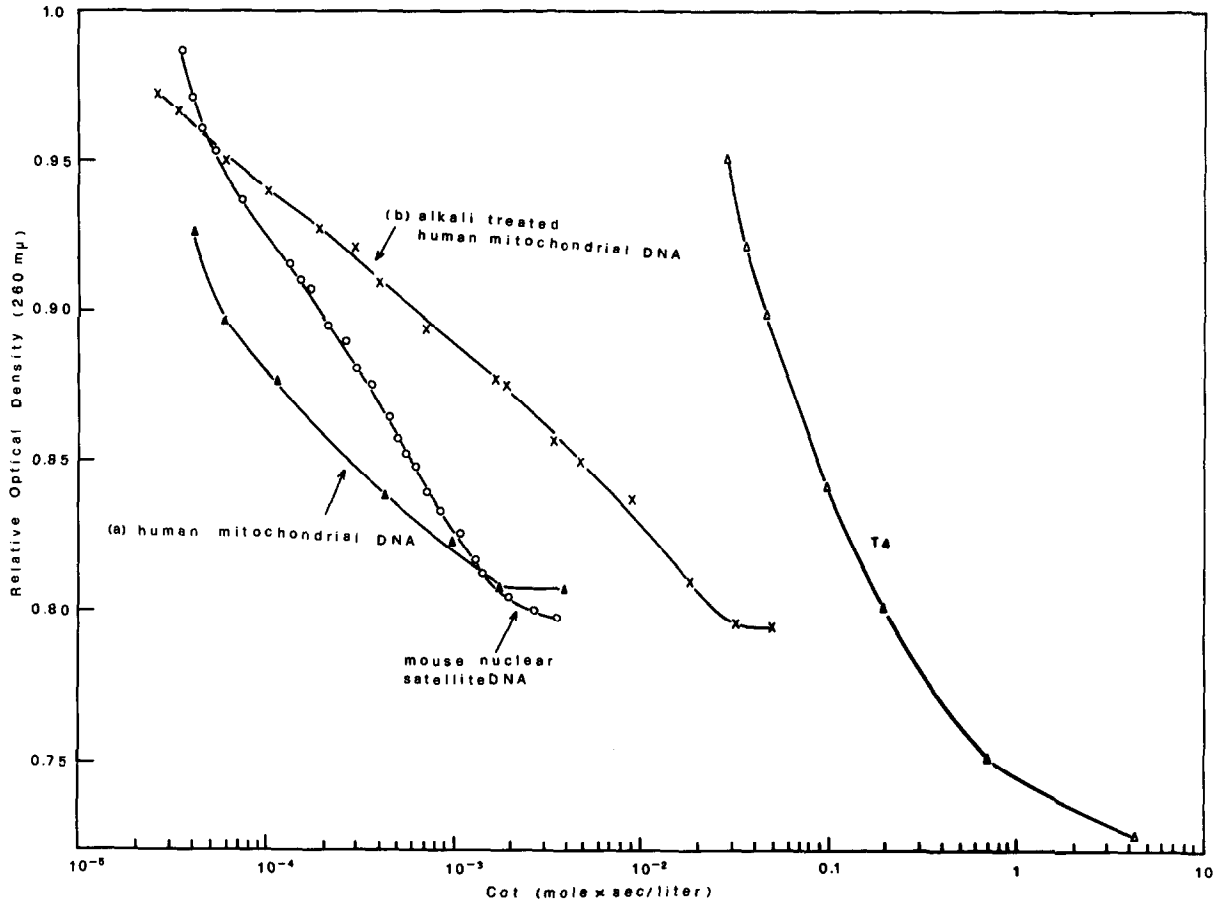


Fig. 2. Optical-reassociation curves of human mitochondrial DNA: (a) '$C_0t$' curve of a mixture of circular (39 S) and nicked (27 S) molecules as shown in fig. 1; (b) the same after treatment with alkali (pH 12.3) for 18 hr (sedimentation value of mitochondrial DNA = 7 S). The '$C_0t$' curves of mouse satellite DNA and T4 phage DNA are reported as reference. The ratio of the optical density at 60° to the initial value measured at 98°, reported on the left scale, is plotted against the '$C_0t$', being the product of DNA molar concentration and time of incubation expressed in seconds. Mitochondrial DNA samples and mouse satellite DNA were reassociated at a salt concentration of 0.04 M NaCl. T4 phage DNA was reassociated at a salt concentration of 0.18 M NaCl. All the curves were normalized to give the rate that would be observed at 0.18 M sodium ion concentration according to the plot reported by Britten [29].

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