THE MITOCHONDRIAL DNA OF HAMSTER–MOUSE HYBRID CELLS

George L. ELICEIRI
Departments of Pathology and Microbiology, St. Louis University School of Medicine, St. Louis, Mo. 63104, USA

Received 15 July 1973

1. Introduction

The synthesis of both hamster- and mouse-mitochondrial ribosomal RNA (mit-rRNA) has been recently reported in hamster–mouse hybrid cells [1]. Hybrids with a majority of chromosomes of one species contained mainly cytoplasmic-ribosomal RNA (cyt-rRNA) of the same species [2] and synthesized mostly, or only, mit-rRNA of the same species [1]. An exception was hybrid 10A, with a small excess of mouse chromosomes, a large excess of mouse cyt-rRNA, but synthesizing mainly hamster mit-rRNA. Recently it has been shown that human mitochondrial-DNA (mit-DNA) can be distinguished from that of mouse or rat by DNA–RNA hybridization [3]. The present report shows that by DNA–RNA hybridization, it is possible to differentiate mouse-mit-DNA from Syrian hamster mit-DNA; and that in these hamster–mouse hybrids, the hybrid which has mostly chromosomes of one species has a mitochondrial genome which is also mainly of the same species. Conversely, hybrid 10A has a predominantly mouse nuclear genome and a predominantly hamster mitochondrial genome.

2. Materials and methods

The hamster–mouse mononucleate hybrid clones, derived from Syrian hamster kidney cells (T6a) and mouse fibroblasts (3T3-4E), have been previously described [4]. Conditions of cell growth were as indicated earlier [2]. Mitochondria were prepared from Syrian hamster- and mouse-liver, and their DNA extracted by published techniques [5, 6]. Close-circular DNA (component I) was isolated from ethidium bromide–CsCl gradients [7]. The dye was removed with isopropanol [8], the sample treated with ribonuclease, and then the DNA was purified over a column of methylated albumin kieselguhr, followed by banding in a CsCl gradient. Whole cell DNA was extracted as described by Brown and Weber [9].

Complementary RNA (cRNA) was prepared with *Escherichia coli* K-12 RNA polymerase from the Miles Laboratories (Kankakee, Illinois, USA). The conditions of in vitro cRNA synthesis on a mit-DNA template, and purification were described previously [10, 11]. In this report the [^{3}H]cRNA synthesized on a hamster or mouse liver mit-DNA template will be referred to as hamster [^{3}H]cRNA or mouse [^{3}H]cRNA, respectively. To test the purity of the [^{3}H]cRNA preparations with respect to contaminations with nuclear DNA sequences, they were annealed with purified nuclear DNA, and negligible amounts of hybridization were obtained. RNA was hybridized to DNA immobilized on filters in 50% formamide, at 40°C as described by Dawid [11].

3. Results and discussion

Filters loaded with various hamster/mouse ratios of liver mit-DNA were incubated in one vial with hamster [^{3}H]cRNA. Another set of identical filters was simultaneously incubated with mouse [^{3}H]cRNA. Fig. 1 shows that with varying percentages of hamster and mouse mit-DNA in the filters, different proportions of hamster and mouse [^{3}H]cRNA counts hybridize to the filters. A sufficient evolutionary difference exists between these two rodent mit-DNA's to provide a calibration curve. It can also be seen that

North-Holland Publishing Company – Amsterdam

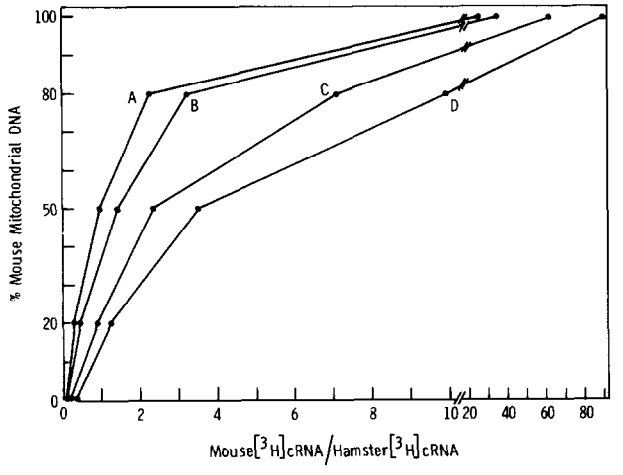

Fig. 1. Hybridization of mouse and hamster liver mit-DNA with mouse [^{3}H]cRNA and hamster [^{3}H]cRNA. The conditions of purification of mit-DNA and DNA–RNA hybridization are mentioned or quoted under Materials and methods. Each filter was loaded with known mixtures of hamster and mouse mit-DNA with a total amount of 50 ng of mit-DNA per filter. Identical sets of filters were incubated with various amounts of hamster [^{3}H]cRNA and mouse [^{3}H]cRNA, separately. The concentrations of [^{3}H]cRNA ranged between 9.4×10^5 and 1.26×10^5 cpm/ml of annealing mixture. The ratios of cpm concentrations in vials with mouse [^{3}H]cRNA *versus* those with hamster [^{3}H]cRNA were 0.93(A), 2(B), 3.75(C), and 7.5(D).

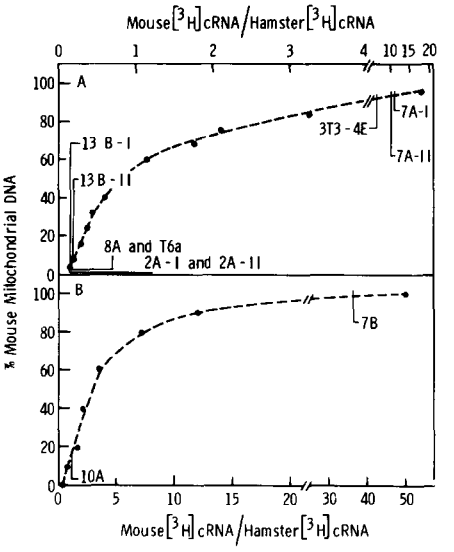

Fig. 2. Hybridization of whole cell DNA from hybrids and their parental cells, with mouse [^{3}H]cRNA and hamster [^{3}H]cRNA. For each hybridization hamster [^{3}H]cRNA was incubated with a series of duplicate filters with known mixtures of hamster and mouse mit-DNA (circles and calibration curve), plus filters with whole cell DNA from 3T3-4E, T6a, and/or hybrid cells (vertical intersections on the calibration curve). An identical set of duplicate filters was incubated with mouse [^{3}H]cRNA. Each filter was loaded with a total amount of 50 ng of mit-DNA or 50 μg of whole cell DNA. In the annealing mixture the concentration of hamster [^{3}H]cRNA was 5×10^5 cpm/ml in (A) and 8.9×10^4 cpm/ml in (B); the concentration of mouse [^{3}H]cRNA was 4.6×10^5 cpm/ml in (A) and 6.1×10^5 cpm/ml in (B). Each point represents the average ratio of two sets of duplicate filters. The average scatter of cpm in duplicate filters was about 9%. Inscriptions like 2A-I and 2A-II indicate that two preparations of hybrid 2A DNA were assayed, from cells harvested on different dates.

similar curves are obtained when the ratio of input hamster and mouse [^{3}H]cRNA is varied (fig. 1).

A series of quadruplicate filters was prepared, loaded with various ratios of hamster and mouse liver mit-DNA for a calibration curve, and whole cell DNA from T6a, 3T3-4E, and/or hybrid cells. One half of the set was annealed with hamster [^{3}Hc] RNA and the other identical half with mouse [^{3}H]cRNA. Fig. 2A shows that [^{3}H]cRNA synthesized on a template of liver mit-DNA of one species hybridizes quite similarly to liver mit-DNA or cultured cell whole cell DNA of the same species. That is, the mouse/hamster ratio of [^{3}H]cRNA hybridized to filters loaded only with hamster liver mit-DNA is quite similar to that of T6a whole cell DNA filters, and that of filters loaded only with mouse liver mit-DNA is similar to the one of 3T3 4E whole cell DNA filters (fig. 2A). Similar results were obtained by Coon et al. [3] for human, mouse and rat DNA. It is then possible to analyze the mit-DNA of hamster–mouse hybrids by simply annealing their whole cell DNA. In hybrid clones with a majority of hamster chromosomes most, if not all, of the mit-DNA is of hamster type (8A, 2A-I, 2A-II, 13B-I, and 13B-II in fig. 2A). Conversely, in hybrids having mainly mouse chromosomes most, if not all, of the mit-DNA is of mouse type (7A-I and 7A-II in fig. 2A, 7B in fig. 2B). The exception is hybrid 10A, with a majority of mouse chromosomes and mouse cyt-rRNA [2], which synthesises mainly hamster mit-rRNA [1], and contains mostly hamster mit-DNA (fig. 2B). The data do not allow to distinguish between a cell having none or a

small percentage of mit-DNA of one species, e.g. the values for T6a and 3T3-4E do not exactly coincide with the values for 100% hamster and 100% mouse liver mit-DNA filters, respectively (fig. 2A). Coon et al. [3] have shown the simultaneous presence of mit-DNA from both parental species in human–mouse and human–rat hybrids.

These results indicate that hybrid 10A transcribes mainly hamster mit-rRNA because most of its mitochondrial template is of hamster type. In mammalian cells it has not been established yet to what extent the nuclear DNA is involved in coding for the mitochondrial ribosomal proteins. It would be interesting to learn if the various mitochondrial ribosomal proteins of hybrid 10A are of mouse or hamster type.

Acknowledgements

It is a pleasure to acknowledge the advice of Drs. Ivan Horak and Igor B. Dawid on some of the methods used. This work was supported by grants from the U.S. Public Health Service and the Damon Runyon Fund, and a Research Career Development Award from the U.S. Public Health Service.

References

[1] Eliceiri, G.L. (1973) Nature New Biol. 241, 233.
[2] Eliceiti, G.L. (1972) J. Cell Biol. 53, 177.
[3] Coon, H.G., Horak, I. and Dawid, I.B., submitted for publication.
[4] Basilico, C., Matsuya, Y. and Green, H. (1970) Virology 41, 295.
[5] Dawid, I.B. and Wolstenholme, D.R. (1967) J. Mol. Biol. 28, 233.
[6] Borst, P. (1972) Ann. Rev. Biochem. 42, 333.
[7] Radloff, R., Bauer, B. and Vinograd, J. (1967) Proc. Natl. Acad. Sci. U.S. 57, 1514.
[8] Cozzarelli, N.R., Kelly, R.B. and Kornberg, A. (1968) Proc. Natl. Acad. Sci. U.S. 60, 992.
[9] Brown, D.D. and Weber, C.S. (1968) J. Mol. Biol. 34, 661.
[10] Reeder, R.H. and Brown, D.D. (1970) J. Mol. Biol. 51, 361.
[11] Dawid, I.B. (1972) Developmental Biol. 29, 152.