Mitochondrial DNA in the mouse preimplantation embryo

K. M. Ebert, H. Liem* and N. B. Hecht*

*Department of Anatomy and Cellular Biology, Tufts University, School of Veterinary Medicine, North Grafton, Massachusetts 01536 and *Department of Biology, Tufts University, Medford, Massachusetts 02155, U.S.A.*

Summary. Total DNA was extracted from mouse embryos that were collected from CD-1 random-bred females on Day 1 of pregnancy and cultured for up to 4 days *in vitro*, or from the reproductive tracts of pregnant females on Days 1, 3, 4 and 5 of pregnancy. Southern blot analyses with a cloned mouse mitochondrial DNA probe were performed to determine the relative levels of mitochondrial DNA in the zygote, morula, blastocyst and early egg cylinder stage embryos. The results indicated that the total amount of mitochondrial DNA does not change during development of the mouse embryo up to the egg cylinder stage and is not altered during in-vitro culture of the fertilized one-cell embryo to the blastocyst stage.

Keywords: mitochondria; DNA; mouse

Introduction

It is estimated that the mouse oocyte contains 100 000 mitochondria (Piko & Matsumoto, 1976) with each mitochondrion believed to contain between 2 and 10 copies of the mitochondrial genome (Nass, 1969; Borst & Kroon, 1969). The number of genomes per mitochondrion differs in diverse cell types and organs and is likely to be dependent upon physiological conditions. Studies using electron microscopy to determine total numbers of mitochondria in the mammalian oocyte suggest that the mitochondria do not increase in number during the preimplantation stage of development (Szollosi, 1965). However, no quantitative information measuring relative levels of mitochondrial DNA during early embryogenesis is available. The purpose of this study was to determine whether, during the active nuclear synthetic intervals of the preimplantation stages of embryo development, the relative number of mitochondrial genomes also increases.

Materials and Methods

Zygotes were collected from CD-1 (Charles River) random-bred females between 09:00 and 12:00 h on Days 1, 3, 4 and 5 of pregnancy. Naturally mated females were selected for oestrus and placed with males between 15:00 and 18:00 h. The presence of a vaginal plug the following morning was considered to indicate Day 1 of pregnancy. Embryos were collected according to Brinster (1972). The oviducts were excised, and zygotes liberated from the oviducts by tearing open the ampulla region and dispersing the cumulus mass by incubating with hyaluronidase (1 mg/ml) in medium containing pyruvate, lactate and glucose (PLG) with Hepes salts (Ebert & Brinster, 1983) for 10 min; hyaluronidase was removed with 3 washes of PLG and Hepes medium. After incubation, the embryos were cultured *in vitro* in PLG medium under oil as previously described (Brinster, 1972). Embryos from 3, 4, and 5 days of pregnancy were collected from the oviducts or uterus by excising the reproductive tract and flushing with PLG and Hepes medium.

Isolation of DNA from embryos. Washed embryos were resuspended in a lysis buffer containing 0.02 M-Tris (pH 8.0), 0.01 M dithiothreitol (Sigma, St Louis, MO, U.S.A.), 0.15 M-NaCl, 0.01 M-EDTA (Sigma) and 1% sodium dodecyl sulphate (Sigma) (75 embryos/ml) and disrupted by homogenization in a 1-ml glass homogenizer with a Teflon pestle. The lysate was incubated with proteinase K (100 µg/ml) for 2 h and DNA was purified as previously described (Hecht *et al.*, 1984). Recovery of total DNA from each stage was monitored by adding 60 pg (Exp. 1) or 60 ng (Exp. 2) of pBR322 plasmid DNA as a carrier before cell disruption.

Quantitation of mitochondrial DNA. Total purified DNA from each pooled group of mouse embryos was digested with the restriction enzyme, Eco-R1, and mitochondrial DNA was analysed as previously described (Hecht *et al.*, 1984). After digestion with Eco-R1, the DNA was analysed by the technique of Southern blotting (Southern, 1975) using ^{32}P -labelled nick-translated plasmid 501-1, a clone of the whole mouse mitochondrial genome (Bibb *et al.*, 1981). The relative intensities of autoradiographic bands were compared by densitometry. Comparison of the ratio of intensities of each mitochondrial DNA band to the exogenous pBR322 DNA band was used to assess relative amounts of purified mitochondrial DNA recovered from the different developmental stages of the preimplantation embryos.

Results

Experiment 1

The relative concentrations of mitochondrial DNA from three developmental stages of the mouse preimplantation embryo were determined. Total DNA extracted from embryos representing the zygote, morula and blastocyst stages was digested with Eco-R1, run on an agarose gel and blotted onto nitrocellulose. Hybridization of the Southern blot with the mouse mitochondrial DNA probe inserted in pBR322 detected 2 mitochondrial DNA fragments of 14.7 and 1.6 kb (Fig. 1, Bands 1 and 3). Since pBR322 DNA contains one Eco-R1 site, it migrates as a single band of linear DNA of intermediate electrophoretic mobility (Fig. 1, Band 2). Densitometry measurements of the Southern blots gave a ratio of mitochondrial DNA (Band 1) to pBR322 DNA (Band 2) of 5.85, 5.96 and 5.60 for the zygote, morula and blastocyst preparations, respectively.

Experiment 2

This experiment was performed to determine whether the concentration of mitochondrial DNA in preimplantation embryos was altered during in-vitro development. Additionally, embryos at the egg cylinder stage were analysed to determine whether mitochondrial DNA was increased as a result of the initiation of implantation. The egg cylinder stage embryos were intact and consisted of the embryonic and extraembryonic layers. It was assumed that they were free of maternal tissue as they were flushed at the earliest stage of implantation. Embryos were cultured *in vitro* from the zygote stage for 3, 4 or 5 days resulting in embryos at the morula, early blastocyst and expanded blastocyst stages, respectively. For each sample population 75 embryos were collected. In addition, 75 embryos from pregnant females were collected on Days 1, 3, 4 and 5 *post coitum*, representing the zygote, morula, blastocyst and the early egg cylinder stages. After the addition of 60 ng pBR322 DNA, samples were extracted as described above. The results are shown in Fig. 2. Using densitometry tracings of the 14.7 kb fragment of mitochondrial DNA (Band 1) and the pBR322 DNA (Band 2), ratios of relative mitochondrial DNA per sample were calculated. The cultured embryos had ratios of 0.067, 0.071 and 0.041 for the morula, early blastocyst and expanded blastocyst (Lanes A, B and C) respectively. These ratios do not reflect a major increase or decrease in the amount of mitochondrial DNA that was originally present in the zygote. The mitochondrial to pBR322 DNA ratios of zygotes, morulae and the early egg cylinder stage embryos *in vivo* were 0.037, 0.063 and 0.073 (Lanes E, F and G) respectively. We conclude that embryos grown *in vitro* and *in vivo* had similar amounts of mitochondrial DNA at comparable morphological stages even though there is a 24-h delay in development during in-vitro culture of the zygote.

Discussion

Determining the amount of mitochondrial DNA that exists in the mammalian embryo in the early preimplantation stages is hindered by the low numbers of cells in the early embryo (1–128 cells) and therefore large numbers of embryos are needed to measure accurately mitochondrial DNA synthesis and decay by standard radioactive labelling techniques. However, the use of radiolabelled DNA probes has been successfully used to measure the total amount of mitochondrial DNA in small

Fig. 1. Southern blot of mitochondrial DNA. Total DNA was extracted from samples of 40 mouse zygotes (A), morula (B) and blastocysts (C) and digested with the restriction enzyme, Eco-R1. Bands 1 and 3 represent two fragments of mouse mitochondrial DNA of 14.7 and 1.6 kb. Band 2 represents the linearized form of the pBR322 carrier DNA. The blot was hybridized with 0.2 µg of nick-translated mouse mitochondrial DNA probe p501-1 at a specific activity of 10^7 c.p.m./µg as previously described (Hecht *et al.*, 1984).

numbers of differentiating mouse testicular cell types by Southern blot hybridization (Hecht *et al.*, 1984). The results presented here show that a ^{32}P -labelled mitochondrial DNA probe can also be used to estimate relative concentrations of mitochondrial DNA in early preimplantation embryos.

These studies demonstrate that the steady state level of mitochondrial DNA in the mouse embryo, whether grown *in vivo* or *in vitro*, does not increase or decrease significantly during the preimplantation stages of development. The zygote, morula, blastocyst and early egg cylinder stage embryos all contained approximately equal amounts of mitochondrial DNA, suggesting mitochondrial DNA is not synthesized in the preimplantation embryo. Due to limited material available in these early embryonic stages, we cannot directly measure mitochondrial DNA synthesis but must rely upon the Southern blot technique to quantitate mitochondrial DNA

Fig. 2. Southern blot of mitochondrial DNA extracted from mouse embryos. Total DNA was extracted from samples of 75 mouse embryos that were cultured *in vitro* from the zygote to the morula (A), early blastocyst (B) or expanding blastocyst (C) or recovered *in vivo* at the zygote (E), morula (F), and egg cylinder stage (G). Lane D is an Eco-R1 digested pBR322 control. The blot was hybridized as described in Fig. 1. Band 1 is the 14.7 kb fragments of the mitochondrial genome following Eco-R1 digestion. Band 2 is pBR322 DNA. A longer exposure of this autoradiogram beyond that required for linearity for quantitation of bands 1 and 2 reveals the second mouse mitochondrial DNA band of 1.6 kb.

accumulation. Considering that the half life of mitochondrial DNA has been demonstrated to be from 3 to 14 days in metazoan somatic and germ cells, DNA turnover is not likely to influence these estimates significantly (Gross & Rabinowitz, 1969; Chase & Dawid, 1972; Hecht & Liem, 1984).

The lack of mitochondrial DNA accumulation may be of importance when answering questions such as: what happens to the paternal mitochondrial DNA that enters the oocyte at fertilization, or what will be the fate of newly introduced mitochondrial DNA by microinjection techniques? Without extensive replication during the early embryonic stages, mitochondrial DNA from selected mitochondria may be partitioned during cellular division of the developing embryo.

This work was supported by NIH Grant HD11878 (N.B.H.).

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Received 7 April 1987