Assaying the probabilities of obtaining maternally inherited heteroplasmy as the basis for modeling OXPHOS diseases in animals

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Received 26 December 2005; received in revised form 2 May 2006; accepted 13 May 2006

Available online 19 May 2006

Abstract

Gross alterations in cell energy metabolism underlie manifestations of hereditary OXPHOS (oxidative phosphorylation) diseases, many of which depend on proportion of mutant mitochondrial DNA (mtDNA) in tissues. An animal model of OXPHOS disease with maternal inheritance of mitochondrial heteroplasmy might help understanding the peculiarities of abnormal mtDNA distribution and its effect on pre- and postnatal development. Previously we obtained mice that carry human mtDNA in some tissues. It co-existed with murine mtDNA (heteroplasmy) and was transmitted maternally to the progeny of animals developed from zygotes injected with human mitochondria. To analyze the probability of obtaining heteroplasmic mice we increased the number of experiments with early embryos and obtained more specimens from F1. About 33% of zygotes injected with human mtDNA developed into post-implantation embryos (7th – 13th days). Lower amount of such developed into neonate mice (ca. 21%). Among post-implantation embryos and in generations F0 and F1 percentages of human mtDNA-carriers were ca. 14–16%. Such percentages are sufficient for modeling maternally inherited heteroplasmy in small animal groups. More data are needed to understand the regularities of anomalous mtDNA distribution among cells and tissues and whether heart and muscles frequently carrying human mtDNA in our experiments are particularly susceptible to heteroplasmy.

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Keywords: Energy metabolism; OXPHOS diseases modeling; Human mtDNA; mtDNA transfer; Transgenic mice; Maternal inheritance

1. Introduction

Protracted alterations of cellular energy metabolism should result in cell death. In fact, disintegration of mitochondria that provide cells with energy is a frequent component of apoptosis. However, a decrease in energy metabolism may be moderate, under which condition a cell does not switch to the programmed death, yet accumulation of energy-starving cells in a tissue or an organ causes its gradual degeneration. Manifestations of the latter are evidenced in ageing tissues/organs, but are even more vivid in case of the so-called OXPHOS diseases (from ‘oxidative phosphorylation’). In patients the continuous reduction of mitochondrial energy production goes much more rapidly than it occurs in normal individuals at ageing, hence, symptoms of degeneration in the most energy-dependent tissues sometimes become visible already in infancy, early death being the outcome in quite a few cases. OXPHOS diseases associated with mutations in mitochondrial DNA (mtDNA) are inherited along the maternal lineage as a developing mammalian embryo gets the great majority of its mitochondria, if not all, from the oocyte. Despite that OXPHOS diseases are known since early 1960s [1], the linkage of their occurrence to mutations in mtDNA was discovered much later [2]. Mutations are often fatal for the function of mtDNA as they result in synthesis of invalid protein subunits of several respiratory complexes in mitochondria [3,4]. Some aspects of this hereditary pathology are poorly understood, one of the reasons being the absence of an adequate animal model for studying the inborn defects of energy metabolism.

mtDNA may undergo several replications within a cell cycle and, possibly, due to peculiar segregation of mutant mtDNA copies during cytokinesis these copies are accumulated in certain cell lineages. When a cell accumulates the critical concentration of invalid mtDNA, its energy production becomes insufficient. In
response, the cell produces more mitochondria and more mtDNA, but the share of mutant copies usually increases or at least remains the same. Co-existence of wild-type and mutant mtDNA within a cell or a mitochondrion is termed ‘heteroplasmy’ and is a crucial requirement to an animal model of OXPHOS diseases.

A prospective approach to obtaining such a model seems to be the introduction of foreign or in vitro modified mtDNA-carrying mitochondria into murine zygotes. Experiments of this kind mostly included the delivery of mitochondria of one murine strain to oocytes of another [5–8]. We have shown recently that injection of human mitochondria into mouse zygote results in persistence of foreign mtDNA in the tissues of the animal through its prenatal and postnatal development [9,10]. Evidence that human mtDNA is not eliminated from the mouse embryo during its pre-implantation development had been collected [9,11] by the time when we confirmed the suggestion that it is transmitted along the maternal lineage [10,12].

In this way we collected evidence that the two prerequisite features of OXPHOS diseases, i.e., maternal inheritance and heteroplasmy can be reproduced in experiments on mice carrying human mtDNA. In our experiments the latter was playing a role of anomalous mitochondrial genome distributed among the tissues of a patient with hereditary disorders of energy metabolism.

To move further on the way to regulated modeling of OXPHOS diseases one had to know if there are chances to obtain as many heteroplasmic animals as one needs and whether there are regularities in occurrence of heteroplasmy in sequential generations.

We collected substantially more data on the early development of mouse embryos injected with human mtDNA and some additional data on “human mtDNA/mouse mtDNA” heteroplasm in generation F1. Additionally, F2 was obtained with transmitochondrial animals in it, which supported our data on maternal inheritance of heteroplasmy, but the amount of data collected so far is not sufficient to be included in calculations.

Careful statistical evaluation of the results obtained in this and previous studies was needed to look for indications at some algorithm of distribution of xenogeneic mtDNA among organs/tissues, at the specific impact of manipulations with murine zygotes upon the mortality of embryos and neonates, at the existence of any regularity in occurrence of heteroplasmy in sequential generations of mice. However, the first and main goal of this work was to learn: (a) what is the mean quota of surviving animals in experiments with mtDNA transfer that we and other groups keep carrying out; (b) what is the share of transmitochondrial animals (i.e., foreign mtDNA-carriers) that can be obtained at least in the two first generations. Answering these simple questions would help us concluding what amount of experimental animals should be sufficient to make the modeling of maternally inherited OXPHOS diseases.

2. Materials and methods

2.1. Isolation of mitochondria

Human mitochondria were isolated from cultured HepG2 cells as described [10]. Their functional activity evidencing the overall integrity of organelles was assayed by treatment with MitoTracker Red (Molecular Probes, USA) for 30 min at +4°C dissolved in PBS (phosphate buffer saline) to the final concentration 100 nM. Red fluorescence was viewed under Leica DM RXA (Germany) fluorescent microscope evidencing the integrity of the mitochondrial membranes. Fertilized murine zygotes were prepared as described in our previous paper [9].

Human mitochondria (5–10 pl of suspension) were microinjected using micro-manipulator into zygotes after the latter had been immersed for 20–40 min at 37 °C into HT6 medium containing cytochalazin B (5 mg/ml). Microinjections were performed at room temperature using glass injection pipettes with internal diameter 4–6 mm [9]. The subsequent procedure is described in [10]. About 500 human mitochondria were delivered into a zygote by a microinjection.

To evaluate the effect of manipulation on subsequent development of an embryo some zygotes were microinjected at room temperature with buffer solution containing no mitochondria and left for culturing at 37 °C along with the others.

2.2. Transplantation of microinjected zygotes to the pseudopregnant mice

Murine zygotes carrying human mitochondria were transplanted to the C57Bl pseudopregnant mice soon after microinjection of mitochondria. Each female received 10–15 zygotes transplanted into the oviduct under ether anaesthesia. Species-specific primers were constructed previously [9]. We selected human mtDNA site 13959–13976 [13] and murine mtDNA site 13437–13455 [14] as non-homologous sequences suitable for discriminative analysis. The corresponding human (A) and murine (B) 5′-primers looked as:

\[ \text{A} \ 5′-\text{CTCTTACAGACCTAAAC-3′} \]
\[ \text{B} \ 5′-\text{ACTCTTACACAAACATTA-3′} \]

To construct 3′-primers human mtDNA site 14190–14207 and murine mtDNA site 13584–13601 were selected. These two sequences are highly homologous (15 bp of 18 coincide). The corresponding human (A) and murine (B) 3′-primers looked as:

\[ \text{A} \ 5′-\text{CGTTGTACCTTACACACATAA-3′} \]
\[ \text{B} \ 5′-\text{CTGGTGTACCTTACACATTA-3′} \]

Details of PCR performance with these primers are described in [10].

2.3. DNA isolation for control

Human nuclear [15] and mitochondrial [16] DNA specimens were purified from HepG2 cells and used as control. Mouse total DNA to be used as control was isolated and purified from the liver of animals [17].

2.4. Isolation of DNA from tissues of embryos and neonate animals

Total DNA was isolated after the lysis of pre-implantation embryos at 2- and 4-blastomere stages [9]. Embryos of post-implantation stages were taken out on the 7th, 8th, 9th, 10th, 11th and 13th days of intrauterine development after the females had been sacrificed by dislocation of cervical vertebrae. Lysis of entire 7- to 11-day embryos and isolation of their DNA or isolation of the latter from organs of 13-day embryos and neonate animals were accomplished as we described earlier [10].

2.5. Statistical evaluation

For each experimental group sampling fraction of the total number of animals or embryos in that group was calculated. A sampling fraction is the quota of animals or embryos that carries a feature under examination (e.g., transgenic organisms or embryos developing to a certain stage). An error was calculated for each sampling fraction. All values were expressed percent to facilitate elaboration of Results and Discussion. Significance of the difference between various sampling fractions was calculated using criterion \( \zeta \) that is analogous to Student’s criterion for binomial distribution. It was also assayed using \( \chi^2 \), and the precise double-side Fisher’s criterion \( p \) [18].

To evaluate the validity of the difference between a sampling fraction and the general fraction we used criteria \( \zeta \) and \( \chi^2 \). By general fraction we term a portion of...
animals or embryos carrying a feature of interest. That portion can be obtained in all the experiments of such kind (i.e., at infinitely large sampling).

“Survival curves” in various experimental and control groups were compared by method of Mantel/Haenzel [19]. Calculation of the values for precise double-side Fischer’s criterion was accomplished using computer program StatXact-4.

3. Results

21 zygote of 73 microinjected with human mitochondria developed to blastocysts when cultured in vitro, which is 28.8±5.3% (see Fig. 1). In the control group where zygotes were left intact prior to culturing 19 embryos of 34 reached the blastocyst stage, which is 55.9±8.5%. The difference between the shares of blastocysts in the control and experimental groups is significant at 1% level (ζ > 2.58, χ² = 7.4, P = 0.099).

After injecting PBS into 27 zygotes 8 of those developed to blastocysts (see Fig. 1), which is 29.6±8.8%. The difference between this and the control group was significant at 5% level (ζ > 1.96, χ² = 4.5, P = 0.04).

In all early-stage embryos developing after a microinjection with human mitochondria PCR would reveal human mtDNA. It is worth noticing that examination of separate blastomeres showed that not each of those contained human mtDNA. For example, of 156 embryos analyzed at the 2-blastomere stage in 102 human mtDNA was detected in both cells, while in 54 it was contained only in one blastomere. All blastocysts developed from microinjected zygotes contained human mtDNA.

Human mtDNA was revealed by PCR also in the tissues of mouse embryos on the 7th–13th day of the intrauterine development. Upon microinjection with human mitochondria 270 murine zygotes were transplanted to 23 pseudopregnant mice, which allowed obtaining 89 post-implantation embryos. Human mtDNA was detected in 13 of those, which is 14.6±3.7%. The same samples contained also murine mtDNA as revealed by PCR with mouse mtDNA-specific primers (see Materials and methods).

In subsequent experiments 105 mouse zygotes into which human mitochondria had been injected were transplanted to 8 pseudopregnant mice. The total litter contained 22 animals of which 8 died soon after birth. Organs/tissues were sampled from 18 neonate mice either on the day of their death or on the 10th day after birth when the animals were sacrificed. In three animals human mtDNA was found. All three were analyzed on the 10th day after birth and it seems important that their size and weight were noticeably lower than those of the littermates with no human mtDNA (Table 1; Fig. 2).

Fig. 1. In vitro development of embryos in the control and experimental groups.

| Body masses of mice born after injection of human mitochondria into murine zygotes (F₀) and of their progeny (F₁) |
|---|---|---|
| Control group | Body mass (g) on the 7th day | Body mass (g) on the 10th day |
| 2.42±0.31 | 3.08±0.41 |
| F₀ | Non-transgenic mice⁠a | 1.98±0.30⁠b | Non-transgenic mice |
| Human mtDNA carriers | 1.59; 1.63; 1.68 | Human mtDNA carriers |
| F₁ | Non-transgenic mice | 2.39±0.33⁠c | Non-transgenic mice |
| Human mtDNA carriers | 1.73; 1.78; 1.84; 1.89 | Human mtDNA carriers |

⁠a Animals in whose tissues no human mtDNA was detected.  
⁠b Mean of 16 separate values (for details, see Results).  
⁠c Mean of 29 separate values.
In F₁, i.e., in the progeny of six females born from zygotes into which human mitochondria had been injected 29 animals were analyzed by PCR. Human mtDNA was found in four animals, which is 13.8 ± 6.4%. However, it is worth noticing that the shares of transgenic animals in different progenies varied. One line contained two transgenic mice of ten born by the same female. If the proportion of transmitochondrial animals is calculated only for this progeny it will be 20.0 ± 12.7%. Another line contained four mice in F₁, two of whom carried human mtDNA. In the next generation (F₂) of the same line three of five animals belonging to the progeny of the same female were carrying human mtDNA. Meanwhile, not a single animal in other lines was carrying human mtDNA.

Human mtDNA was unevenly distributed among the organs of 10-day mice that developed from zygotes injected with human mitochondria (F₀). One animal carried foreign mtDNA in heart and skeletal muscles, the second had it in heart and testicles, in the third mouse it was detected in brain, bladder, skeletal muscles and stomach (Table 2).

The first transmitochondrial mouse of F₁ carried human mtDNA in heart, skeletal muscles, stomach, ovaries, lungs and kidneys. In the second animal it was detected only in ovaries and the third one carried it only in heart. Meanwhile the fourth mouse had human mtDNA in brain, lungs, uterus and skeletal muscles.

The three transgenic animals of F₂ carried human mtDNA in one organ each, i.e., in heart, stomach and liver.

It should be mentioned that in the 13-day embryos human mtDNA was also found in heart, skeletal muscles and in digestive tract.

### 4. Discussion

Percentage of mouse embryos developed without malformations and in due time from intact zygotes was compared to the respective proportions of embryos in the group where zygotes had been injected with human mitochondria and in that with sham injections of PBS. Histogram in Fig. 1 shows that proportions of normally developing embryos were lower (ca. 80%) in the groups of both mitochondria- and PBS-injected zygotes than in the control group (ca. 90%) already on the third day. This difference becomes highly significant by the stage of blastocyst. We have shown previously [10] that about 4% of embryos in the two groups with injections developed to blastocysts some 24 h later than other 96% and than all the intact embryos in the control group.

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**Table 2**

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>13-day embryos</th>
<th>Mice in F₀</th>
<th>Mice in F₁</th>
<th>Mice in F₂</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Skeletal muscles</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>–</td>
<td>5</td>
</tr>
<tr>
<td>Stomach</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Lungs</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Brain</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Ovaries</td>
<td>–</td>
<td>–</td>
<td>2</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Testicles</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Bladder</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Kidneys</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Uterus</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Intestine</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
</tbody>
</table>

*a* Anatomical discrimination between stomach and intestine in 13-day embryos was not always precise, therefore, this finding may be equally attributed either to stomach or to intestine.
We used Mantel–Haenzel method to analyze the differences among the ‘survival curves’ that reflect the pace with which the number of normally developing embryos decreased at every developmental stage. Firstly, the group of zygotes injected with PBS was compared to that in which the ova were injected with human mitochondria. Since it had been proved that no significant difference existed between these two groups, we combined the data and compared the unified group to the control group of intact zygotes. The obtained result $\chi^2=5.15$ confirms that the pace of decrease of the normally developing embryos’ number differs significantly between the control group and the experimental groups. It should be noticed that using such logics (when data of similar experiments are combined) in calculations of the precise double-side Fischer’s criterion increases noticeably the significance of the difference ($P=0.022$).

This result confirms our earlier conclusion [10] that microinjection itself and the accompanying manipulations rather than the content of the needle (e.g., human mitochondria) evoke a decrease of the percentage of normally developing embryos. It is also in line with the evidence obtained by another group [20].

We demonstrated more than once that foreign mtDNA is never detected in all blastomeres of an early-stage mouse embryo developing in vitro [9,10]. Our present study together with the previous data speaks in favor of a possible mosaicism of mtDNA that occurs already at the first egg splittings. On the other hand, these data allow suggesting that xenogeneic mitochondria are distributed non-randomly at every division of an embryo. The amount of mitochondria injected was sufficiently high (ca. $10^2$), hence, in case of their stochastic distribution in the course of an embryo partition the probability of their distribution among both daughter cells is very high. Meanwhile, we have shown that of 156 embryos having undergone the first splitting 54 (which is 34.6±3.8%) carried human mtDNA only in one blastomere. This can be explained either by aggregation of mitochondria in clusters in the course of zygote splitting, or by the existence of some minimum threshold amount of foreign organelles that is necessary for their fixation in a daughter cell. If it was not the first splitting, one might speculate on the mtDNA segregation bottleneck that prevents free selection of mitochondrial genome copies during cell division as those are clustered in nucleoids and attached to the inner mitochondrial membrane [21]. That does not seem to be the case, since foreign mitochondria that we injected into mouse zygotes could be traced for some 2–3 splittings if tagged with MitoTracker or another fluorescent probe [9], which indicates that at least their major part preserved the structural integrity. It remains unclear whether human organelles could bind to cytoskeleton as described for the native mitochondria [22–24]. Hence, this mechanism regulating their behavior in the process of egg splitting cannot be relied upon as an explanation of uneven distribution of human mtDNA that we observed. Moreover, experiments of another group [25] demonstrated that shortly after introduction of foreign mitochondria into a cell they do not get united with the native mitochondria in a common network, but rather co-exist as two homoplasmic populations of organelles. It seems likely that human mitochondria in our experiments co-existed with murine organelles during the first splittings. Therefore, it remains unclear which mechanism governs their uneven distribution among blastomeres.

Of 270 zygotes injected with human mitochondria and transplanted to pseudopregnant mice 89 developed to the age of 7–13 days, which is 33.0±2.9%. This share is close to the percentage of embryos that reached the stage of blastocyst when cultured in vitro upon injection (see Fig. 1). It is also similar to the share of embryos developed upon injection into mouse zygotes of mitochondria obtained from another murine line, which was 33% [26].

We suggested that the general set of all embryos surviving after our manipulations is 33%. To test this hypothesis, we calculated the significance of the difference between the general set and the share of embryos developed to blastocysts from zygotes injected with human mitochondria (28.8±5.3%). Obtained coefficients $\zeta$ and $\chi^2$ were so small that our suggestion is not refuted even at very low levels of significance.

Percentage of embryos that survived an injection of human mitochondria and developed to term (i.e., to neonate animals) was 21.0±4.0% (22 mice vs. 105 zygotes). With 95% probability ($\zeta=1.96$) this quota is lower than the percentage of embryos that survived after injection and developed to 7th–13th day. This can be explained by an increased mortality of manipulated embryos in late prenatal development, but also by peculiarities of carrying the transplanted embryos by each particular female. The fact that 8 neonate mice died within the first days after birth favor the first notion. The second one is partly confirmed by the fact of successful delivery only by four of eight pseudopregnant mice to whom zygotes were transplanted upon injection of human mitochondria.

The share of transmitochondrial embryos at the age of 7–13 days among 270 embryos transplanted to 23 pseudopregnant mice after injection of human mitochondria into zygotes was 14.6±3.7%, as mentioned above. Raising on this percentage one can assert that a possible general set of transmitochondrial embryos achieving this stage of development with 95% probability will be between 7.35% and 21.85% (coefficient $\zeta=1.96$). This result can be relied upon in case of planning similar experiments. In those one has all grounds to expect a high probability of obtaining transmitochondrial mice even when small groups of animals are taken. In particular, our data may be useful for correct modeling the maternally inherited OXPHOS diseases.

Percentage of transmitochondrial mice in $F_0$ was 16.7±8.8% (3 of 18 animals analyzed). It was 13.8±6.4% in $F_1$ (4 of 29 analyzed mice). These numbers fall within the predicted limits for the possible value of the general set of transmitochondrial embryos (see above). This is indicative of the probable regularity in the reproduction of a certain quota of transmitochondrial animals in similar experiments.

In contrast to 100% blastocysts that carry human mtDNA the percentage of transmitochondrial embryos by the 7th–13th day of development goes down to 14.6±3.7%. This share remains more or less stable both in the neonate animals of $F_0$ (16.7±8.8%) and in mice of $F_1$ (13.8±6.4%). An apparently quick drop in the share of transmitochondrial organisms needs a short comment.

Firstly, we did not determine the percentage of cells in a blastocyst that carried foreign mtDNA. Based on our observation that sometimes not every blastomere in a 2- and 4-cell embryo carries foreign mtDNA, it can be suggested that not all the cells of a
blastocyst are transmitochondrial. Since the entire blastocyst was used to isolate DNA, one could expect exactly what we observed, i.e., exclusively positive answer in a test for human mtDNA, unless the latter is eliminated at an earlier stage of embryo development, which was not the case.

Secondly, part of the cellular mass of a blastocyst containing foreign mtDNA might not give the origin to tissues/organisms of post-implantational embryo (e.g., they might be used to form the covering membranes). We cannot speculate on the input of this factor in our results, since the regularities underlying the distribution of cells in early prenatal development are poorly understood.

Lastly, the conservation of a stable quota of imported mtDNA in generations of cells and organisms can be explained by the peculiarities of mtDNA segregation through a “bottleneck” [27], the main events of which take place during oocytes’ maturation. It has been shown that transmission of a quota of imported mtDNA (that can resist elimination) to the next generation of germline cells results in appearance of the same or very similar quota in daughter cells [25]. In our case indirect evidence in favor of the latter phenomenon is the uneven frequency of transgenic animals in various murine lineages founded by females developed from ova injected with human mitochondria. In some lineages no transmitochondrial mice were revealed either in F1 or F2. In other lineages human mtDNA was detected in half of the litter or more (2 of 4 animals in F1 and 3 of 5 in F2).

However, the bottleneck model proposed by Shoubridge and collaborators [25] presumes that the stable share of imported mtDNA (i.e., the percentage of heteroplasmy) should be conserved virtually in all organs of a transmitochondrial animal. We were unable to detect foreign mtDNA in all the tissues of transmitochondrial mice, which is not fully in line with that model based mainly upon thorough investigation of mtDNA segregation in oogenesis [25,27]. One should conclude that more transmitochondrial zygotes and subsequently more embryos are needed to study the segregation of the heteroplasmy.

Careful examination of the distribution of human mtDNA among various organs of 10-day mice and in 13-day embryos allows noticing that the most frequent targets for foreign mtDNA are heart and skeletal muscles (Table 2). Due to the strong dependence on energy production these organs are mostly affected in various OXPHOS diseases, but more data are needed to understand whether we observed a coincidence or these tissues are really more susceptible to heteroplasmy.

Acknowledgements

This work was partly supported by RFBR grants No 04-04-49157, No 06-04-49564 and the Presidential Program ‘Leading Scientific Schools’ Grant 1730.2003.4.

References


[16] A. Torroni, T.G. Schurr, C.-C. Yang, E.J.E. Szathmany, R.C. Williams, M.S. Schanfield, G.A. Troup, Native American mitochondrial DNA analysis – the Amerind of the Nadene populations were founded by two independent migrations, Genetics 130 (1992) 153–162.


[20] A. Torroni, T.G. Schurr, C.-C. Yang, E.J.E. Szathmany, R.C. Williams, M.S. Schanfield, G.A. Troup, Native American mitochondrial DNA analysis indicates that the Amerind of the Nadene populations were founded by two independent migrations, Genetics 130 (1992) 153–162.


[22] M. Tourte, C. Besse, J.-C. Mounolou, Cytochemical evidence of an organized microtubular cytoskeleton in Xaenopus laevis oocytes: involvement in the


