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Nuclear Counterparts of the Cytoplasmic Mitochondrial 12S rRNA Gene: A Problem of Ancient DNA and Molecular Phylogenies

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Abstract. Monkey mummy bones and teeth originating from the North Saqqara Baboon Galleries (Egypt), soft tissue from a mummified baboon in a museum collection, and nineteenth/twentieth-century skin fragments from mangabeys were used for DNA extraction and PCR amplification of part of the mitochondrial 12S rRNA gene. Sequences aligning with the 12S rRNA gene were recovered but were only distantly related to contemporary monkey mitochondrial 12S rRNA sequences. However, many of these sequences were identical or closely related to human nuclear DNA sequences resembling mitochondrial 12S rRNA (isolated from a cell line depleted in mitochondria) and therefore have to be considered contamination. Subsequently in a separate study we were able to recover genuine mitochondrial 12S rRNA sequences from many extant species of nonhuman Old World primates and sequences closely resembling the human nuclear integrations. Analysis of all sequences by the neighbor-joining (NJ) method indicated that mitochondrial DNA sequences and their nuclear counterparts can be divided into two distinct clusters. One cluster contained all temporary cytoplasmic mitochondrial DNA sequences and approximately half of the monkey nuclear mitochondriallike sequences. A second cluster contained most human nuclear sequences and the other half of monkey nuclear sequences with a separate branch leading to human and gorilla mitochondrial and nuclear sequences. Sequences recovered from ancient materials were equally divided between the two clusters. These results constitute a warning for when working with ancient DNA or performing phylogenetic analysis using mitochondrial DNA as a target sequence: Nuclear counterparts of mitochondrial genes may lead to faulty interpretation of results.

Key words: Nuclear integrations — Mitochondrial 12S rRNA gene — Ancient DNA — primates

Introduction

Mitochondria are energy-producing cellular organelles which contain their own genome. The size of this genome is approximately 16–17 kb in vertebrates and up to 10,000 copies may be present per cell. The organization of the mitochondrial (mt) genome is remarkably efficient, resembling bacterial and viral genomes. In total it codes for 22 tRNAs, 2 rRNAs, and 13 mRNAs; other proteins present in mitochondria are encoded by the nuclear genome. The endosymbiont hypothesis proposes that mitochondria (and other cellular organelles, like plant chloroplasts) originate from bacteria which were incorporated into a nucleus-containing host cell. (For a review see Gray 1989.) Recent evidence suggests that the most likely bacterial ancestor of the mitochondrion is an ancestor of *Mycoplasma capricolum,* or a close relative, as mitochondria appear to be the sister group of this species (Cardon et al. 1994). If the mitochondrion was a bacterium, its present-day coding strategy shows that part(s) of the original genome has been relocated to the nucleus in the past and others have probably been lost.

Copies of mitochondrial genes have been observed in the nuclear genome of many species. Sequences resem-

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bling mitochondrial rRNA genes have been observed in the insect *Locusta migratoria* (Gellissen et al. 1983), the rat (Hadler et al. 1983), and humans (Nomiyama et al. 1985), and cytochrome b gene sequences have been seen in rodents (Smith et al. 1992). Two human nuclear clones, one containing part of a copy of the 16S rRNA gene and the other the 16S, $tRNA^{val}$, and part of the 12S gene, have been sequenced (Nomiyama et al. 1985). Homologies with contemporary human mtDNA were 84% and 80%, respectively, indicating that the sequences have diverged considerably since the integration events, which were estimated to have occurred about 12 and 15 million years ago. The high mutation rate was as expected for a pseudogene. No repeats were present at the flanking sequences, indicating that integration was random. Kamimura et al. (1989) sequenced a human genomic clone containing adjacent parts of the mitochondrial genes for 12S rRNA, cytochrome oxidase I, and two NADH dehydrogenases (ND4L/ND4). The nuclear 12S rRNA fragment had a 92% similarity to its cytoplasmic counterpart.

Recently, a 7.9-kb fragment of the mitochondrial genome has been detected in nuclear DNA of the domestic cat *Felis catus* (Lopez et al. 1994). This fragment occurs as a tandem repeat, has been amplified 38–76 times, and contains both rRNA genes; the ND1, ND2, and CO I genes; and part of the D-loop and CO II gene. It has a relatively low amount of substitutions and is estimated to have been integrated, and subsequently amplified, in the nuclear genome of an ancestor of the genus *Felis* approximately 1.8–2.0 million years ago.

Mitochondrial DNA is a favorite tool for those studying ancient DNA and molecular phylogenies because of its high rate of evolution, its high copy number, and its mainly maternal inheritance. In this paper, we show that caution should be taken when analyzing sequences obtained by PCR amplification using conserved primers for the mt 12S rRNA gene, as several sequences with high homology to this gene are present in the nuclear DNA of primate species, including humans.

Materials and Methods

Samples. Ancient organic samples were obtained from the following sites or institutions: Seven primate bones were collected at the North Saqqara Baboon Galleries (Saqqara, Egypt): six left femurs and a right humerus from different monkey species (possibly baboons, African green monkeys, or Barbary macaques), approximately 2,000–2,400 years old (Perizonius et al. 1993; Van der Kuyl et al. 1994). Also two teeth were collected from the skull of a male baboon. Abdomenal tissue samples were obtained from an Egyptian mummified baboon (number AAM15, Anastasy Collection 1829 A.D.) of unknown provenance, kept at the National Museum of Antiquities (Leiden, The Netherlands). Additional samples were obtained from the skins of red-capped mangabeys (*Cercocebus torquatus torquatus,* numbers #220372, #220092, #220354), dating to 1917–1918 A.D., and sooty mangabeys (*Cercocebus torquatus atys,* numbers #63305, #220953, and #481748), dating to 1896–1971 A.D., all from the Smithsonian Institution (Washington, D.C.).

Human skin fibroblasts, cultured in the continuous presence of 50 ng ethidium bromide (EB)/ml medium for at least 10 cell generations (Jakobs et al. 1994), were used as a source of nuclear DNA. These cells are completely depleted of mtDNA, as intercalation of EB in mtDNA causes inhibition of mtDNA replication and transcription.

Serum or blood cells were obtained from the following Old World primates: *Gorilla gorilla* (lowland gorilla), *Macaca sylvanus* (Barbary macaque), *Mandrillus sphinx* (mandrill), *Papio hamadryas* (hamadryas or sacred baboon), *Colobus guereza* (Abyssinian black-and-white colobus), *Cercocebus aterrimus* (black mangabey), *Cercopithecus aethiops pygerythrus* (vervet), *Cercopithecus aethiops tantalus* (tantalus monkey), *Cercopithecus aethiops sabaeus* (green monkey), *Cercopithecus patas* (patas monkey), and *Cercopithecus nictitans* (spot-nosed guenon). Origins of the samples were as published previously (Van der Kuyl et al. 1995).

DNA Extraction, PCR Amplification, and Sequencing. Total DNA was extracted from serum, cells, or ancient tissue/bone powder by a procedure using silica and GuSCN (Boom et al. 1990). For the ancient samples, initial DNA extractions were done in L6 buffer (containing GuSCN; Boom et al. 1990) for 24 h at 37°C, and appropriate precautions were taken to prevent contamination with contemporary DNA. PCR amplifications were performed with a 5′-shortened primer, L01373 5′AGAAATGGGCTACATTTTCT 3′ (Thomas et al. 1989), in combination with H01478 (Kocher et al. 1989), which amplify a fragment of approximately 104 bp of the mitochondrial 12S rRNA gene. Alternatively, the upstream primer L01091 (Kocher et al. 1989) was used in combination with H01478 to amplify a larger fragment of approximately 390 bp. Numbering of the primers refers to the position of the 3′ nucleotide in the human mitochondrial sequence of Anderson et al. (1981). PCR amplifications were performed using the following protocol: denaturation 5 min 95°C, amplification 35 cycles of 1 min 95°C, 1 min 55°C, 2 min 72°C, followed by an extension of 10 min 72°C. PCR products were cloned using the TA Cloning Kit from Invitrogen. Sequencing of the clones was done in both directions using an Applied Biosystems 373A automated DNA sequencer, following the manufacturer's protocols.

Sequence Analysis. Alignment of the sequences was done using Clustal (Higgins and Sharp 1988) and adjusted by hand. The cluster analysis was done using the neighbor-joining method as implemented in the MEGA package (Kumar et al. 1993); 100 bootstrap replicates were analyzed. The distance matrix was made using Tamura-distances (Tamura 1992).

Results

In the PCR reactions, performed with primers L01373/H01478 designed for the cytoplasmic mitochondrial 12S rRNA gene, six out of 16 ancient monkey samples were consistently negative (including one bone, one tooth, and four of the six mangabey skins), although primer-dimers could be observed (indicating that no inhibition of the polymerase had occurred due to the ancient DNA extract). Höss and Pääbo (1993) have argued that the extraction method used is the most efficient for old samples, suggesting that negative results were not due to the extraction method. The age of the samples ranges from approximately 400 B.C. to 1971 A.D., but negative PCR results were not correlated with increasing age. Sequence analysis showed that in many cases, PCR fragments were obtained from contemporary human DNA contaminants

Table 1. PCR and sequence results of ancient samples

Sample	Species	PCR amplification[a]	Human mtDNA contamination[c]	Other sequences
Femur 1	Baboon	+/+[b]	Yes	—
Femur 2	Monkey	+/+	Yes	Femur 2.1; femur 2.2; femur 2.3
Femur 3	Monkey	+/–	Yes	Femur 3.1
Femur 4	Monkey	–/–	—	—
Femur 5	Monkey	+/+	No	Femur 5.1
Femur 6	Baboon	+/+	Yes	Femur 6.1; femur 6.2
Humerus	Baboon	+/+	No	Humerus 1
Tooth	Baboon	+	Yes	Tooth 1.1; tooth 1.2
Tooth	Baboon	–	—	—
Abdominal tissue	Baboon	+	Yes	Tissue 1; tissue 2; tissue 3
Skin (#220372)	Mangabey	–	—	—
Skin (#220092)	Mangabey	+	Yes	Skin 1; skin 2
Skin (#220354)	Mangabey	–	—	—
Skin (#63305)	Mangabey	–	—	—
Skin (#220953)	Mangabey	+	N.d.[d]	N.d.[d]
Skin (#481748)	Mangebey	–	—	—

[a] PCR amplification of a 104-bp fragment with primers for the mtDNA 12S rRNA gene
[b] Two samples from each bone were analyzed
[c] Human cytoplasmic mtDNA contamination, determined by sequence analysis after cloning of PCR products
[d] N.d. = not determined

as indicated by the recovery of cytoplasmic human mtDNA sequences (Table 1). Although precautions have been taken, and extraction and PCR controls were negative, materials have often been handled by museum curators and archaeologists. Table 1 summarizes the PCR and sequence results of the ancient samples. Sequences other than readily identifiable human mtDNA were also obtained. They could easily be aligned with the mt 12S rRNA gene, but were only distantly related to contemporary monkey mt 12S rRNA gene sequences (Fig. 1). Mutations can be expected in ancient DNA as chemical breakdown of nucleotides occurs normally. However, the large amount of nucleotide change observed in these sequences prompted us to look for another DNA source. None of the sequences could be aligned with known mt sequences from other vertebrate species, so we decided to clarify whether they could be attributed to a human nuclear source.

PCR amplification of total DNA from human skin fibroblasts depleted in mtDNA with mitochondrial primers L01373/H01478 resulted in several clones with high homology or identity to our "ancient" clones. Eight different sequences were obtained out of a single PCR amplification (Fig. 1); three of them were found twice. Sequence identity of "ancient" clones femur 3.1, skin 2, tissue 3, tooth 1.2, tissue 2, and skin 1 to nuclear human clones identified these DNA fragments as human contamination. In some cases, DNA amplification from ancient samples resulted in the generation of (suspected) human nuclear clones (and no human mtDNA clones) only, suggesting wrongly that no contamination had occurred. If the phenomenon of nuclear mitochondrial-derived sequences is widespread, and does not only occur in humans, problem might also be expected when these sequences are amplified for phylogenetic purposes. Recently, we sequenced 183 mitochondrial clones obtained from 82 animals belonging to 26 species of African primates (Van der Kuyl et al. 1995). Close inspection of the clones, generated with primers L01091/H01478 from total DNA, showed that from 10 animal samples 18 sequences had been amplified with a considerable homology to the human nuclear clones (Fig. 1). Frequently, not only mutations but also deletions and insertions were present in these sequences compared to the mt 12S rRNA sequence of the primate species. At first, these clones were put aside as PCR artifacts, but now we believe that they are real and that caution should be taken when analyzing only one or a few clones from a single individual of a species. Direct sequencing of the obtained PCR products will probably circumvent the problem in most cases. However, the amount of mtDNA isolated can vary between samples, as from a particular *Colobus guereza* serum only two out of the six clones sequenced gave the cytoplasmic mitochondrial 12S rRNA sequence. Larger 390-bp nuclear fragments could not be amplified from human skin fibroblasts. Possibly there are less integrations containing a large part of the 12S rRNA gene, compared to the smaller 3′ end clones, and/or the primer sites have been mutated. This is not the case in the monkey species sequenced, and might reflect a difference in the number or evolution of mitochondrial integrations. Cluster analysis of all sequences obtained using the NJ method based upon a Tamura distance matrix (Fig. 2) shows that the sequences can be divided into two clusters with low bootstrap values, labeled A and B for practical purposes. Most sequences, including all contemporary monkey mtDNA sequences, are present in cluster A. "Ancient" sequences femur 2.3, femur 5.1

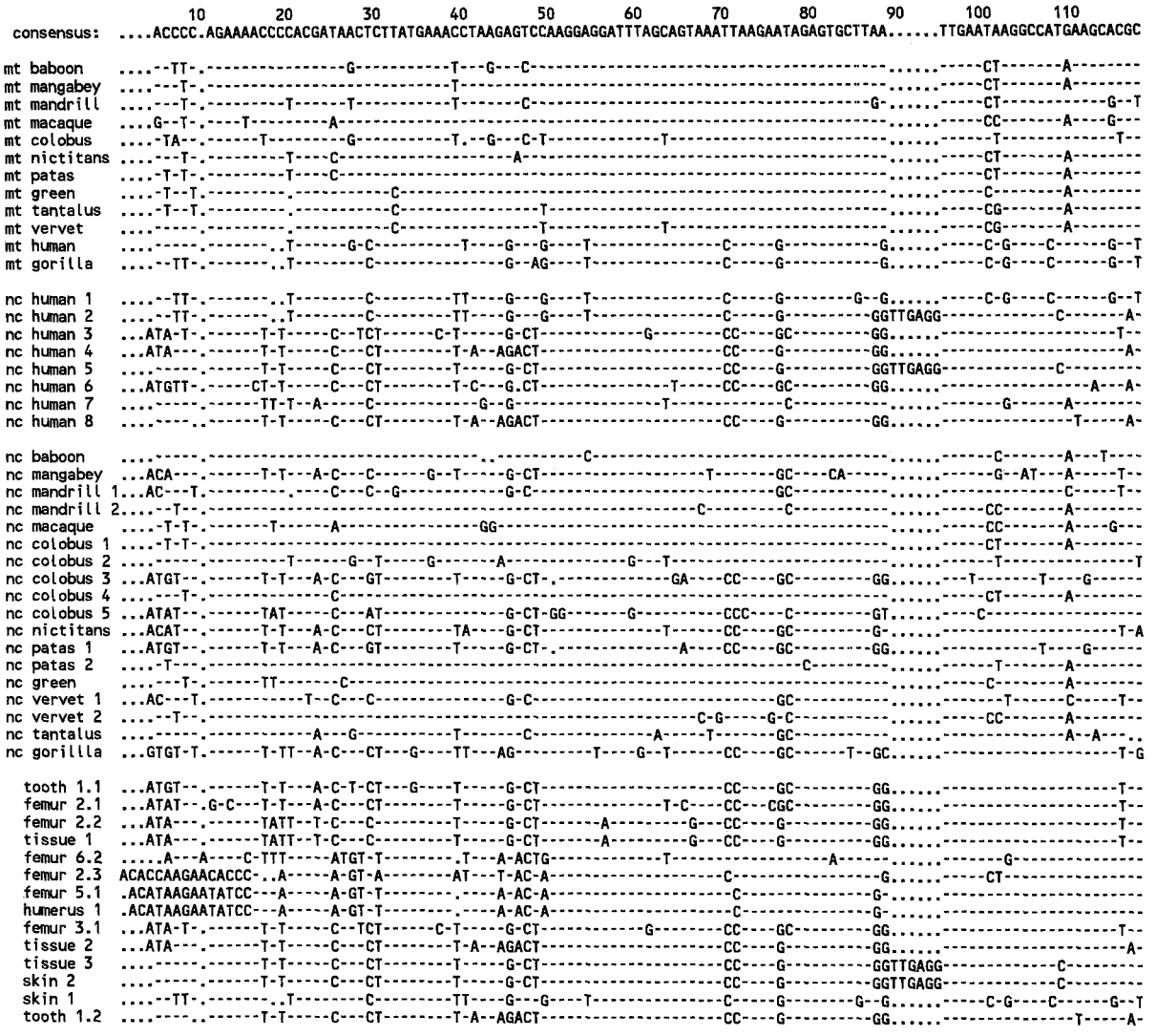

Fig. 1. Alignment of mitochondrial and nuclear sequences obtained by amplification of DNA with primers L01373/H01478 specific for the mt 12S rRNA gene. A consensus sequence obtained for all sequences is shown in the *upper line.* The sequences are divided into four groups: mitochondrial primate sequences, nuclear human sequences, primate presumed nuclear integrations, and sequences obtained from ancient samples. (See also Table 1.) Mitochondrial sequences are indicated with "*mt,*" nuclear sequences with "*nc,*" and sequences derived from ancient materials are labeled according to the source from which they were isolated. Identity of sequences obtained from ancient sources to other sequences (human nuclear sequences or each other) is as follows: *femur 3.1* = nc human 3; *tooth 1.2* = nc human 8; *tissue 2* = nc human 4; *skin 1* = nc human 1; *skin 2* = tissue 3 = nc human 5; *femur 5.1* = humerus 1; *tissue 1* = femur 2.2. Gaps introduced for optimal alignment are indicated by *dots.* Identical nucleotides are indicated by *dashes.*

(=humerus 1), and femur 6.2 form a more distant group with a high bootstrap value in cluster A, suggesting that they stem from another "family" of mtDNA-like sequences. The second cluster (B) contains most of the human nuclear sequences, as well as eight monkey-and-ape-derived presumed nuclear integrations. In this cluster, a separate branch leads to the human and gorilla mtDNA sequences, together with two human nuclear sequences. These latter might reflect relatively recent integrations expected to have occurred before the human/great ape split (approximately 5–7 million years ago) but after the ape/monkey separation. Also, three of the ancient sequences are present in this cluster, indicating that these probably originate from human contamination. Clustering of several nuclear-derived monkey sequences is not according to accepted phylogenetic placement of the species, indicating that many integrations could be ancient, predating species separation. Some integrations are probably amplified from the same locus in different species. (Compare clusters *nc vervet 1 / nc mandrill 1; nc vervet 2 / nc mandrill 2; nc colobus 3 / nc patas 1.*) In cluster B, sequences *nc colobus 3* and *nc patas 1* have similarities to human nuclear integrations, suggesting that an identical integration is present in monkey and human nuclear DNA. The monkey and ape sequences yielded a comparative phylogeny when the larger (390 bp) 12S rRNA fragments were analyzed with the neighbor-joining method (result not shown), enhancing the confidence in our phylogeny based upon smaller fragments.

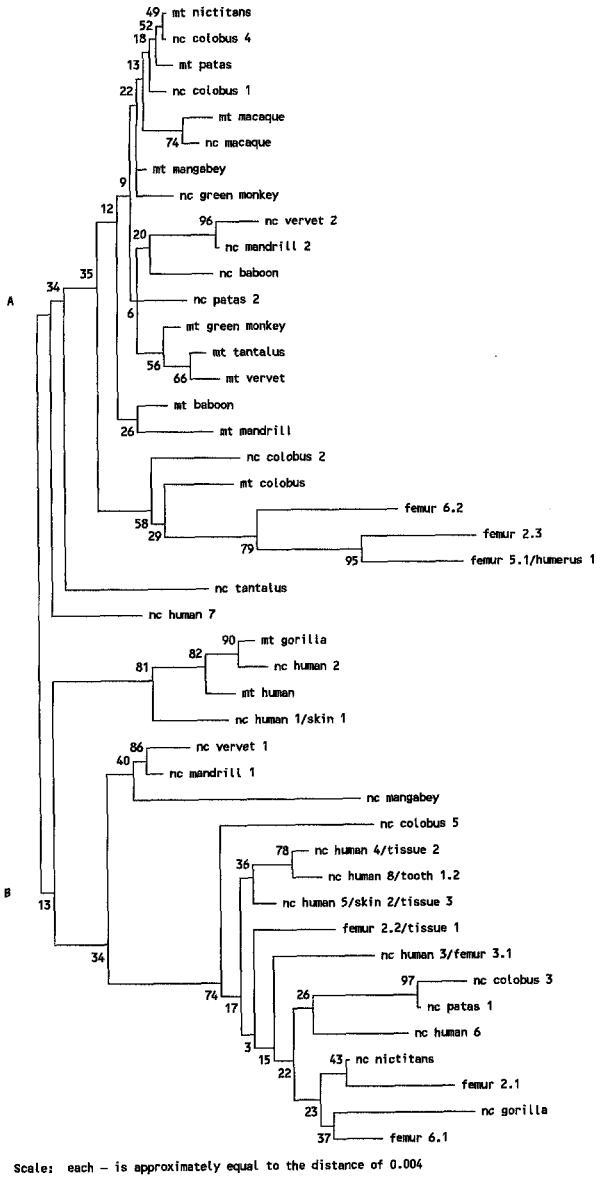

Fig. 2. NJ tree of the sequences shown in Fig. 1. Mitochondrial sequences, nuclear sequences, and sequences obtained from ancient sources are labeled as in Fig. 1. The two clusters have been named *A* and *B*. Bootstrap values (node reproduction frequencies out of 100 trees) are represented from bootstrap analysis.

Discussion

In this paper we have demonstrated the existence of nuclear sequences resembling part of the mt 12S rRNA gene in humans and other primates. It is unlikely that the amplified sequences are derived from a few aberrant mitochondrial molecules, as up till now not much variation has been observed in the mt 12S rRNA gene. Only 7 nt substitutions have been detected so far in the complete gene in humans, not occurring together in the same individual (Marzuki et al. 1991). Between individuals of a certain primate (sub)species, we have never observed insertions and deletions in this gene.

The first indications for the presence of nuclear integrations of mitochondrial rRNA genes in humans came from Tsuzuki et al. (1983b), who accidentally isolated several clones from a human cDNA library, showing homology to mt rRNA genes. Subsequent screening of a human genomic library with a probe homologous to the mt 16S rRNA gene led to the isolation of two clones: one showing homology to the mt 16S rRNA gene, and a longer clone containing parts of both rRNA genes and the tRNA gene separating them (Tsuzuki et al. 1983a). Sequencing of these clones and their flanking sequences (Nomiyama et al. 1985) suggested that the fragments are derived from nuclear integrations of part of the mitochondrial genome. In one clone a small part of the 3′ end of the 12S gene was present, with a remarkable homology to its mitochondrial counterpart. For instance, there is only a single substitution in the H01478 primer sequence. However, the fragment is too small to account for any of the sequences observed in our experiments, suggesting that more integrations exist. Also, the nuclear 12S rRNA gene fragment sequenced by Kamimura et al. (1989) cannot account for the sequences obtained by us, as it represents a more upstream part of the gene.

Out of a single PCR amplification of human nuclear DNA, eight different nuclear 12S rRNA–like clones were found. Additionally, some sequences were found more than once, suggesting that the number of integrations does not greatly exceed this number. NJ analysis of all sequences showed that some integrations are deeply rooted in the primate lineage, while others are more recent. The relatively high amount of substitutions observed between sequences presumably amplified from the same locus in different primate species is not unexpected for pseudogenes.

Amplification of total DNA extracted from both old and modern material showed that obtaining nuclear sequences instead of the aimed mitochondrial 12S rRNA gene is not unlikely. If similar integrations are not only present in primates but also in other species, contamination of ancient materials may become even more difficult to spot, suggesting that the study of nuclear genes is more appropriate. Contaminating modern DNA is generally amplified with preference above the fragmented and chemically modified ancient DNA (if present at all in the sample), explaining why we could not obtain genuine monkey clones. The relatively large amount of nuclear sequences amplified in contaminated ancient materials could be explained by the fact that the contaminating material will consist mainly of saliva and shedded skin cells, which contain relatively low amounts of mitochondria. In contrast, serum or blood cells were used as a DNA source when amplifying our contemporary monkey samples. Generally, blood cells are supposed to contain less nuclei and more mitochondria than the average cell (e.g., red blood cells lose their nucleus, but not their mitochondria), suggesting that the ratio of mitochondrial DNA to nuclear DNA favors the amplification of cytoplasmic mtDNA sequences in this case.

The evolution of nuclear sequences (genes and pseudogenes) is generally different from that of mtDNA, so anciently integrated mtDNA sequences can resemble contemporary mtDNA sequences from other species by chance or by convergent evolution. Also, in molecular phylogenetic trees, accidental introduction of a nuclear sequence might lead to unexpected placement of the species. Other mt fragments also have counterparts in the nuclear genome. Fukuda et al. (1985) showed that human nuclear DNA contains many sequences hybridizing to different parts of the mitochondrial genome.

In conclusion, we have demonstrated that it is possible to amplify by PCR primate nuclear sequences closely resembling the mitochondrial 12S rRNA gene, which could interfere with experiments designed to obtain exclusively cytoplasmic mitochondrial sequences.

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References

Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJH, Staden R, Young IG (1981) Sequence and organization of the human mitochondrial genome. Nature 290:457–465

Boom R, Sol CJA, Salimans MMM, Jansen CL, Wertheim-van Dillen PME, van der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495–503

Cardon LR, Burge C, Clayton DA, Karlin S (1994) Pervasive CpG suppression in animal mitochondrial genomes. Proc Natl Acad Sci USA 91:3799–3803

Fukuda M, Wakasugi S, Tsuzuki T, Nomiyama H, Shimada K, Miyata T (1985) Mitochondrial DNA-like sequences in the human nuclear genome. Characterization and implications in the evolution of mitochondrial DNA. J Mol Biol 186:257–266

Gellissen G, Bradfield JY, White BN, Wyatt GR (1983) Mitochondrial DNA sequences in the nuclear genome of a locust. Nature 301: 631–634

Gray MW (1989) The evolutionary origins of organelles. Trends Genet 5:294–299

Hadler HI, Dimitrijevic B, Mahalingam R (1983) Mitochondrial DNA and nuclear DNA from normal rat liver have a common sequence. Proc Natl Acad Sci USA 80:6495–6499

Higgins DG, Sharp PM (1988) CLUSTAL: a package for performing multiple sequence alignments on a microcomputer. Gene 73:237–244

Höss M, Pääbo S (1993) DNA extraction from Pleistocene bones by a silica-based purification method. Nucleic Acids Res 16:3913–3914

Jakobs BS, van den Bogert C, Dacremont G, Wanders RJA (1994) β-Oxidation of fatty acids in cultured human skin fibroblasts devoid of the capacity for oxidative phosphorylation. Biochim Biophys Acta 1211:37–43

Kamimura N, Ishii S, Liandong M, Shay JW (1989) Three separate mitochondrial DNA sequences are contigious in human genomic DNA. J Mol Biol 210:703–707

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci USA 86:6196–6200

Kumar S, Tamura K, Nei M (1993) MEGA: molecular evolutionary genetics analysis, version 1.0. The Pennsylvania State University, University Park, PA

Lopez JV, Yukhi N, Masuda R, Modi W, O'Brien SJ (1994) *Numt*, a recent transfer and tandem amplification of mitochondrial DNA to the nuclear genome of the domestic cat. J Mol Evol 39:174–190

Marzuki S, Noer AS, Lertrit P, Thyagarajan D, Kapsa R, Utthanapol P, Byrne E (1991) Normal variants of human mitochondrial DNA and translation products: the building of a reference data base. Hum Genet 88:139–145

Nomiyama H, Fukuda M, Wakasugi S, Tsuzuki T, Shimada K (1985) Molecular structures of mitochondrial DNA-like sequences in human nuclear DNA. Nucleic Acids Res 13:1649–1658

Perizonius WRK, Attia MAM, Smith HS, Goudsmit J (1993) Monkey mummies and North Saqqara. Egyptian Archaeol 3:31–33

Smith MF, Thomas WK, Patton JL (1992) Mitochondrial DNA-like sequence in the nuclear genome of an akodontine rodent. Mol Biol Evol 9:204–215

Tamura K (1992) Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Mol Biol Evol 9:678–687

Thomas RH, Schaffner W, Wilson AC, Pääbo S (1989) DNA phylogeny of the extinct marsupial wolf. Nature 340:465–467

Tsuzuki T, Nomiyama H, Setoyama C, Maeda S, Shimada K (1983a) Presence of mitochondrial-DNA-like sequences in the human nuclear DNA. Gene 25:223–229

Tsuzuki T, Nomiyama H, Setoyama C, Maeda S, Shimada K, Pestka S (1983b) The majority of cDNA clones with strong positive signals for the interferon-induction-specific sequences resemble mitochondrial ribosomal RNA genes. Biochem Biophys Res Commun 114: 670–676

Van der Kuyl AC, Dekker J, Attia MAM, Iskander N, Perizonius WRK, Goudsmit J (1994) DNA from ancient Egyptian monkey bones. Ancient DNA Newslett 2:19–21

Van der Kuyl AC, Kuiken CL, Dekker JT, Goudsmit J (1995) Phylogeny of African monkeys based upon mitochondrial 12S rRNA sequences. J Mol Evol 40:173–180