J Forensic Sci, July 2006, Vol. 51, No. 4
doi:10.1111/j.1556-4029.2006.00176.x
Available online at: www.blackwell-synergy.com

David R. Foran,[1] *Ph.D.*

Relative Degradation of Nuclear and Mitochondrial DNA: An Experimental Approach*

ABSTRACT: Single copy nuclear loci often cannot be amplified from degraded remains, necessitating the analysis of mitochondrial DNA (mtDNA). The success in analyzing mtDNA is generally thought to result from its higher copy number in the cell; however, other factors, such as cellular location or molecular features, may be equally or more important in the superior preservation of mtDNA. To explore and compare mtDNA and nuclear DNA degradation, mouse tissues (muscle, liver, and brain) were allowed to degrade at different temperatures, and the relative degradation of a mitochondrial gene, a single copy nuclear gene, and a multi-copy nuclear gene was assayed using real-time polymerase chain reaction. The tissues were also homogenized, allowing the three loci to degrade in the same cellular environment. Gene copy number and cellular location both influence DNA recovery. In some instances, multi-copy loci could be recovered when the single copy locus could not; however, the pattern of relative DNA degradation changed between whole and homogenized tissues. The overall results indicate that DNA degradation is influenced by multiple factors—including cellular location, chromatin structure, and transcriptional activity—factors that could be used to exploit loci for more robust forensic analysis from degraded biological material.

KEYWORDS: forensic science, DNA typing, DNA degradation, mitochondrial DNA, nuclear DNA, real-time polymerase chain reaction, mouse tissues, cytochrome b, 28S ribosomal RNA gene, myelin basic protein gene

State-of-the art forensic methods for DNA-based identification involve nuclear DNA analysis of short tandem repeat (STR) loci using the polymerase chain reaction (PCR). One important advantage that STR and other PCR-based analyses have over earlier DNA typing techniques is that they are more likely to be successful on degraded DNAs, which are often encountered in forensic situations. Over time, however, unless frozen, desiccated, or otherwise preserved, DNA in a cell will degrade, and in many circumstances even PCR-based testing of nuclear DNA will no longer be successful. In these situations, forensic scientists often turn to mitochondrial DNA (mtDNA) analysis (1,2). MtDNA has been effectively isolated and amplified from aged and degraded forensic samples including skeletal remains (reviewed in (3)), fingernails (e.g., (4)), and shed hairs (e.g., (5)), where nuclear DNA testing is often unsuccessful. Indeed, mtDNA analysis has become the method of choice for many anthropologists working on ancient skeletal material. The Armed Forces DNA Identification Laboratory routinely analyzes mtDNA for identification of military personnel whose remains are recovered from earlier overseas conflicts as well as from aircraft crashes and other mass disasters, and the FBI mtDNA caseload has increased to the extent that regional mtDNA laboratories have recently been established.

Owing to its circular structure, mtDNA can uniquely be isolated away from nuclear DNA using cesium chloride density centrifugation and an intercalating dye such as perpidium iodide or ethidium bromide (6). While the advent of PCR has largely made this method obsolete, being able to purify mtDNA in this way meant that the molecule could be studied in detail long before most nuclear loci, which had to await cloning techniques for their isolation and individual characterization. Yet, in spite of the head start molecular biologists have had in mtDNA analysis, the reason(s) for its successful analysis from aged/degraded material when STRs or other nuclear DNA markers fall short remains unclear.

The most obvious factor that could influence positive mtDNA analysis from forensic samples is copy number. For simplicity, forensic scientists examine single copy loci for identification purposes, as these allow straightforward interpretation and statistical calculations (although some quantification methods do assay multi-copy DNA). In contrast, because eukaryotic cells contain multiple mitochondria, and because each mitochondrion contains one or more mtDNAs, most cells have multiple, basically identical copies of their mtDNA. Robin and Wong (7) estimated that mammalian cells house 80–680 mitochondria (dependent on cell type) and 200–1700 mtDNAs, or about 2.6 DNAs/mitochondrion. Certainly, mtDNA PCR amplification success should be better than that of single copy loci in compromised forensic samples, and countless authors attribute mtDNA typing success to copy number.

Beyond copy number, additional fundamental differences exist between mtDNA and nuclear DNA. Foremost among these is that each is located in a different cellular organelle. Bermudes and Margulis (8) proposed that mitochondria stem from externally derived, prokaryote-like ancestors that now exist within the eukaryotic cell. The hypothesis was supported on many levels, including the mitochondrial circular chromosome, different genetic code, bacteria-like ribosomal RNAs, and the lack of a nucleus. Today, based on extensive protein and DNA sequence analyses, the mitochondrial precursor seems to have been eubacterial, with the closest extant group being α-proteobacteria. This ancient mitochondrial ancestor apparently entered, fused with, or was engulfed by archaea (perhaps a methanogen), leading to modern eukaryotes (9–11). The actual mechanisms for this, and the primitive organisms involved, are under constant debate (12–14) but the vastly different origins of the modern eukaryotic mitochondrion and nucleus are widely accepted. Interestingly, of the

[1]Forensic Science Program, School of Criminal Justice and Department of Zoology, Michigan State University, East Lansing, MI 48824.

*A portion of this work was presented at the Mid-Atlantic Association of Forensic Scientists annual meeting in Williamsburg, VA.

Received 14 May 2005; and in revised form 5 Sept. 2005; 28 Jan. 2006; accepted 18 Mar. 2006; published 21 June 2006.

α-proteobacteria for which genome sequences are available, the closest to mtDNA is *Rickettsia prowazekii* (15), the causative agent of typhus. *Rickettsia* are obligate intracellular parasites (the typhus agent is spread between humans by lice, fleas, etc.) and depend on their hosts for some proteins, as well as ATP early in the life cycle; however, the electron transport chain is comparable to that in the most similar mitochondria.

It is intriguing to consider how these bacteria have lost their ability to live beyond their hosts, much as mitochondria have, and are resistant to host cell destruction. Clearly, the progenitor of the modern mitochondrion was able to forgo host cell destruction as well, a characteristic that may be at least partially retained by mitochondria today. In contrast, the host cell, with its DNA organized within a structure that presumably evolved into the modern nucleus, would not need to be resistant to such insult. Indeed, the nuclear membrane is itself highly porous, allowing passage of large macromolecules such as mRNAs.

Numerous other differences exist between mitochondria and nuclei, and their DNA, one or more of which could influence its degradation. The linearity of nuclear DNA might make it susceptible to exonucleases that would not digest a circular molecule. Chromatin structure may have an influence (negative or positive) on nuclear DNA that would not be found with mtDNA. Several enzymes are located in each organelle, including polymerases that have varying nuclease activities. Likewise, different tissues demonstrate different levels of postmortem DNA degradation (e.g., (16,17)), presumably owing to dissimilar autolytic or putrefying processes, although characteristics of the tissue (e.g., its natural ability to regenerate), or the DNA itself could come into play. Unfortunately, *in vivo* experiments addressing differential DNA stability relevant to forensics are somewhat limited beyond the standard observation that larger amplicons are more difficult to generate or show reduced peak heights in degraded samples, and mtDNA results are easier to obtain than nuclear ones. Regarding this latter point, Ito et al. (18) showed that mtDNAs isolated from postmortem mouse tissues stored at 4°C for a month or more were able to undergo replication and transcription when transferred to mtDNA-less cells.

To begin examining the relative degradation of mtDNA and nuclear DNA, and to address the influence of DNA copy number and cellular location on their degradation, a series of controlled experiments was performed on freshly harvested tissues from an inbred mouse strain, which were allowed to decompose at varying temperatures. Levels of a single-copy gene (myelin basic protein [MBP]), a medium copy-number gene existing at several hundred copies per cell (similar to mtDNA) forming large repeat units at multiple loci (the 28S ribosomal RNA gene [28S]) (19), and a region of mtDNA (the cytochrome b gene [Cyt b]), were assayed using real-time PCR. Amplicons were of similar size (ca. 200 bp), and multiple animals and PCR replicates were tested. DNA degradation was assayed in harvested whole tissues stored at different temperatures, as well as after cellular disruption via tissue homogenization, which placed both mitochondrial and nuclear loci into the same cellular milieu, assessing the influence of cellular location.

Materials and Methods

Tissue Degradation

Preliminary experiments related to tissue selection, real-time PCR amplification parameters (reagent concentrations and cycling settings, such that a single, clean PCR product was produced), and tissue incubation conditions that would generate usable results (e.g., all DNA was not completely degraded) were conducted on multiple mice; details can be found in Yoshida (20). Experiments outlined below (including animals/tissues, DNA preparations, and PCR procedures) stem from methods and parameters found to give informative results in preliminary trials.

For the examinations of relative DNA degradation, three adult C57BL/6 mice were obtained from Jackson Laboratories (Bar Harbor, ME). All animals were utilized with University approved procedures, and were sacrificed by cervical dislocation immediately preceding organ removal. Tissue samples (brain, liver, rear leg muscle) were isolated and approximately one-third of each sample was immediately frozen on dry ice (and subsequently stored at −20°C until processing) as a zero time point control. Another third was kept on ice as whole tissue. The remaining third was homogenized on ice using a 1 mL Kontes (Vineland, NJ) glass homogenizer and 200 μL of sterile isotonic saline, in order to place all DNA types into the same cellular milieu during degradation. Homogenization was carried out for the minimum amount of time required to make the tissue into a uniform slurry. Whole and homogenized tissues were then divided into sterile 1.5 mL microcentrifuge tubes for incubation at −20, 4, 24, and 37°C for 1 week, a length of time found not to result in total DNA degradation in preliminary experiments. Samples at 24 and 37°C were covered with Parafilm with needle holes punched through to allow air to circulate. Because preliminary experiments showed that samples at 37°C became desiccated, these were placed in a tray that was lined with water-saturated paper towels and loosely covered with a plastic wrap (relative humidity ca. 45%).

DNA Isolation

Following tissue incubation, DNA was isolated using a standard organic extraction. Briefly, whole tissues were minced using a sterile scalpel, and all samples were incubated overnight at 56°C in 400 μL of 10 mM Tris, pH 8, 100 mM NaCl, 50 mM EDTA, and 0.5% SDS, with addition of 2 μL of 20 mg/mL proteinase K. Samples were extracted with an equal volume of phenol/chloroform/isoamyl alcohol (25/24/1), and the aqueous layer was precipitated with 1/10 volume of 3 M NaAcetate and two volumes of 95% ethanol. DNAs were centrifuged, vacuum dried, and resuspended in 100 μL of TE (10 mM Tris, pH 8, 1 mM EDTA).

Real-Time PCR

The three loci were amplified using a Cepheid SmartCycler (Sunnyvale, CA). All PCR reactions were run in triplicate in a 25 μL reaction volume. Reactions were set up using a master mix containing the appropriate DNA sample (equivalent to 2 μL of DNA/reaction), to ensure that the same quantity of DNA from a tissue was in each reaction. The master mix consisted of 3.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, 200 μM each dNTP, and 1.5 U Taq polymerase (Promega, Madison, WI). 1 × SYBR Green (Molecular Probes, Eugene, OR) (equivalent to a 1:10,000 dilution) was also added. Aliquots of the master mix were distributed to new tubes and primers (2 μM) added. The primer sequences were as follows: Cyt b (172 bp) forward TGTTCGCAGTCATA GCCACAG, reverse TCGGGTCAAGGTGGCTTTGTCTA; 28S (212 bp) forward CGGCGAGTGAACAGGGAAGAGCC, reverse GCATTCCCAAGCAACCCGACACC; MBP (188 bp) forward TGTTCCGAGCACACAAAAGAG, reverse GGAGGGCACAGG AAACAAAAA. The cycling parameters for Cyt b and MBP were 95°C for 3 min, followed by 50 cycles of 94°C for 30 sec, 56°C for

1 min, and 72°C for 30 sec. More stringent conditions were required for 28S amplification owing to the tandemly repeated nature of the loci. The parameters were 95°C for 3 min, followed by 50 cycles of 95°C for 40 sec, 72°C for 10 sec, and 75°C for 30 sec. Nine samples (three replicates of three loci/tissue sample) plus positive (high molecular weight mouse DNA) and negative (DNA replaced with sterile water) controls were amplified at a time. A threshold (C_t value) of 20 relative fluorescent units was used to compare samples; the faster the threshold is reached (i.e., the fewer PCR cycles required to reach the threshold) the more the DNA is present for that locus. Melt temperatures were measured during all reactions to confirm that a single PCR product was being produced.

Data Analysis

The C_t values from the triplicate PCR reactions for each locus of each tissue were averaged, and the values were then averaged among the replicate mice. Among all experiments, a single tissue that gave a result substantially different from the others was considered as an outlier, and was removed from data interpretation (see Results). Loci data were analyzed in a relative, not absolute manner; that is, for any given tissue and incubation temperature, results for one locus were compared with the other two loci. For each tissue, results for each marker from the frozen controls were compared, and then these values were compared with marker values for degraded samples. Changes in how a locus degraded relative to the other loci (again, beginning with the frozen controls) were noted.

Results

The design of the degradation experiments—comparing the three loci with each other within a treatment—meant that the resultant quantitative data were examined in a relative, not absolute, manner. An advantage of this was that the amount of starting DNA added to a PCR reaction was not critical; the relative levels of the three loci within a sample (and the change in these ratios as samples degraded) were the important factor. The frozen tissues represented the original relative copy number of the three loci, generating a starting C_t value for each comparison. From this starting value, the relative degradation of the three loci at warmer temperatures was examined. If all three loci degraded equally over time and temperature, their relative ratios would remain the same (e.g., if 50% of the total DNA was lost, all three loci would decline by 50%, and the relative difference in C_t values would not change). However, if loci degraded differently (e.g., one lost 10% of its copies in a certain period while another lost 50%), then the C_t values would become closer together or farther apart, reflecting that difference.

The large amount of raw data generated (several hundred PCR reactions for the results presented here, and many more while designing the final procedures) can be found in (20). A summation of the data is shown in Table 1. Overall, there was consistency among replicate runs and within tissue types among animals; thus, averages are shown in the table. Not surprisingly, as samples were allowed to degrade, some variation among animals was seen, although consistency within PCR replicates remained high. In all instances, negative controls did not produce an amplification product. Likewise, a single melt peak was produced during the PCR process, indicating that extraneous product was not amplified. This was confirmed by agarose gel electrophoresis, which showed a single band.

TABLE 1—*Relative degradation of loci among tissue and treatment types.*

	–20°	4°	24°	37°
W. muscle				
Cyt b	12.4	14.8	12.3	11.4
MBP	26	25.4	24.2	23.9
28S	26.2	27.2	27.5	26.9
W. liver				
Cyt b	9.4	11.4	11.8	13.7
MBP	18.4	22.4	22	24.7
28S	16.3	28.1	27.8	24.8
W. brain				
Cyt b	9.6	11.6	10.3*	18.6*
MBP	18.9	19.4	21.6*	33.5*
28S	24.6	26.6	29.9*	28.8*
H. muscle†				
Cyt b	12.5	13.7	16	20.5
MBP	25	25.4	26.1	26.8
28S	27	25.4	24.4	26.8
H. liver				
Cyt b	10.5	10.7	12.7	18.2
MBP	20.4	21.9	23.8	26.3
28S	26.6	27.3	27.6	24.9
H. brain				
Cyt b	10.9	11.1	10.7‡	11.6
MBP	19.97	21.4	23.6	23.8
28S	26.6	24.7	23.5	26

*The locus was too degraded to amplify in at least one animal (see text).
†All loci at 37°C were too degraded to amplify in one animal.
‡Outlier (one sample that amplified only after a very large number of cycles) existed in this experiment that strongly skewed the final results; it is not included in these data.

Average real-time PCR C_t values for whole (W.) and homogenized (H.) tissues are displayed; a lower C_t value indicates that fewer PCR cycles were required to meet the threshold. DNA levels of the three loci should be compared within a sample, not among them. Each value resulted from three replicate amplifications from a tissue from three different animals.

PCR, polymerase chain reaction; Cyt b, cytochrome b; MBP, myelin basic protein; 28S, 28S ribosomal RNA.

DNA Degradation in Whole Tissues

Whole tissue samples stored at −20°C generated the baseline locus copy values. For all frozen tissues, Cyt b reached the 20 RFU C_t value first, after 9.6 and 9.4 cycles for the brain and the liver, respectively, and 12.4 cycles for muscle (average values among runs and animals). As expected the single copy locus, MBP, required several more cycles to reach the threshold: 18.9 for the brain, 18.4 for the liver, and 26.0 for muscle. The multi-copy 28S amplification required 24.6, 16.3, and 26.2 cycles, respectively, although it should be noted that 28S amplification used far different cycling parameters, so these values must be viewed in that context; their change relative to the other loci is the critical factor.

There was little change in relative C_t values for whole tissues stored at 4°C when compared with those at −20°C. The exception was the 28S loci in the liver, which appeared to be degrading much faster than either MBP or Cyt b. This trend was maintained in whole liver samples incubated at higher temperatures (24 and 37°C), where it consistently degraded faster than Cyt b. At these temperatures, the MBP locus also degraded faster than Cyt b, although the difference was not as pronounced as with 28S.

Whole muscle tissue was similar to the liver in that 28S loci degraded faster than MBP and Cyt b at warmer temperatures, although the difference was not nearly as extreme as the liver. In contrast, Cyt b degraded faster than MBP in muscle; however, this difference was slight.

Whole brain seemed to have the most overall DNA degradation, with all loci at 24 and 37°C failing to amplify in one sample. In the other two samples, MBP degraded more relative to the other loci, including not amplifying at 24°C in one nor at 37°C in the other. For the remaining 24 and 37°C MBP trials, a greater number of PCR cycles was needed to reach C_t values as well. 28S degraded to a greater extent than Cyt b.

DNA Degradation in Homogenized Tissues

The goal of homogenizing tissues was to put all DNAs into the same cellular milieu, thus diminishing any protection from degradation afforded by organellar membranes or other localized factors. Not surprisingly, frozen homogenized samples generally had relative amplification of the three loci similar to those found in whole tissues. However, incubation of homogenized samples above freezing resulted in strikingly different degradation patterns.

Liver samples homogenized and incubated at 4, 24, and 37°C showed a complete and temperature-dependent (more extreme at warmer temperatures) change in the relative order of DNA degradation compared with −20°C, with Cyt b and MBP degrading substantially faster relative to 28S. The degradation of Cyt b and MBP was similar at the lower two temperatures, while at 37°C Cyt b degraded slightly faster. It should be noted, however, that in homogenized liver subsequently stored at −20°C, 28S may have degraded early even though homogenization was conducted over wet ice for the minimal amount of time necessary (see Discussion). Still, the locus amplified at all temperatures.

Homogenized muscle showed a similar trend, with Cyt b and MBP degrading faster than 28S. In one sample, no loci amplified after the 37°C incubation. The change in degradation was again temperature dependent, with Cyt b increasing in degradation relative to the other loci as temperature increased, although there was no increase in degradation of MBP relative to 28S from 24 to 37°C.

In homogenized brain, 28S again had slightly slower degradation compared with the other loci (most apparent at 24°C), although in brain, Cyt b degradation was not as extreme as in the other two tissues. MBP showed more degradation than the other loci, although this was much lower than in whole brain.

Discussion

The goal of these experiments was to examine the relative degradation of mtDNA and nuclear DNA, in an attempt to increase our understanding of why mtDNA can often be isolated from old or degraded biological samples when nuclear DNA cannot. Two primary hypotheses were tested. The first explains successful mtDNA analysis in poor or compromised samples through its higher cellular copy number when compared with single copy loci, such as the STRs currently used for human identification. The second accounts for successful mtDNA analysis through its location in the mitochondrion, resulting in a higher level of "protection" than the nucleus affords chromosomal DNA. If the first hypothesis is correct, examining multi-copy nuclear markers in degraded tissues should be as successful as mtDNA analysis, and potentially more so as some nuclear genes exist in copy numbers far exceeding mtDNA. In this regard, given the vast size of the nuclear genome, identifying high copy number markers could potentially lead to genetic identification schemes that are equal to or more successful than mtDNA analysis, while offering far more resolution than mtDNA. On the other hand, if the second hypothesis is correct and mtDNA is differentially protected inside the mitochondrion, then mtDNA is unique in its ability to endure tissue degradation, and there will possibly be few alternatives to its use in poor or compromised samples, although attempts to augment standard STR-based identification have been made, with varying success (21).

The results from the degradation experiments presented here do not (not surprisingly) wholly negate either hypothesis. When samples were allowed to degrade, there were two instances, both from whole brain tissue, in which the high copy number loci could be amplified while the single copy locus could not, and never an instance when the opposite was true. Clearly, copy number influences the ability to recover specific loci from degraded samples. Further, in no instance could Cyt b be amplified when 28S could not, or vice versa. However, there were samples (one whole brain and one homogenized muscle) in which all three markers could not be amplified, and higher copy number itself did not assure successful DNA amplification. Interestingly, the brain is sometimes considered to be an organ with relatively low levels of DNA degradation (16,17), although the opposite was seen in this study. It is possible that the removal of the skull and subsequent brain sectioning, with the resultant loss of the blood–brain barrier, may have increased brain DNA degradation in these experiments.

Cellular location, on the other hand, seems to have an extreme influence on DNA degradation, in particularly affecting the degradation of mtDNA. In all whole tissues, Cyt b degraded less than the nuclear loci in general, and far less than 28S (which seemed most susceptible to degradation; see below). This result was reversed in homogenized tissues. Once put into the same cellular milieu, Cyt b generally degraded faster than the nuclear markers, with the most pronounced difference existing between it and 28S. The fact that the cellular environment plays a key role in mtDNA survival and successful analysis is, perhaps, to be expected. It is known, for instance, that as hair shafts develop and keratinization occurs, the nuclei of these cells are lost while mitochondria, although fragmented, still exist, presumably resulting in the ability to obtain mtDNA results from telogen hairs when nuclear DNA analysis is impossible (22). However, the reversal in degradative vulnerability observed in the experiments detailed above was striking, in that the mtDNA in homogenized samples did not simply reach par with the nuclear loci, but actually surpassed them in its level of degradation. In this regard, it is possible that the nucleosomes that make up chromatin have a feature of their own that influences DNA's susceptibility to degradation, or that some other characteristic of the nucleus or nuclear DNA functions in protecting it from degradation when placed in the same environment as mtDNA.

It is also possible that cellular homogenization removed nuclear DNA from components that preferentially led to its degradation. For instance, nuclear DNA separated from the nucleus would no longer be as exposed to the multiple polymerases that exist there, most of which have 5′ and/or 3′ exonuclease activity. This would not directly account for the mtDNA's higher level of degradation in homogenized tissues, as both mtDNA and nuclear DNA should be equally exposed to nucleases, but it might come into play if the lack of chromatin structure, or some other factor(s) of mtDNA, makes it more vulnerable to DNases. Regardless of the mechanism, these results show that when mtDNA and nuclear DNA are placed in the same environment, mtDNA not only degrades as fast as nuclear DNA, it may degrade even faster. The cellular environment, as well as DNA makeup, have an influence on DNA degradation.

The final finding of these experiments, and at first glance the most surprising, is that the two nuclear genes studied, the multi-

copy 28S ribosomal gene and the single copy MBP gene, did not degrade similarly in either whole or homogenized tissues. Indeed, when compared with 28S, MBP behaved more like Cyt b in general (the difference being that, with the exception of homogenized brain, MBP degraded more in whole tissues and Cyt b degraded more in homogenized ones). One can surmise why 28S and MBP behave differently, with the most likely reason involving their role in the cell itself, and in particular expression of each gene. MBP is a single copy gene that is transcriptionally active in the nervous system, and is relatively inactive in other tissues (23). It is likely that, with the exception of the brain, the MBP gene exists in a histone-bound state in the tissues examined here. This could account for the very different degradation profile seen for MBP in whole brain (and to a lesser extent homogenized brain), where it degraded fastest relative to liver or muscle.

Likewise, the 28S gene (found at multiple loci) is associated with the nucleolus in all protein-producing tissues, and is actively transcribed throughout; thus, it may exist in a uniquely vulnerable state regarding DNA degradation. Its quick degradation was most apparent in whole liver but was seen in the other whole tissues as well, a trait lost upon homogenization. The extreme 28S degradation level in whole liver is based on a low starting C_t value for the control ($-20^{\circ}C$) tissue, although this low value was obtained from all mice. If liver 28S is extremely susceptible to degradation, even at $4^{\circ}C$, it is possible that the bulk of it degraded during the homogenization process, resulting in the higher $-20^{\circ}C$ C_t found there. Neither possibility substantially affects the findings presented, and the overall high level of 28S degradation observed in this study indicates that, while existing in higher copy numbers, it would make a particularly poor forensic marker.

Conclusions

The experiments described here show that successful locus recovery from degraded tissues involves multiple components. Certainly, a higher gene copy number, as exists for both 28S and Cyt b, is an important factor in obtaining typing results as tissues and DNA decay. However, other factors appear to influence substantially the ability to amplify DNA markers in degrading material, including cellular location and perhaps transcriptional activity. While a unique cellular location will continue to be the sole domain of mtDNA in animals, locus type and gene expression may be worthwhile features to consider when selecting nuclear DNA markers for forensic analyses. In this regard, loci that are downregulated or not expressed in tissues or body fluids of forensic interest, including blood, semen, bone, etc., might very well offer the best choice for analysis from degraded material. Overall, taking gene copy number, tissue type, and the function of DNA loci into account could improve our ability to analyze markers in degraded forensic material.

Acknowledgments

The author wishes to thank Ms. Kari Yoshida and Ms. Leah Ford for their efforts and involvements with this research. Thanks are also due to the two anonymous reviewers who made several worthwhile additions to this manuscript.

References

1. Holland M, Parsons T. Mitochondrial DNA sequence analysis—validation and use for forensic casework. Forensic Sci Rev 1999;11:22–50.
2. Wilson M, Stoneking M, Holland M, DiZinno J, Budowle B. Guidelines for the use of mitochondrial DNA sequencing in forensic science. Crime Lab Dig 1993;20(4):68–77.
3. O'Rourke D, Hayes M, Carlyle S. Ancient DNA studies in physical anthropology. Ann Rev Anthro 2000;29:217–42.
4. Cline R, Laurent N, Foran D. The fingernails of Mary Sullivan: developing reliable methods for selectively isolating endogenous and exogenous DNA from evidence. J Forensic Sci 2003;48:328–33.
5. Melton T, Nelson K. Forensic mitochondrial DNA analysis: two years of commercial casework experience in the United States. Croation Med J 2001;42:298–303.
6. Hudson B, Upholt W, Devinny J, Vinograd J. The use of an ethidium analogue in the dye-buoyant density procedure for the isolation of closed-circular DNA: the variation of the superhelix density of mitochondrial DNA. Proc Natl Acad Sci USA 1969;62:813–20.
7. Robin E, Wong R. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells. J Cell Physiol 1988;136: 507–13.
8. Bermudes D, Margulis L. Symbiosis as a mechanism of evolution: status of the symbiosis theory. Symbiosis 1985;1:101–24.
9. Gupta R, Golding G. The origin of the eukaryotic cell. Trends Biochem Sci 1996;21:166–71.
10. Hartman H, Fedorov A. The origin of the eukaryotic cell: a genomic investigation. Proc Natl Acad Sci USA 2002;99:1420–5.
11. Horiike T, Hamada K, Kanaya S, Shinozawa T. Origin of eukaryotic cell nuclei by symbiosis of Archaea in bacteria is revealed by homology-hit analysis. Nat Cell Biol 2001;3:210–4.
12. Searcy DG. Metabolic integration during the evolutionary origin of mitochondria. Cell Res 2003;13:229–38.
13. López-García P, Moreira D. Metabolic symbiosis at the origin of eukaryotes. Trends Biochem Sci 1999;24:88–93.
14. Gray M, Burger G, Lang BF. The origin and early evolution of mitochondria. Genome Biol 2001;2:1018.1–.5.
15. Andersson S, Zomorodipour A, Andersson JO, Sicheritz-Ponten T, Alsmark UC, Podowski R, et al. The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. Nature 1998;396:133–40.
16. Bar W, Kratzerm A, Machler M, Schmid W. Postmortem stability of DNA. Forensic Sci Int 1988;39:59–70.
17. Ludes B, Pfitzingers H, Mangin P. DNA fingerprinting from tissues after variable postmortem periods. J Forensic Sci 1993;38:686–90.
18. Ito S, Inoue K, Yanagisawa N, Kaneko M, Hayashi J. Long-term postmortem survival of mitochondrial genomes in mouse synaptosomes and their rescue in a mitochondrial DNA-less mouse cell line. Biochem Biophys Res Commun 1998;247:432–5.
19. Worton R, Sutherland J, Sylvester J, Willard H, Bodrug S, Dube I, et al. Human ribosomal RNA genes: orientation of the tandem array and conservation of the 5′ end. Science 1988;239:64–8.
20. Yoshida K. Forensic analysis of mitochondrial vs nuclear DNA degradation. Thesis, George Washington University, Washington, DC, 2001.
21. Biesecker L, Bailey-Wilson J, Ballantyne J, Baum H, Bieber F, Brenner C, et al. DNA identifications after the 9/11 World Trade Center attack. Science 2005;310:1122–3.
22. Linch C, Whiting D., Holland M. Human hair histogenesis for the mitochondrial DNA forensic scientist. J Forensic Sci 2001;46:844–53.
23. Foran D, Peterson A. Myelin acquisition in the central nervous system of the mouse revealed by an MBP-LacZ transgene. J Neurosci 1992;12: 4890–7.

Additional information and reprint requests:
David R. Foran, Ph.D.
Forensic Science Program
Michigan State University
560 Baker Hall
East Lansing, MI 48824
E-mail: foran@msu.edu