TECHNICAL NOTE

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Degeneration of Nuclei and Mitochondria in Human Hairs

ABSTRACT: It is generally accepted that nuclei degrade in developing hair shafts but the point at which such occurs has not been investigated. The fate of mitochondria in the keratinizing hair shaft has been less clear. This study uses transmission electron microscopy to investigate when nuclei and mitochondria are no longer visible in the developing hair shaft. Serial sections were obtained from anagen head hairs absent follicles in order to determine the sequence of degradation of nuclei and mitochondria in the hair shaft by starting at the root bulb and proceeding toward the hair tip. It was demonstrated that nuclei and mitochondria become invisible in the keratinizing hair shaft at about the same time. This was found to occur fairly early in the process at the level of the hair shaft where the hair cuticle becomes permanent.

KEYWORDS: forensic science, hair DNA, hair mitochondria, hair nuclei, keratinization

The ability to recover mitochondrial DNA (mtDNA) from small pieces of hair is probably possible due to the high copy number of mtDNA molecules per cell (1–6). Nuclear DNA (nuDNA) is more difficult to recover from hair shafts and efforts in that regard continue (7). It is presently unclear how a visible nucleus or a visible mitochondrion affects the ability to obtain nuDNA or mtDNA from a particular tissue type. This study investigates whether the intact mitochondrion may contribute to the successful recovery of mtDNA from keratinized hair shafts. A prior publication, in which this author was a contributor, states that during keratinization "nuclei lose their DNA" and that "presumably the mitochondrion affords protection for mtDNA molecules" (8). It may rather be that nuDNA loses its nucleus and the mitochondrion affords no protection for mtDNA molecules. Transmission electron microscopy (TEM) of serial sections from hair roots was used to determine at which stage of human hair shaft development the mitochondrion and nucleus could no longer be observed.

The nucleus and mitochondrion are the only human cell organelles that have bilayer membranes separated by an intermembrane space (9). Each of the two membrane layers of the nucleus are composed of phospholipids containing many different types of proteins. The mitochondrial outer membrane is composed of *c*. 50% lipid and 50% protein, some of which are porins (9). This membrane is similar to the outer membrane of Gram-negative bacteria and the mitochondrion is about the size of the bacterium *Escherichia coli*. The mitochondrial inner membrane is composed of *c*. 20% lipid and 80% protein, making it much less permeable than the outer membrane (9). The inner membrane forms the cristae, or infoldings, that make mitochondria easily recognizable at the ultrastructural level (9). The rod-shaped mitochondria can be *c*. 1–2 μ long and *c*. 0.1–0.5 μ wide (9).

An atlas and text of hair follicle ultrastructure by Dr. K. Morioka (10) demonstrates that mitochondria in the keratinizing neonatal rat hair shaft degrade either by lysosomal digestion or self-destruction (apoptosis). Micrographs in that text (10) show mitochondria and other cytoplasmic components of the hair cortical and cuticular cells possibly being degraded within vesicles (lysosomes). Neonatal hairs are excellent for TEM studies as fixatives and resins more readily penetrate the thin, softer hairs (10). Adult hairs are one of the most difficult tissues to prepare for TEM observation. Cells in the lower hair root bulb rapidly divide, differentiate into cuticle, cortex, or medullary cells, and quickly begin to produce trichohyalin granules and keratin microfilaments (10). These dense keratin fibers are refractory to TEM resin and fixative infiltration, thus possibly preventing cell fine structure preservation (10). The details of human hair development and structure have been previously described (8).

Materials and Methods

Transmission Electron Microscopy

Brown Caucasian, anagen head hair shafts without follicles were obtained from one individual by plucking. They were immediately placed in a fixative solution of 3% paraformaledyde and 1.5% glutaraldehyde in phosphate buffer for at least 1 day at 4°C. After postfixation in 1% osmium tetroxide, the hairs were dehydrated in a series of alcohols and propylene oxide was used as a transition solvent prior to embedment in 100% epoxy resin. Two hairs were embedded in blocks with orientation for horizontal (cross) sectioning and two hairs were embedded for longitudinal sectioning. After the resin blocks containing individual hairs were polymerized at 60°C over night, the hair roots were sectioned serially at a thickness of *c*. 88–90 nm. The sections were placed on copper grids and stained with uranyl acetate and lead citrate. The sections were examined using a Hitachi H-7650 TEM (Hitachi High Technologies America, Inc., Pleasanton, CA).

Scanning Electron Microscopy

A plucked anagen head hair shaft absent follicle was mounted on an aluminum stub, sputtered coated with gold, and examined with a Hitachi S-3400N SEM (Hitachi High Technologies America, Inc.).

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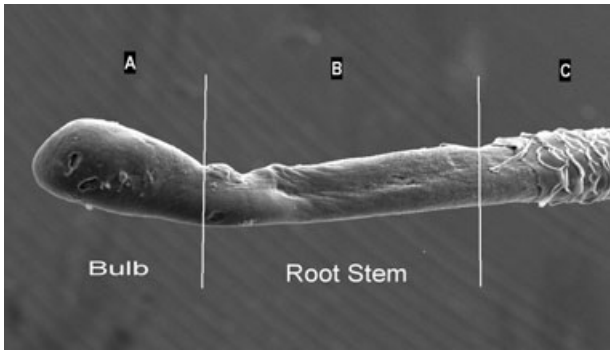

FIG. 1—*Anagen head hair absent follicle. This is the type of hair used in the study and serial sections were taken from area A (root bulb) to area C where the cuticle is visible. SEM (original magnification 210×).*

Results and Discussion

Figure 1 represents the type of anagen hair root absent follicle that sections were taken from. It has been the experience of this author that these types of hairs can produce full nuDNA profiles after 10 years of storage while mounted in Permount® on glass microscope slides. This hair root form is distinguishable from telogen roots by the crook at the bulb/root stem junction (Fig. 1). Although the hairs were from only one individual, that individual's hairs were included in a recent mtDNA study using five persons with red hair, five persons with dark brown hair, and five persons with gray hair (6). When hair pieces were cut from the same position in those hair shafts, and cut with the same length and diameter, no significant differences could be seen in recovered mtDNA amounts when using PCR product gels (6). One would expect that the greatest individual variable in hair root cell ultrastructure would include the amount and type of melanosomes rather than the number of nuclei or mitochondria.

The hairs were serially sectioned from the root bulb toward the tip within the different areas labeled A, B, and C in Fig. 1. Figures 2–4 represent cellular changes in the hair shaft as one proceeds from the hair root bulb (area A, Fig. 1) toward the hair tip. The tissue of area A, Fig. 1, is composed of metabolically active cells containing melanosomes that are specifically eumelanosomes in this instance, as the hairs that were used were dark brown (Fig. 2*A*). Some Fig. 2*A* nuclei show condensed chromatin (numerous, small dark images in the nucleus) and a distinct nucleolus (larger, single inclusion in nucleus). Numerous mitochondria and a normal nucleus are seen in the precortical cell in Fig. 2*B*. Faint cristae can be seen in the lightly stained, rod-shaped mitochondria.

In area B, Fig. 1, the keratin synthesis has progressed and the keratin bundles can be seen in cross-section in Figs. 3*A* and 3*B*. The mitochondrial cristae are not as distinct in the area B samples and the nuclei appear to be degenerative. Rod-shaped mitochondria measuring 0.3–0.4 μ are indicated at the right of Fig. 3*A*. The cytoplasmic matrix appears granular while the keratin bundles appear circular, gray, and relatively featureless. The nuclei in Fig. 3*B* show a pasty, evenly stained appearance, and an amorphous contour, as described by Morioka as nucleus degeneration in neonatal rat hairs (10). The chromatin has diffused evenly into the nucleoplasm to form a structure-less nucleus (10). The dark melanosomes appear unchanged from their appearance in Fig. 2*A*. Further disintegration of chromosomal structures can be seen by the "zebra" chromatin pattern in the flattened nuclei of cuticle cells in Fig. 5*B*. The image in Fig. 5*B* also came from area B of Fig. 1. Areas suggestive of lysosomal digestion of mitochondria are indicated by asterisks in

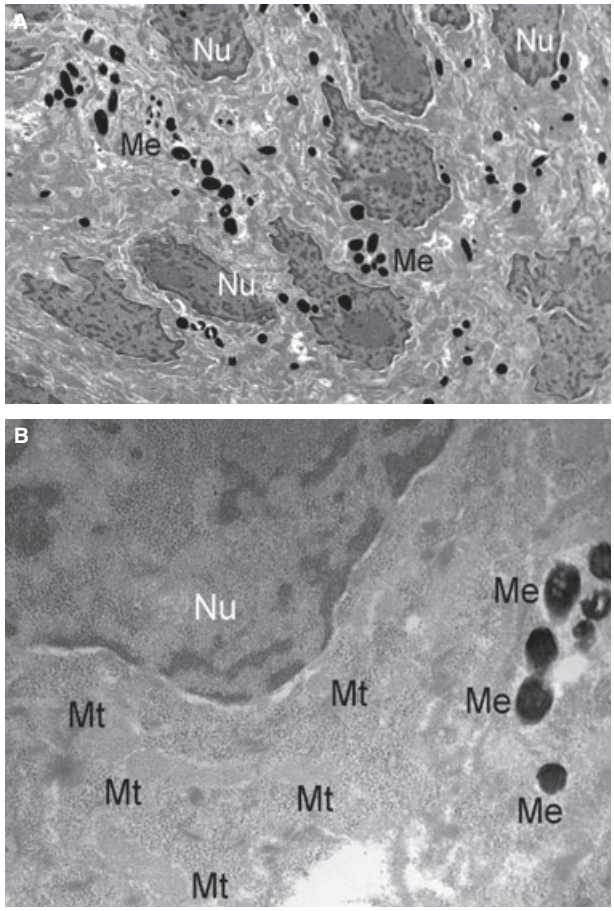

FIG. 2—*Images from area A, Fig. 1. (A) Longitudinal section of hair root matrix. TEM (original magnification 2000×). (B) Cross-section of precortical cell. TEM (original magnification 10,000×). Mt, mitochondria; Nu, nucleus; Me, melanosome; Ke, keratin.*

Figs. 3*A* and 3*B* (10). Degeneration of nuclei and mitochondria is evident in distal sections of area B, Fig. 1. The cytoplasm in the Fig. 3 images is about to be completely filled by keratin tonofilament bundles.

Images in Fig. 4 were obtained from sections in area C of Fig. 1. The cuticle is fully formed in the Fig. 4*A* cross-section and the cortex appears amorphous. Figure 4*B* shows the interdigitation of the hard keratin bundles in longitudinal section. The Fig. 4*C* longitudinal section demonstrates how organelles are forced into an orientation that is parallel to the keratin bundles. Melanosomes are the only recognizable cytoplasmic organelle at this level of the hair shaft. No nuclei or mitochondria were observed in tissue sections from area C of Fig. 1. This is about the level in the hair root stem where the cuticle has become fully keratinized.

Figure 5 illustrates the progressive metamorphosis that occurs as the hair cuticle becomes fully keratinized. Figure 5*A* is from area A, Fig. 5*B* is from area B, and Fig. 5*C* is from area C of Fig. 1. Each cuticle layer in Fig. 5*C* was an individual cell as seen in Fig. 5*A*. Cornification of the cuticle starts later than cornification of the cortex as seen in Fig. 5*B* (10).

It could be argued that the nucleus and mitochondria in the hair shaft were not properly fixed and therefore became invisible in the more keratinized sections of the hair shafts. The cuticle cells have the most exposure to the TEM fixative as they are the outer layer of the hair. One would expect the best cytoplasmic preservation in the cuticle and cortex next to the cuticle, which are shown to be

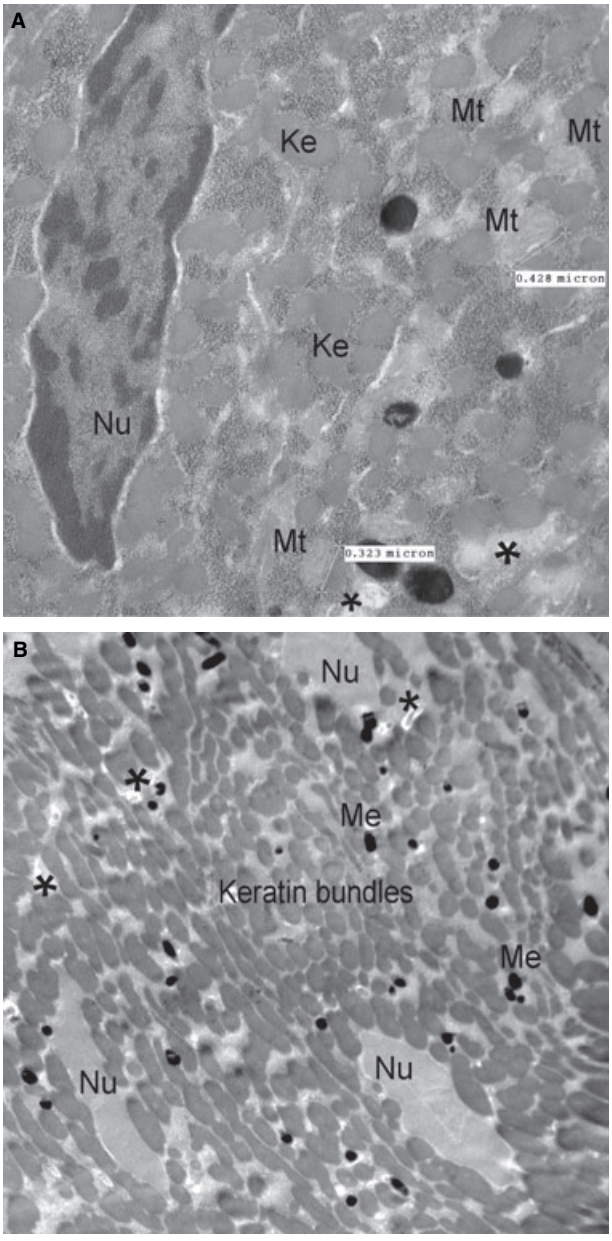

FIG. 3—*Images from area B, Fig. 1. (A) Cross-section of cortical cell. TEM (original magnification 8000×). (B) Cross-section of hair cortex. TEM (original magnification 2500×). Mt, mitochondria; Nu, nucleus; Me, melanosome; Ke, keratin. *Possible degenerating mitochondria.*

devoid of nuclei and mitochondria in Figs. 4*A* and 5*C*. It could also be argued that membranous structures like mitochondria and nuclear envelopes would not survive lysosomal scavenging or dehydration during terminal differentiation (cornification) of the keratinocyte. These ultrastructural findings were consistent with findings in neonatal rat hairs that are easier to fix for electron microscopy due to their smaller diameters and soft texture (10). It has been reported in neonatal rat studies that the degradation of mitochondria occurs fairly rapidly during the early keratinization process (10).

The stratum corneum cells of the epidermis are keratinized and nonnucleated yet DNA examiners are able to obtain nuDNA from shed skin samples. The lower nuDNA copy number and higher degree of keratinization in hair shafts are probably the major factors in hair shaft nuDNA extraction failures. Keratinization and

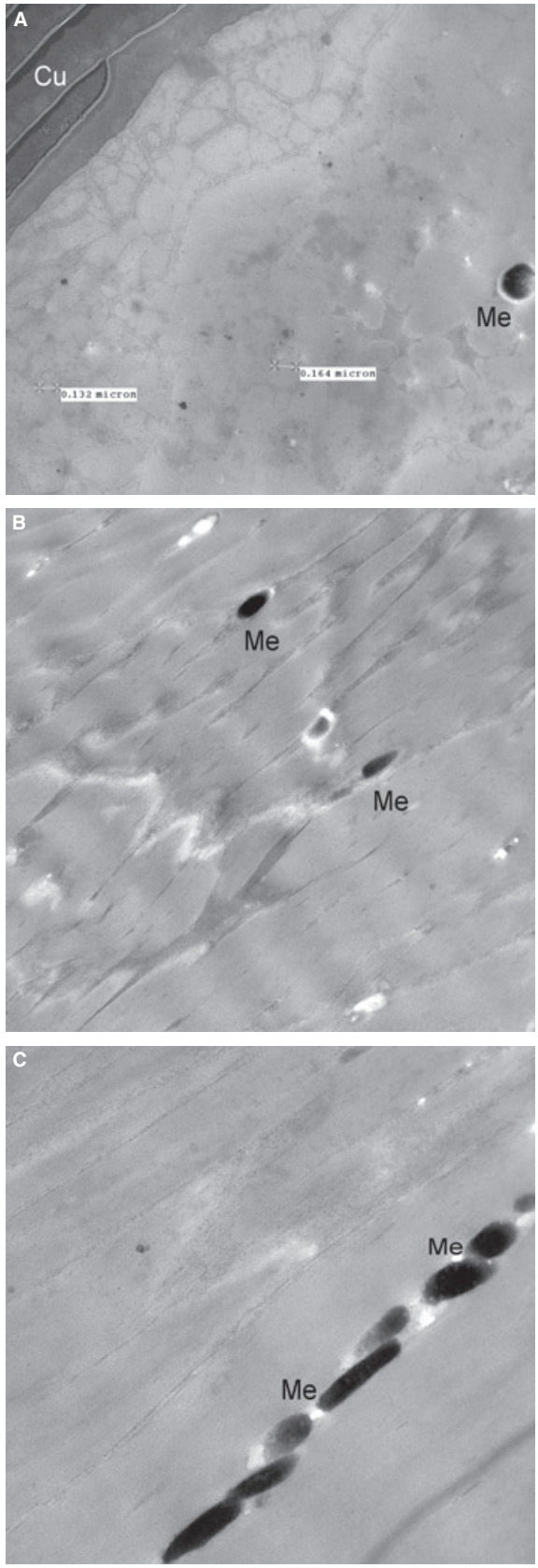

FIG. 4—*Images from area C, Fig. 1. (A) Cross-section of hair cuticle/cortex junction. TEM (original magnification 8000×). (B) Longitudinal section of hair cortex. TEM (original magnification 6000×). (C) Longitudinal section of hair cortex. TEM (original magnification 10,000×). Cu, cuticle; Me, melanosome.*

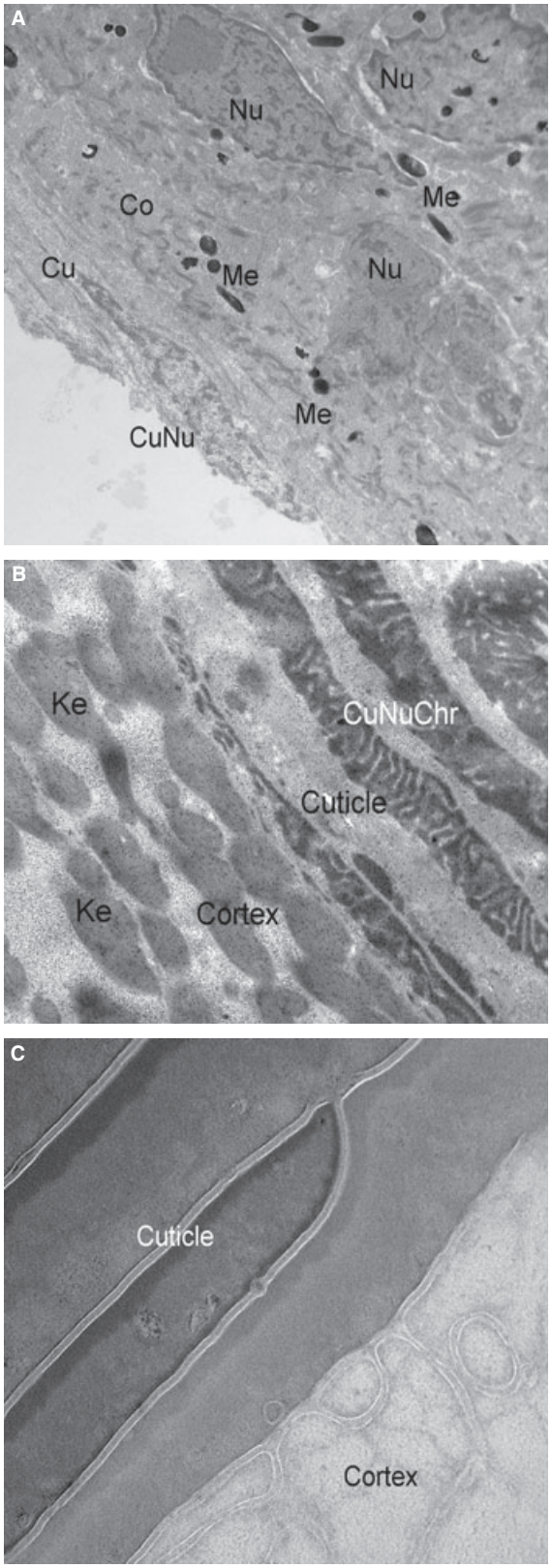

FIG. 5—*Metamorphosis of the hair cuticle during terminal differentiation. (A) Longitudinal section from area A, Fig. 1. TEM (original magnification 3000×). (B) Longitudinal section from area B, Fig. 1. TEM (original magnification 7000×). (C) Cross-section from area C, Fig. 1. TEM (original magnification 25,000×). Cu, cuticle; CuNu, cuticle cell nucleus; Co, cortex; Nu, nucleus; Me, melanosome; Ke, keratin bundles; CuNuChr, cuticle nuclear chromatin "zebra pattern."*

loss of nuclei and other organelles are connected events in terminal differentiation of skin and hair cells (11). Morioka suggested that this reorganization and scavenging of the cytoplasm are necessary to provide additional amino acids and nutritional components for the keratin fiber synthesis (10).

Conclusion

Nuclei and mitochondria were not observed in areas of the hair shaft where a cuticle was fully formed. This location in the hair root is observable with transmitted light microscopy and Permount® slide preparations. It may in fact be more proper to state that nuDNA loses its nucleus rather than nuclei lose their DNA in keratinizing tissues. MtDNA in hair shafts is easily recovered several centimeters distal to the location in the keratinized hair shaft where mitochondria are no longer visible (6). Thus it appears that the mitochondrion provides no additional protection for mtDNA in the keratinized hair shaft. Keratin in the skin and hair of mammals provides a first line of defense against the effects of weathering that includes ultraviolet radiation exposure. MtDNA molecules appear to be protected by the embedment within the keratin macrofibrils of a hair shaft.

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