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Original article

RNA Synthesis in the Tracheal Epithelial Cells of Aging Mice as Revealed by Electron Microscopic Radioautography

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ABSTRACT

For the purpose of studying the aging changes of macromolecular synthesis in the tracheal epithelial cells of experimental animals, we studied 10 groups of aging mice during development and aging from fetal day 19 to postnatal month 24. They were injected with 3H-uridine, a precursor for RNA synthesis, sacrificed and the tracheal tissues were taken out, fixed and processed for light and electron microscopic radioautography. On many radioautograms the localization of silver grains demonstrating RNA synthesis in tracheal epithelial cells in respective aging groups were analyzed qualitatively and quantitatively. The number of mitochondria per cell, the number of labeled mitochondria with silver grains and the number of silver grains in each cell in respective aging groups were analyzed quantitatively in relation to the aging of animals. The results revealed that the RNA synthetic activity as expressed by the incorporations of RNA precursor, i.e., the number of silver grains in cell nuclei, cell organelles, changed due to the aging of animals. The number of mitochondria, the number of labeled mitochondria and the mitochondrial labeling index labeled with silver grains were counted in each tracheal epithelial cell. It was demonstrated that the number of mitochondria increased from embryonic day 19 to postnatal newborn day 1, 3, 9, 14, adult month 1, 2, reaching the maximum and kept plateau, while the number of labeled mitochondria increased from embryonic day 19 to postnatal adult month 2, reaching the maximum but the labeling indices showing RNA synthesis increased from embryonic day 19 to postnatal newborn day 14 and adult month 1 and decreased to year 2, indicating the aging changes. Based upon our findings, available literature on macromolecular synthesis in mitochondria of various cells are reviewed.

1. Introduction

The trachea of a mouse is a thin tube lined with the ciliated pseudostratifiedcolumnar epithelium covered with connective tissues, smooth muscle cells and hyaline cartilages. We studied the macromolecular synthesis of the aging mouse trachea at various ages by means ofelectron microscopic radioautography. We first studied the DNA synthesis of mouse tracheal and pulmonary cells in aging by LM and EM RAG using 3H-thymidine (Sun et al.1997a,b) [1, 2]. The tracheal cells of 8 groups of aging mice from prenatal day 18 to postnatal year 2 were observed. The radioautographs revealed that the DNA synthesis incorporating 3H-thymidine, a DNA precursor, appearing as silver grains were observed in the nuclei of both ciliated and noncilated cells. The labeling indices of respective cell types were analyzed. As the results, the indices of the epithelial cells showed their maxima on fetal day 18, then fell down from postnatal day 3 to year 2. The ciliated cells could not synthesize DNA and proliferate in the postnatal stages. They are supposed to be derived from the cell division and proliferation of the basal cells in the epithelia. On the other hand, the incorporation of sulfuric acid, 35SO4, was also studied in the tracheal epithelial cellsof 9 groups of aging mice from prenatal day 19 to postnatal

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year 1(Nagata 2000)[3]. As the results, silver grains indicating the incorporation of radiosulfate was mainly found over the cartilage matrix in the hyaline cartilage of the tracheae of the prenatal and postnatal newborn mice. The grain density as analyzed by grain densitometry was the maximum at the fetal day 19, then decreased to postnatal day 1, 3, 9, 14 and month 1 when it reached 0 to month 2, 6 and 12 at senescence. The results indicated that the glycoprotein constituting the cartilage matrix was synthesized from perinatal stage to month 1 (Nagata 2000)[3].

Formerly, intramitochondrial nucleic acid syntheses, both DNA and RNA, in mammalian and avian cells were first demonstrated morphologically by the present author and associates by means of electron microscopic radioautography in primary cultured cells of the livers and kidneys of mice and chickens in vitro (Nagata et al. 1967a,b)[4,5] and then in some other established cell lines such as HeLa cells (Nagata 1972a,b)[6,7] or mitochondrial fractions prepared from in vivo cells (Nagata and Usuda 1985, Nagata et al. 1986a,b,c)[8, 9, 10,11]. It was later commonly found in various cells and tissues not only in vitro obtained from various organs in vivo (Nagata 1972a,b, 1974, 1992, 1993a,b, 1996, 1997, 1999, 2001, 2002, 2003, 2007a,b, 2008a,b, 2009a,b,c,d,e, 2010a, 2011a, Nagata et al. 1967, 1976, Nass 1966, Nass and Nass 1963, Gahan and Chayen1965, van Bruggen 1966, Schatz1970, Guttes and Guttes1964, Schuster1965, Stone and Miller1965, Chévremont1963, Salpeter et al. 1969, Nadler1971, Uchida and Mizuhira 1971)[12-51], but also in vivo cells of various organs such as the salivary glands(Nagata et al. 2000)[52], the liver(Nagata et al. 1979, 1982a,b, Ma and Nagata 1988a,b, Ma et al. 1994, Nagata 2003, 2006, 2007a,b,c,d,e, Nagata and Ma 2005)[53-64], the pancreas(Nagata et al. 1986)[65], the trachea (Sun et al. 1997)[66], the lung (Nagata and Sun 2007)[67], the kidneys (Hanai and Nagata 1994)[68], the testis (Gao et al. 1994, 1995), the uterus (Yamada and Nagata 1992, 1993)[71,72], the adrenals (Ito 1996, Ito and Nagata 1996, Liang et al. 1999, Nagata 2008a,b)[73-75], the brains (Cui et al 1996)[76], and the retina (Gunarso 1984, Gunarso et al. 1996,1997, Kong and Nagata 1994, Nagata 2006)[77-81] of mice, rats and chickens. The relationship between the intramitochondrial DNA synthesis and cell cycle was formerly studied and it was clarified that the intramitochondrial DNA synthesis was performed without any nuclear involvement (Nagata 1972a,b)[8,10]. However, the relationship between the aging of individual animals and the DNA synthesis in respective cell types in these organs has not yet been clarified. Recently, the relationship of both the DNA and RNA synthesis to the aging of animals was first demonstrated in the hepatocytes of mice (Ma and Nagata 1988a,b, Ma et al. 1994, Nagata 2003, 2006, 2007a,b,c, Nagata and Ma 2005, Nagata et al. 1979, 1982a,b, 1986, 1988a,b, 1994)[56-72]. Later, the relationship of the DNA synthesis to the aging of animals in the adreno-cortical cells (Ito 1996, Ito and Nagata 1996, Nagata 2008)[73,74,75], then of the RNA synthesis (Liang et al. 1999, Nagata 2010b)[76,93] were also clarified. However, the relationship of the RNA synthesis to the aging of animals in the tracheal epithelial cells has not yet been clarified. This paper deals with the relationship between the RNA synthesis and the aging in the tracheal ciliated columnar cellsof mice in vivo at various developmental stages from fetal day 19 to postnatal month 2 and further to adult and senescent stages up to month 24 (year 2) during aging by means of electron microscopic radioautography as a part of serial studies on special cytochemistry (Nagata 2001)[17] and radioautographology (Nagata 2002)[12].

2. Materials and Methods

2.1. The experimental animals

The tracheal tissues were obtained from 10 groups of aging normal ddY strain mice, each consisting of 3 litter mates of both sexes, total 30, from prenatal embryo day 19 to newborn postnatal day 1, 3, 7, 14, adult at month 1, 2, 6, 12 (year 1) to month 24 (year 2). All the animals were housed under conventional conditions and bred with normal diet (mouse chow Clea EC2, Clea Co., Tokyo, Japan) with access to water ad libitum in our laboratory. They were administered with 3H-uridine, an RNA precursor, and the tracheal tissues were taken out, fixed and processed for electron microscopic radioautography. All the procedures used in this study concerning the animal experiments were in accordance with the guidelines of the animal research committee of Shinshu University School of Medicine as well as the principles of laboratory animal care in NIH publication No. 86-23 (revised 1985).

2.2. Procedures of electron microscopic radioautography

All the animals were injected intraperitoneally with 3H-uridine (Amersham, England, specific activity 877 GBq/mM) in saline, at 9 a.m., one hour before sacrifices. The dosage of injections was 370 KBq/gm body weight. The animals were perfused at 10 a.m., one hour after the injection, via the left ventricles of the hearts with 0.1 M cacodylate-buffered 2.5% glutaraldehyde under Nembutal (Abbott Laboratories, Chicago, ILL, USA) anesthesia. The central portion of the trachea was taken out from each animal, excised and 3 small pieces of the tracheal tissues (size 1mm x 1mm x 1mm) were immersed in the same fixative at 4˚C for 1 hr., followed by postfixation in 1% osmium tetroxide in the same buffer at 4˚C for 1 hr., dehydrated in graded series of ethanol and acetone, and embedded in epoxy resin Epok 812 (Oken, Tokyo, Japan).

For electron microscopic radioautography, semithin sections at 0.2μm thickness, thicker than conventional ultrathin sections in order to shorten the exposure time, were cut in sequence on a Porter-Blum MT-2B ultramicrotome (Dupont-Sorvall, Newtown, MA, USA) using glass knives. The sections were collected on collodion coated copper grid meshes (VECO, Eerbeek, Netherlands), coated with Konica NR-H2 radioautographic emulsion (Konica, Tokyo, Japan) by a wire-loop method (Nagata 1992, 1993a,b)[5, 6, 7]. They were stored in dark boxes containing silica gel (desiccant) at 4˚C for exposure. After the exposure for 10 months, the specimens were processed for development in freshly prepared gold latensification solution for 30 sec at 16˚C and then in fresh phenidon developer for 1 min at 16˚C in a water bath, rinsed in distilled water and dried in an oven at 37˚C overnight, stained with lead citrate solution for 3 min, coated with carbon for electron

microscopy. The electron microscopic radioautograms(EMRAG)were examined in a JEOL JEM-4000EX electron microscope (JEOL, Tokyo, Japan) at accelerating voltages of 400 kV for observing thick specimens.

2.3. Quantitative analysis of electron micrographs

For quantitative analysis of electron micrographs, twenty EM radioautograms showing cross sections of ciliated tracheal epithelial cells from each group, based on the electron microscopic photographs taken after observation on at least 100 epithelial cells from respective animals were analyzed to calculate the total number of mitochondria in each cell, and the number of labeled mitochondria covered with silver grains by visual grain counting.

On the oter hand, the number of silver grains in the same area size as a mitochondrion outside cells was also calculated in respective specimens as background fog, which resulted in less than 1 silver grain (0.02/mitochondrial area) almost zero. Therefore, the grain count in each specimen was not corrected with background fog. From all the data thus obtained the averages and standard deviations in respective aging groups were computed with a personal computer (Macintosh type 8100/100, Apple Computer, Tokyo, Japan). The data were stochastically analyzed using variance and Student's t-test. The differences were considered to be significant at P value <0.01.

3.Results

3.1. Morphological observations

The tracheal epithelial cells obtained from ddY strain mice at various ages from embryo day 19 to postnatal month 24, consisted of pseudostratified epithelial cells, mainly ciliated cells and some nonciliated cells and basal cells, containing some goblet cells appearing dissesminatedly between the columnar cells (Figs. 1-6). Because the numbers of cells as well as the numbers of mitochondria in therespective ciliated columnar epithelial cells were relatively much more than the other cells, only the ciliated epithelial cells were analyzed in this study.

3.2. Radioautographic Observations

Observing electron microscopic radioautograms, the silver grains were found over the nuclei as well as over the cytoplasm including mitochondria of almost all the ciliated epithelial cells, labeled with 3H-uridine, demonstrating RNA synthesis at respective aging stages from perinatal stages at embryonic day 19 (Fig. 1), to postnatal day 1(Fig. 2) and day 3 and 7 (Fig. 3), and day 14, to adult stage at month 1 (Fig. 4), month 2 and 6, and to senescent stage at month 12 (Fig. 5) and 24 (Fig. 6).

The localizations of silver grains over the mitochondria were mainly on the mitochondrial matrices similarly to other cells such as in the livers (Nagata 2010)[20] or the adrenal glands (Nagata 2011a)[21].

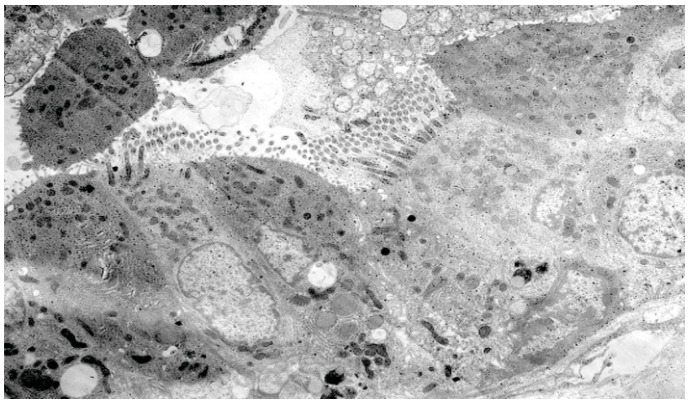


Fig: 1. EM RAG of ciliated tracheal epithelial cells of a prenatal embryo day 19 mouse labeled with 3H-uridine, showing few silver grains in the nucleus and cytoplasm including mitochondria. x3,000.

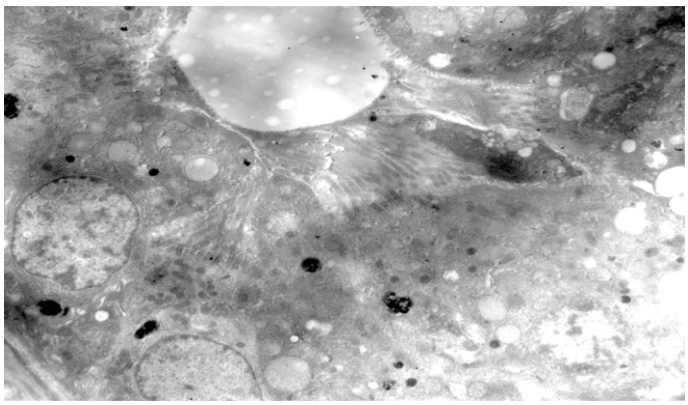


Fig: 2. EM RAG of ciliated tracheal epithelial cells of a postnatal day 3mouse labeled with 3H-uridine, showing a few silver grains in the nucleus and cytoplasm including mitochondria. x3,000.

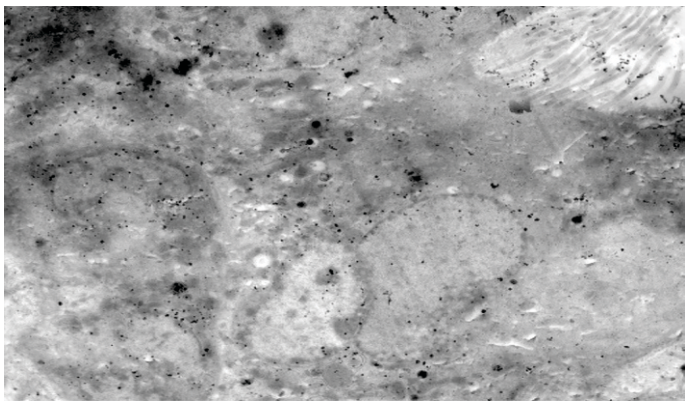


Fig: 3. EM RAG of ciliated tracheal epithelial cells of a postnatal day 14 mouse labeled with 3H-uridine, showing several silver grains in the nucleus and cytoplasm including mitochondria.x3,000.

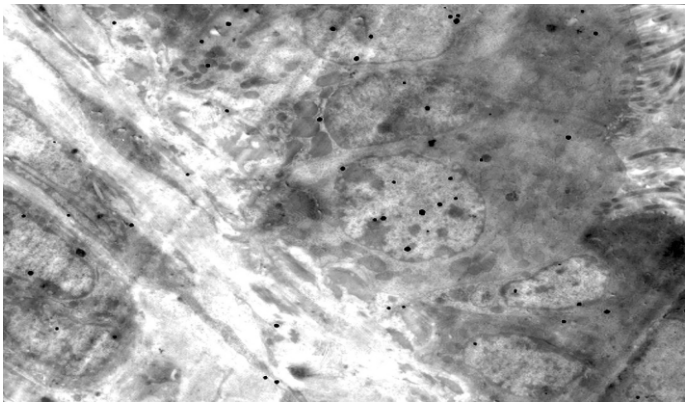


Fig. 4. EM RAG of ciliated tracheal epithelial cells of a postnatal month 1 mouse labeled with 3H-uridine, showing several silver grains in the nucleolus and cytoplasm including mitochondria.x3,000.

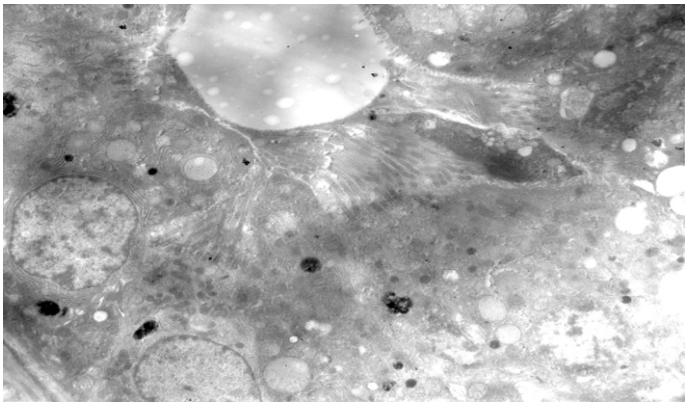


Fig. 5. EM RAG of ciliated tracheal epithelial cells of a postnatal year 1 mouse labeled with 3H-uridine, showing a few silver grains in the nucleus and cytoplasm including mitochondria. x3,000.

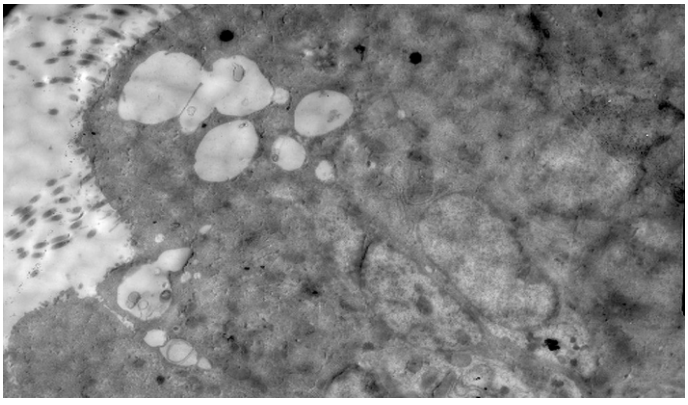


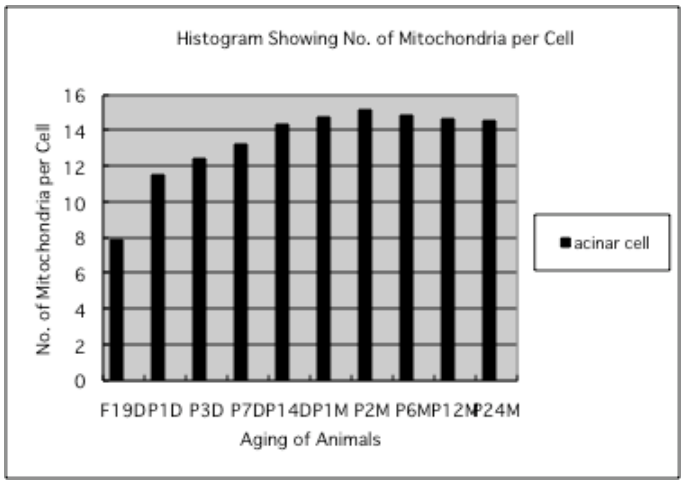
Fig. 6. EM RAG of ciliated tracheal epithelial cells of a postnatal year 2 mouse labeled with 3H-uridine, showing a few silver grains in the nucleus and cytoplasm including mitochondria. x3,000.

3.3. Quantitative Analysis

3.3.1. Number of mitochondria per cell

Preliminary quantitative analysis on the number of mitochondria in 10 ciliated columnar epithelial cells whose nuclei were labeled with silver grains and other 10 cells whose nuclei were not labeled in each aging group revealed that there was no significant difference between the number of mitochondria and the labeling indices ($P<0.01$). Thus, the number of mitochondria and the labeling indices were calculated regardless whether their nuclei were labeled or not. The results obtained from the number of mitochondria in ciliated columnar epithelial cells of respective animals in 10 aging groups at perinatal and newborn stages, from prenatal embryo day 19 to postnatal day 1, 3, 7, 14, and adult and senescent stages at month 1, 2, 6, 12, and 24, seemed to show an gradual increase from the prenatal day 19to postnatal month 24. Counting the number of mitochondria per cell at respective aging stages, it increased from prenatal embryo around 4.7/cell in average, to 5.4 at postnatal day 1, to 8.2 at day 3, to 9.6 at day 7, to 10.5 at day 14, to 11.8 at month 1, to 13.1 at month 2, then 13.3 at month 6, 13.2 at month 12 and finally 12.9 at month 24 as shown in Fig. 7. All the data from embryonic day 19 to postnatal month 24, were stochastically analyzed using variance and Student's t-test. The increases of mitochondrial numbers in the tracheal epithelial cells from embryonic day 19 to adult stage at postnatal month 2 were considered to be significant at P value <0.01. However, the slight changes at the senescent stage from month 6 to 24 were considered to be not significant at P value <0.01.

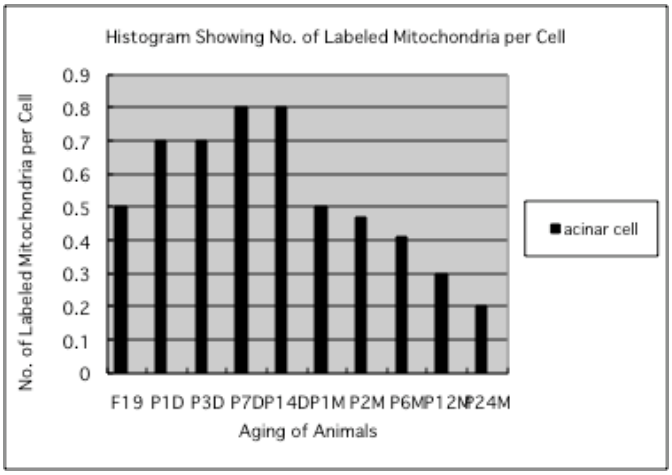
3.2. Mitochondrial RNA synthesis



The results of visual counting on the number of mitochondria labeled with silver grains obtained from 10 tracheal ciliated epithelial cells of each animal labeled with 3H-uridine demonstrating RNA synthesis in 10 aging groups at perinatal stages, from prenatal embryo day 19 to postnatal day 1, 3, 7 and 14, to adult stages at month 1, 3, and 6, 12 and 24, increased gradually from prenatal day 19 (1.8/cell) to day 1 (2.1/cell), to day 3 (3.4/cell) to day 7 (4.3/cell) and day 14 (4.9/cell), to month 1 (5.5/cell), to month 2 (5.7/cell), reaching the maximum, then

decreased gradually to month 6 (5.5/cell), to month 12 (5.3/cell) and month 24 (5.1/cell) as shown in Fig. 8. The data were stochastically analyzed using variance and Student's t-test. The increases of the numbers of labeled mitochondria from embryo day 19 to postnatal month 2,were stochastically significant (P <0.01). However, the decreases from month 2 to month 24 were not significant.

3.3. The labeling index



Finally, the labeling indices of mitochondrial RNA synthesis in pancreatic acinar cells at respective aging stages were calculated from the number of labeled mitochondria (Fig. 8) dividing by the number of total mitochondria per cell (Fig. 7) which were plotted in Fig. 9.

The results showed that the labeling indices gradually increased from prenatal day 19 (38.3%) and postnatal newborn day 1 (38.9%), to postnatal day 3 (41.5%), to day 7 (44.8%), to day 14 (46.7 %) and adult stages at month 1 (46.6%), reaching the maximum, and then decreased to month 2 (43.5 %), month 6 (41.4%), month 12 (40.2%) and 24 (39.5%) as shown in Fig. 9. From the results, the increases of the mitochondrial labeling indices in tracheal ciliated columnar cells from embryo day 19 and newborn postnatal day 1 to postnatal day 7, 14 and month 1, as well as the decreases from month 2 to 6, 12 and 24 were stochastically significant (P <0.01).

Fig. 7. Histogram showing the number of mitochondria per cell in respective aging groups.

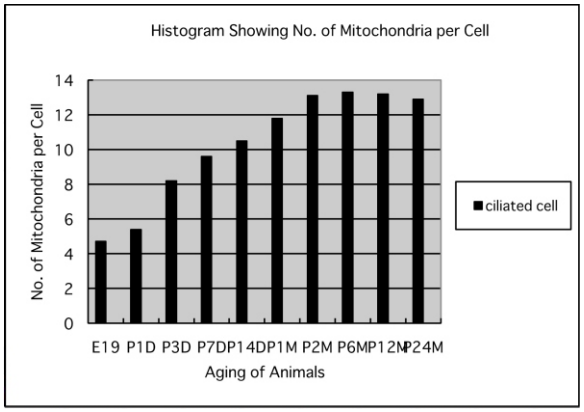


Fig. 8. Histogram showing the number of labeled mitochondria per cell in respective aging groups labeled with 3H-uridine.

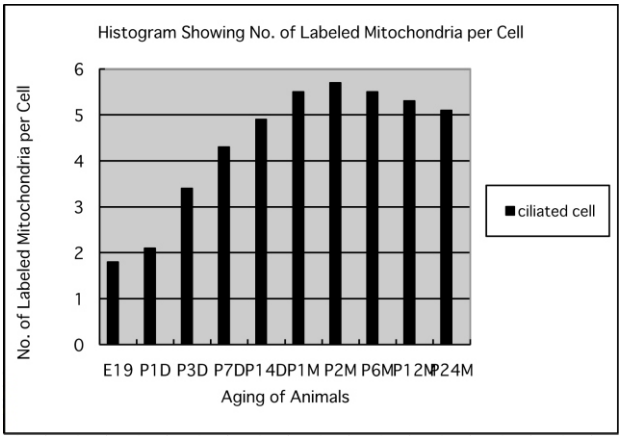
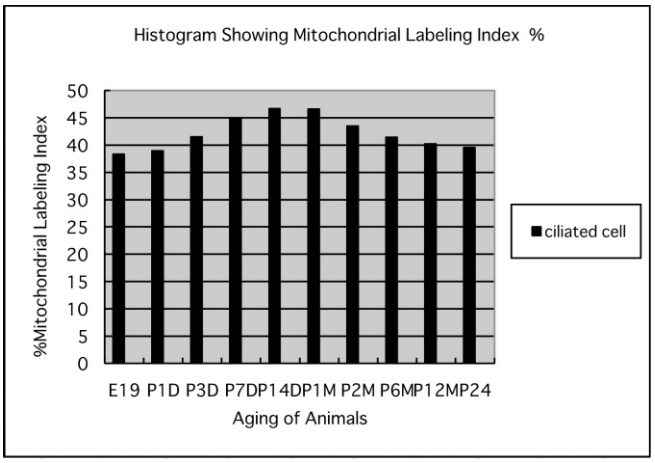


Fig. 9. Histogram showing the average labeling index in aging groups labeled with 3H-uridine.



4. Discussion

From the results obtained in the present study on the tracheal ciliated columnar epithelailcells of ddY aging mice at various ages in 10 groups from perinatal stages at embryo day 19, to newborn day 1, 3, 7, 14, and young adult at postnatal month 1, 2, 6 as well as the senescent adult at postnatal month 12 and 24, it was shown that intramitochondrial RNA synthesis was observed in these cells of all the aging stages from prenatal embryos to postnatal newborn, young juvenile and adult stages and the number of mitochondria per cell showed increases due to aging, while the number of labeled mitochondria per cell and the labeling indices showed increases and decreases due to aging. These results demonstrated that intramitochondrial RNA synthesis in the tracheal ciliated columnar epithelial cells revealed variations due to aging of individual animals depending upon the cellular activities at respective aging stages.

With regards the macromolecular synthesis in various cells in various organs of experimental animals observed by light and electron microscopic radioautography, it is well known that the silver grains due to radiolabeled3H-thymidine demonstrate DNA synthesis (Nagata et al. 1967, Nagata 1999, 2001, 2002, 2010) [1, 4, 6, 10, 12-18]. The previous results obtained from the studies on the hepatocytes of aging mice by light and electron microscopic radioautography revealed that silver grains indicating DNA synthesis incorporating 3H-thymidine were observed over the nuclei of some hepatocytes at perinatal stages from postnatal day 1 to day 14 and decreased due to aging (Nagata et al. 1967, Nagata 1999, 2001, 2002, 2003, 2010)[15-18]. Then, we lately observed the intramitochondrial DNA synthesis in the various organs such as the livers (Nagata 1999, 2001. 2002, 2007a,b, 2009a,b)[12, 13, 19-22] adreno-cortical (Nagata 2008a,b, 2009a, 2011, [14, 23-26] and adreno-medullary cells (Nagata 2009b,c, 2011)[14, 27, 28], at various ages from fetal day 19 to postnatal newborn day 1, 3, 7, juvenile day 14 and to adult month 1, 2, 12 and 24. In the present study, further data obtained from the tracheal ciliated epithelial cells from prenatal to adult senescent animals at postnatal month 12 and 24 were added.

From these studies, the numbers of silver grains showing nuclear RNA synthesis resulting from the incorporations of 3H-uridine into mitochondria indicating mitochondrial RNA synthesis demonstrated the silver grain localization over the mitochondria independently from the nuclei whether the nuclei were labeled with silver grains or not in the tracheal ciliated epithelial cells from prenatal embryo day 19 to postnatal month 24 during the development and aging. The numbers of labeled mitochondria showing RNA synthesis as well as the labeling indices increased from perinatal embryonic day to postnatal newborn and juvenile stages at day 14, to adult postnatal month 1, reaching the maxima, and then slightly decreased to the senescent stages at month 2, 6,12 and 24.

With regards to DNA in mitochondria in animal cells or plastids in plant cells, many studies have been reported in various cells of various plants and animals since 1960s (Nass and Nass 1963, Gibor and Granick1964, Gahan and Chayen 1965, Nass1966, van Bruggen et al. 1966, Sinclair and Stevens1966) [29-34]. Most of these authors observed DNA fibrils in mitochondria which were histochemically extracted by DN'ase. Electron microscopic observation of the DNA molecules isolated from the mitochondria revealed that they were circular in shape, with a circumference of 5-6 μm (Schatz 1970)[35]. It was calculated that such a single molecule had a molecular weight of about 107daltons(Guttes and Guttes1964)[36]. Mitochondria of various cells also contained a DNA polymerase, which was supposed to function in the replication of the mitochondrial DNA (Schuster1965)[37]. On the other hand, the incorporations of 3H-thymidine into mitochondria demonstrating DNA synthesis were observed by means of electron microscopic radioautography in lower organism such as slime mold (Chévremont1963, Stone andMiller 1965)[38, 39], tetrahymena(Salpeter et al. 1969)[40] or chicken fibroblasts in tissue culture under abnormal conditions (Nadler1971)[41]. However, these authors used old-fashioned developers consisting of methol and hydroquinone (MQ-developer) which produced coarse spiral silver grains resulting in inaccurate localization over cell organelles when observed by electron microscopy. All of these authors showed photographs of electron radioautographs with large spiral-formed silver grains (2-3 μm in diameter) localizing not only over the mitochondria but also outside the mitochondria. In order to obtain smaller silver grains, we first used elon-ascorbic acid developer after gold latensification(Nagata et al. 1967, Nagata 1993b)[7, 15], which produced comma-shaped smaller silver grains (0.4-0.8 μm in diameter), then later we used phenidon developer after gold latensification, producing dot-like smaller silver grains (0.2-0.4 μm in diameter) localizing only inside the mitochondria showing ultrahigh resolution of radioautograms(Nagata and Usuda1985, Nagata 1996, 1997, 2002, 2010)[1, 12, 13, 42, 43]. These papers were the first which demonstrated intramitochondrial DNA synthesis incorporating 3H-thymidine with accurate intramitochondrial localization in avian and mammalian cells. With regards the resolution of electron microscopic radioautography, on the other hand, many authors discussed the sizes of silver grains under various conditions and calculated various values of resolutions (Uchida and Mizuhira1971, Nagata 1984, Nagata 1972a,b, Murata et al. 1979)[8, 10, 44-46]. Those authors who used the M-Q developers maintained the resolution to be 100-160 nm (Uchida and Mizuhira1971, Murata et al. 1979)[44, 45], while those authors who used the elon-ascorbic acid developer (Nagata 1984, Nagata 1972a,b)[8, 10, 46] calculated it to be 25-50 nm. When we used phenidon developer at 16˚C for 1 min after gold latensification, we could produce very fine dot-shaped silver grains and obtained the resolution around 25 nm (Nagata 1984, 1996, 1997, 2002, 2010, Nagata and Usuda1985)[1, 12, 13, 42, 43, 46]. For the analysis of electron radioautographs, Salpeter et al. (1969)[40] proposed to use the half-distance and very complicated calculations through which respective coarse spiral-shaped silver grains were judged to be attributable to the radioactive source in a certain territory within a resolution boundary circle. However, since we used phenidon developer after gold latensification to produce very fine dot-shaped silver grains, we judged only the silver grains which were located in the mitochondria which were dot-shaped very fine ones to be attributable to the mitochondria without any problem as was formerly discussed (Nagata1972a,b, 1996, 1997, 2002, 2010)[8, 10, 12, 13, 42, 43].Then we also demonstrated intramitochondrial DNA synthesis incorporating 3H-thymidine in some other established cell lines originated from human being such as HeLa cells (Nagata1972a,b)[8, 10] or mitochondrial fractions prepared from in vivo mammalian cells such as rat and mouse (Nagata 1974, Nagata et al. 1976)[9, 11]. It was later commonly found in various cells and tissues not only in vitro obtained from various organs in vivo such as the cultured human HeLa cells (Nagata et al. 1977b)[47], cultured rat sarcoma cells (Nagata 1977c)[48], mouse liver and pancreas cells in vitro (Nagata et al. 1969, 1977c,d)[48, 50, 51], but also in vivo cells obtained from various organs such as the salivary glands (Nagata et al. 2000)[52], the liver (Nagata et al. 1979, 1982a,b, Ma and Nagata 1988a,b, Ma et al. 1994, Nagata 2003, 2006, 2007a,b,c, Nagata and Ma 2005)[53-64], the pancreas (Nagata et al. 1986)[65], the trachea (Sun et al. 1977)[66], the lung (Nagata and Sun2007)[67], the kidneys (Hanai and Nagata1994)[68], the testis (Gao et al. 1994, 1995)[69,70], the uterus (Yamada and Nagata 1992, 1993)[71,72], the adrenal glands (Ito 1996, Ito and Nagata 1996, Nagata 2008)[73-75], the brains (Cui et al. 1996)[76], and the retina (Gunarso 1984, Gunarso et al.

1996, 1997, Kong and Nagata 1994, Nagata 2006)[77-81] of mice, rats and chickens. Thus, it is clear that all the cells in various organs of various animals synthesize DNA not only in their nuclei but also in their mitochondria.

The relationship between the intramitochondrial DNA synthesis and cell cycle was formerly studied in synchronized cells and it was clarified that the intramitochondrial DNA synthesis was performed without nuclear involvement (Nagata1972a)[8]. However, the relationship between the DNA synthesis and the aging of individual animals and men has not yet been fully clarified except a few papers published by Korr and associates on mouse brain (Korr et al. 1997, 1997, Schmitz 1999a,b)[82-85]. They reported both nuclear DNA repair, measured as nuclear unscheduled DNA synthesis, and cytoplasmic DNA synthesis labeled with 3H-thymidine in several types of cells in brains such as pyramidal cells, Purkinje cells, granular cells, glial cells, endothelial cells, ependymal cells, epithelial cells as observed by light microscopic radioautography using paraffin sections. They observed silver grains over cytoplasm of these cells by light microscopy and maintained that it was reasonable to interpret these labeling as 3H-DNA outside the nuclei, which theoretically belonged to mitochondrial DNA without observing the mitochondria by electron microscopy. From the results, they concluded that distinct types of neuronal cells showed a decline of both unscheduled DNA and mitochondrial DNA syntheses with age in contrast that other cell types, glial and endothelial cells, did not show such age-related changes without counting the number of mitochondria in respective cells nor counting the labeling indices at respective aging stages. Thus, their results from the statistics obtained from the cytoplasmic grain counting seems to be not accurate without observing mitochondria directly. To the contrary, we had studied DNA synthesis in the livers of aging mice (Nagata et al. 1979, 1982a,b, Ma and Nagata 1988a,b, Ma et al. 1994, Nagata 2003, 2006, 2007a,b,c, Nagata and Ma 2005)[53-64] and clearly demonstrated that the number of mitochondria in each hepatocytes, especially mononucleatehepatocytes, increased with the ages of animals from the perinatal stages to adult and senescent stages, while the number of labeled mitochondria and the labeling indices increased from the perinatal stages, reaching a maximum at postnatal day 14, then decreased.

Our previous studies (Nagata 2003, Nagata and Ma 2005)[59, 60] also clarified that the DNA synthesis and cell proliferation by mitosis were the most active in the nuclei of mononucleatehepatocytes at the perinatal stages in contrast that binucleate cells were less active at the perinatal stage but the number of binucleatehepatocytes increased at senescent stages and the results suggest the possibility that the mitochondria in mononucleatehepatocytes synthesized their DNA by themselves which peaked at postnatal day 14 in accordance with the proliferation of mononucleatehepatocytes while binucleatehepatocytes increased after the perinatal stage and did not divide but remained binucleate keeping many mitochondria in their cytoplasm which were more in number than mononucleatehepatocytes at the senescent stage.

Thus, our previous papers were the first which dealt with the relationship between the DNA synthesis and aging in hepatocytes of mice in vivo at various ages by means of electron microscopic radioautography observing the small dot-like silver grains, due to incorporations of 3H-thymidine, which exactly localized inside the mitochondria.

Later we also studied intramitochondrial DNA synthesis in adreno-cortical cells from prenatal day 19 to postnatal day 1, 3, 9, 14, month 1, 2, 6, 12 and 24 (year 2) and found that the numbers of mitochondria in 3 zones, glomerulosa, fasciculate and reticularis, increased reaching the maxima at postnatal month 2 and which kept continued until senescence up to 24 months (2 years). To the contrary, the numbers of labeled mitochondria and the labeling indices increased to postnatal month 2, reaching the maxima, then decreased to month 24 (Nagata 2008a,b, 2009a, b,c, 2010)[23-28].

Later we also demonstrated the results from the RNA synthesis in the livers and adrenal glands of aging mice which also revealed that an increase was observed by direct observation on mitochondria at electron microscopic level and obtaining accurate mitochondrial number and labeling indices in the hepatocytes and adreno-cortical and adreno-medullary cells. In the present study, we also demonstrated the RNA synthesis in the pancreatic acinar cells in 10 groups of developing and aging mice. There was a discrepancy between our results from the hepatocytes(Nagata 2003, Nagata and Ma 2005)[59, 60] and the adrenal cells (Nagata 2008a,b, 2009a,b,c, 2010)[23-28] as well as the pancreatic acinar cells (Nagata 2011a,b)[91,92] and the results from the several types of cells in the brains by Korr et al. (1997, 1998) and (Schmitz et al. 1999a,b)[82-85]. The reason for this difference might be due to the difference between the cell types (hepatocytes, adrenal cells, pancreatic cells from our results and the brain cells from their results) or the difference between the observation by light or electron microscopy, i.e., direct observation of mitochondria by electron microscopy in our results or light microscopy, i. e., indirect observation of mitochondria without observing any mitochondria directly by Korr et al. (1997, 1998) and (Schmitz et al. 1999a,b)[82-85].

Anyway, the results obtained from the pancrearticacinar cells of aging mice at present should form a part of special cytochemistry(Nagata 2001)[17] in cell biology, as well as a part of special radioautographology(Nagata 2002)[12], i.e., the application of radioautography to the pancreas, as was recently reviewed by the present author including recent results dealing with various organs (Nagata 2009f,g,h,I, 2010b, 2011b,c)[86-92]. We expect that such special radioautographology and special cytochemistry should be further developed in all the organs in the future.

5. Conclusion

From the results obtained at present, it was concluded that almost all the ciliated tracheal epithelial cells in the tracheae of mice at various ages, from prenatal embryo day 19 to postnatal newborn, day 1, 3, 7 and 14, and to postnatal month 1, 2, 6, 12 and 24, were labeled with silver grains showing RNA synthesis with 3H-uridine in their mitochondria. Quantitative analysis on the number of mitochondria in respective ciliated tracheal epithelial

cells resulted in increases from the prenatal day to postnatal day 1, 3, 9, 14, to month 1, 2 and 6, reaching the maximum at postnatal month 6, then slightly decreased to month 12 and 24 (year 1 and 2). To the contrary, the numbers of labeled mitochondria with 3H-uridine showing RNA synthesis increased from prenatal day 19 to postnatal day 14, to month 1 and 2, reaching the maximum at postnatal month 2, then decreased to month 6, 12 and 24, while the labeling indices increased from prenatal day 19 to postnatal day 14, reaching the maximum at postnatalday 14, then decreasedto month 1, 2, 6, 12 and 24. These results demonstrated that the number of mitochondria in the ciliated tracheal epithelial cells increased from perinatal stages to postnatal month 2 and 6, keeping the maximum up to month 24, while the activity of mitochondrial RNA synthesis increased to postnatal day 14, to postnatal month 1,reaching the maximum, then decreased to month 24 (year 2) due to aging of animals.

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