Introduction

The mitochondrial genome is an approximately 16 kbp circular DNA (Fig. 1A) encoding 13 electron transport chain subunits, 22 tRNAs, and 2 rRNAs. Each cell contains anywhere from several hundred to several thousand copies of the mitochondrial genome (Fig. 1B), a number which varies depending on the tissue concerned. Though the number of encoding genes is limited, aerobic ATP synthesis by mitochondria is essential for all genes. Because mitochondria account for more than 80% of ATP synthesis by the cells, abnormalities in the mitochondrial genome have a devastating impact on cell survival and function. Specifically, cases of congenital “mitochondrial encephalopathy” attributed to numerous mitochondrial genomic mutations have been reported, and reports of new mutations continue to emerge in ever greater numbers. Recently, an association with mitochondrial genomic abnormalities has been recognized not only in distinctive, congenital families of disorders, but also in more general diseases, and particularly those diseases whose onset and progression are associated with aging. These findings have increased interest in the mechanisms for maintenance of the mitochondrial genome.
Mitochondrial Genomic Disintegrity and Age-Related Diseases

Because mitochondrial DNA lacks histones, it was long believed that mitochondrial DNA did not assume a nucleosomal structure and existed in naked form. This notion was suggested as one major reason why mitochondrial DNA was more susceptible to damage than nuclear DNA. We discovered that transcription factor A (TFAM) is present in an approximately 1,000-fold greater quantity than mitochondrial DNA, by molecular count, and that nearly all TFAM exists in bound form with mitochondrial DNA. In other words, mitochondrial DNA has been shown to be covered by TFAM and is by no means naked.

TFAM belongs to the High Mobility Group (HMG) protein family, but many HMG protein groups function as structural proteins of nuclear DNA. In mitochondrial DNA as well, TFAM is thought to play a major role as a structural protein in nucleoid formation. Given that TFAM knockout mice undergo embryonic death, and TFAM overexpression increases the number of mitochondrial DNA copies, while restriction of its expression reduces the number of mitochondrial DNA copies, TFAM is regarded as essential for a stable presence of mitochondrial DNA.

Mitochondrial genome mutation and repair

The mitochondrial electron transport chain accounts for more than 90% of intracellular oxygen consumption, 1-5% of which is thought to be converted physiologically to reactive oxygen species. As the organelle thus representing the largest intracellular producer of physiologically reactive oxygen species, the mitochondrial genome is expected to sustain intense reactive oxygen species damage. In reality, many reports state that the oxidized form of guanine 8-oxoguanine (8-oxoG), a representative base modified by reactive oxygen species, is present in mitochondrial DNA at levels several 10-fold greater than in nuclear DNA. In addition, the mitochondrial disposition to accumulate fat-soluble cations is known to subject mitochondrial DNA to chemical modification several 10-fold more intense than that of nuclear DNA, for example when cells are exposed to alkylating agents or other such anticancer agents.

Considering this greater susceptibility to DNA damage than in the nucleus, it would be easy to imagine that the incidence of mutation is higher in the mitochondrial genome than the nuclear genome. This notion is supported indirectly by the fact that the evolutionary mutation rate of mitochondrial genes is more than 10-fold greater than that of nuclear genes, and the high incidence of mitochondrial respiration deletion strains of yeast. However, unlike the case of nuclear DNA, there is no method for direct measurement of the mutation rate of the mitochondrial genome, and over a lengthy period, it has not been determined whether the incidence of mutation in the mitochondrial genome of mammals is truly greater than that of the nuclear genome; however, the

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Fig. 1B. Mitochondrial DNA
Mitochondrial DNA of HeLa cells. A large number of mitochondrial DNA nucleoids stain granularly. Tubular structures appearing faintly in the background illustrate mitochondria.

Mitochondrial nucleoids

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Fig. 2. Mitochondrial DNA and TFAM
Bonding of mitochondrial DNA and TFAM provides mutual stabilization.
actual incidence of mutation is reportedly some 100-fold higher than that of the nuclear genome.\textsuperscript{8,9}

Since the 1974 report of Clayton et al.\textsuperscript{10} that pyrimidine dimers, a UV-induced DNA lesion, are not repaired in mitochondria, despite the importance of the mitochondrial genome and its susceptibility to damage, mitochondria were long believed to have no DNA repair mechanisms. DNA repair mechanisms are classified into four major categories: 1. nucleotide excision repair, 2. mismatch repair, 3. base excision repair, and 4. recombination. UV-induced DNA damage is repaired in the nucleus primarily by nucleotide excision, but just as the initial report of Clayton states that UV-damaged mitochondrial DNA is not repaired, mitochondria have no nucleotide excision repair mechanism. With respect to mismatch repair, the presence of a mismatch recognition-related protein MSH1 and its importance in maintaining the mitochondrial genome has been shown in yeast, but there is little basis for the notion that this system plays an important role, at least in mammals. Yeast clearly has a DNA recombination response, but among mammals, mitochondria are believed to have little DNA recombination response. Though some recent reports assert the existence of such a response, there are also contrary reports, and opinion remains divided as to its physiological role. Enzymes corresponding to a base excision repair system are present in mitochondria, and their activity has also been detected. At present, we can state that DNA repair in mammalian mitochondria relies primarily on base excision.

Because base excision repair plays the primary role in repair of DNA damaged by reactive oxygen species, some ascribe a significance to the continued existence of this repair system in mitochondria, the most potent source for production of reactive oxygen species. We therefore provide some additional discussion on enzymes contributing to repair of the oxidized form of guanine 8-oxoguanine (8-oxoG), a representative base modified by reactive oxygen species.

During DNA replication, 8-oxoG in the DNA chain causes a transversion mutation (mutation from a purine base to a pyrimidine base, or from a pyrimidine base to a pyrimidine base; by comparison, mutation from one pyrimidine base to another or from one pyrimidine base to another is termed a transition mutation) from C:G to A:T or from A:T to C:G due to its pairing with adenine at nearly the same frequency as with cytosine in what would be a normal pair. Three enzymes preventing such mutation by 8-oxoG, termed MutM, MutY, and MutT, were first identified in E. coli. MutM is an 8-oxoG DNA glycosylase, an enzyme which cuts 8-oxoG from a C:8-oxoG pair. MutY is an adenine DNA glycosylase which cuts A from an A:8-oxoG pair. MutT has 8-oxo-dGTPase activity, an enzyme which hydrolyzes 8-oxo-dGTP oxidized by reactive oxygen species. During DNA replication, 8-oxo-dGTP is incorporated in lieu of thymine as a partner for adenine, causing a transversion mutation in like fashion.

Human homologues of these three E. coli enzymes have also been identified. The first identified was an MutT homolog cloned in the Sekiguchi laboratory at Kyushu University and termed hMTH1 (human MutT homolog 1). Because the nucleotide pool for nucleic DNA replication is located in the cytoplasm, hMTH1 within the cell is also localized primarily in the cytoplasm. In mitochondria, however, the nucleotide pool for mitochondrial DNA replication is held exclusively in the mitochondrial matrix. Thus, hMTH1 has also been found to be present in the mitochondrial matrix at roughly the same level as in the cytoplasm.\textsuperscript{11} Subsequently, cDNA of hOGG1 and hMYH were cloned as homologues of MutM and MutY.

Both enzymes were found to be present in mitochondria, and not only in the nucleus\textsuperscript{12,13}. Thus, mitochondria have a protective system against 8-oxoG mutation equivalent to that of nuclear DNA. Because of its mutagenicity and quantitative prevalence as a representative oxidatively modified base, as well as of the fact that its corresponding repair enzyme families have been analyzed thoroughly, a very large number of researchers have also pursued intensive research of 8-oxoG in mitochondria. In particular, analysis of knockout mice for these three base excision repair enzymes has shown that oxidation of the nucleotide pool, mediated by insertion of oxidized bases into the mitochondrial genome, is greatly involved in cell death.\textsuperscript{14} However, the majority of congenital mitochondrial DNA mutations are transition mutations from A to G; most somatic cell mutations discovered by recently developed methods are also transition mutations, not transversion mutations definitively attributable to 8-oxoG. What has been surprising is the fact that the relative importance of 8-oxoG to mitochondrial DNA mutations actually observed in the mitochondrial genome has truly not yet been established.

**Parkinson’s disease and the mitochondrial genome**

Congenital abnormalities of the mitochondrial genome accumulate readily in fully differentiated somatic cells such as nerve and muscle cells. Because these cells are also highly reliant on mitochondria for their energy, such dysfunction is also readily apparent in nerves and muscles. Diseases resulting from congenital abnormalities of the mitochondrial genome are therefore termed encephalomyopathies. Because mitochondrial DNA is also readily susceptible to damage, it is conceivable that damage and mutation of the mitochondrial genome accumulate with aging, and in such a scenario, neurons would also be the largest candidate target.

Parkinson’s disease, one of the most frequent neurodegenerative diseases of advanced age, is caused by loss of neurons from the substantia nigra, the cause of which is strongly suspected to be mitochondrial dysfunction and oxidative stress. Decreased functionality of the mitochondrial electron transport chain has been reported in the brain of many Parkinson’s disease patients. Immunostaining with antibodies to HNE, a lipid peroxidase reaction marker, shows intense staining of neurons of the substantia nigra in Parkinson’s disease patients, versus healthy brain tissue used as a control. The fact that other nerve tissues, and even neurons in the same Parkinson’s disease patients, are not stained suggests that neurons of the substantia nigra exist under particularly intense oxidative stress in Parkinson’s disease patients. Immunostaining with antibodies to 8-oxoG produces intense staining of the cytoplasm in neurons of the substantia nigra in Parkinson’s disease patients, indicating oxidative damage to mitochondrial DNA (Fig. 3). Of great interest is the fact that Parkinson’s disease patients show strongly induced expression of hMTH1, which hydroxylizes the oxidative nucleotide 8-oxo-dGTP, suggesting that neurons react defensively to oxidative stress.\textsuperscript{15} A 5 kbp deletion seen frequently in oxidative stress was also reported to be significantly higher in the mitochondrial DNA of substantia nigra neurons in Parkinson’s disease patients versus control brains.\textsuperscript{16}

1-Methyl-4-phenylpyridinium Ions (MPP+) accumulate selectively in neurons of the substantia nigra and cause synthetic Parkinson’s disease-like symptoms. This is the most frequently used representative, experimental Parkinson’s disease model.\textsuperscript{17} The mechanism of action involved is thought to be inhibition
Mitochondrial Genomic Disintegrity and Age-Related Diseases

of mitochondrial electron transport chain complex I, and inhibition of ATP production. We discovered a new action of MPP⁺ whereby this ion selectively inhibits mitochondrial DNA replication without inhibiting synthesis of nuclear DNA. This inhibition of mitochondrial DNA replication causes a reduction in mitochondrial DNA, which could potentially cause mitochondrial dysfunction 35. MPP⁺ is believed to cause rapid loss of newly formed H chains, affecting reactions in the early initiation of replication. MPP⁺ has also shown no inhibitory effect on human DNA polymerase γ, suggesting that the loss of newly formed H chains results from inhibition of replication initiation reactions, and not from inhibition of DNA synthesis. In reality, MPP⁺ indirectly destabilizes the R-loop structure, a precursor to replication initiation, and the D-loop structure, a replication intermediate, thus acting to release newly formed RNA and DNA; this action is the essence of the replication inhibition reaction. MPP⁺ has a previously unknown, entirely new function by which destabilizes the characteristic triple-stranded structure assumed in mitochondrial DNA replication, thereby inhibiting mitochondrial DNA replication 19. Both in Parkinson’s patients and in experimental models, the mitochondrial genome appears to be one target of this ion.

Recent advances in research on mitochondrial selective autophagy (mitophagy) 20,21 show increasingly that this process is also a crucial mechanism for maintenance of the mitochondrial genome, at least in yeast 22. Genes such as Parkin and PINK1, which cause Parkinson’s disease, are a focus for mitochondrial quality control in mammals. Both factors are regulatory factors for mitophagy, and abnormalities in these factors are thought to lead to reduced quality control among mitochondria damaged by aging, causing neuronal death 23.

Myocardium and the mitochondrial genome

Mitochondrial genomic abnormalities naturally have a marked effect on cellular function even among myocardium, another representative, fully differentiated cell containing abundant mitochondria. In the first instance, diminished mitochondrial function causes mitochondrial genomic abnormalities. In this context, Wallace et al. created two knockout mice of great interest. A mitochondrial-type superoxide dismutase (SOD) knockout mouse experienced fatal dilated cardiomyopathy several days after birth 24. This finding showed clearly that production of reactive oxygen species occurring physiologically in mitochondria could somehow manifest potent toxicity in the body in the absence of proper decomposition and removal. Knockout of the ATP/ADP exchange transporter transporting ATP produced by mitochondria to the cytoplasm results in malfunction of the electron transport chain 25. This knockout mouse experiences hypertrophic cardiomyopathy and presents accelerated production of reactive oxygen species and consequent powerful oxidative impairment of the mitochondrial genome. Malfunction of the electron transport chain causes increased production of reactive oxygen species and malfunction of the mitochondrial genome, ultimately manifesting an organic failure of the mitochondrial electron transport system at the organism level.

In a mouse partial myocardial infarction model, compensatory thickening occurs in non-infarcted areas, and eventually, disintegrity resulting from overcompensation is known to occur. Greatly elevated production of reactive oxygen species and a decreased number of mitochondrial genome copies were observed in non-infarcted areas 26. Based on the fact that only electron transport chain complex activity encoded by the mitochondrial genome decreased at such time, decreased electron transport chain activity is thought to result from mitochondrial genomic abnormalities, not direct protein dysfunction caused by
reactive oxygen species. Though even a normal electron transport chain produces reactive oxygen species, there is conceivably a vicious circle in which elevated physiological electron transport chain activity caused by compensatory thickening leads to elevated production of reactive oxygen species, which causes mitochondrial genome malfunction and organic malfunction of the electron transport chain, which in turn causes a further increase in production of reactive oxygen species. It is also surprising that merely such interim elevation in reactive oxygen species production due to increased physiological activity of the electron transport chain causes mitochondrial genomic malfunction. Oxidative stress on the myocardium was also observed in a chronic heart failure model, and this phenomenon also depends on the mitochondrial electron transport chain.

In TFAM overexpression mice expected to demonstrate a protective effect on the mitochondrial genome, mitochondrial DNA abnormalities resulting from partial myocardial infarction and consequent progression of heart failure were strongly inhibited, and survival rates were dramatically increased (Fig. 4). These findings suggest that protection of the mitochondrial genome from oxidative stress may prove an important preventive and even therapeutic strategy for maintenance of tissue or organ function.

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**Fig. 4.** Death rates following myocardial infarction
Death rates among TFAM expression mice following myocardial infarction are dramatically improved.

**TFAM mice and natural aging**

Though the extent is debated, reactive oxygen species are broadly recognized to contribute to natural aging, in other words, age-related structural changes and decreased functionality of various cells, tissues, and organs. One molecular mechanism proposed is accumulated damage of the mitochondrial genome by reactive oxygen species. As discussed, the mutation rate of mitochondrial DNA is up to a hundred times that of nuclear DNA, indicating a potentially high level of point mutations and deletion mutations accumulated in the aging process, which causes cellular dysfunction and senescent changes.

In this context, TFAM expression mice expected to demonstrate a protective effect on the mitochondrial genome were raised naturally for a long duration and compared to a natural aging phenotype of wild-type mice. Investigation of learning and memory ability, a phenotype of typical aging, in a water maze test showed that ability was virtually equal in 24-month-old TFAM mice and 8-week-old wild-type mice (Fig. 5). Results were the same with regard to motor ability. These findings strongly suggest that protection of the mitochondrial genome may inhibit age-related changes.

**Conclusion**

Recent reports suggest an association between mitochondrial genomic mutations and oncogenesis, and disintegrity of the mitochondrial genome may be associated with a surprisingly large number of pathologies. Though the mitochondrial genome is a mere 16 kbp, the full sequence of which has been decoded for 30 years, many maintenance mechanisms of the mitochondrial genome are still not fully understood, namely, those of replication, repair, and copy number regulation. Understanding of the relationships between the mitochondrial genome and aging and disease requires a molecular-level elaboration of the mechanisms maintaining the mitochondrial genome in a normal state, and the mechanisms activated by the damaged mitochondrial genome.

**Conflict of interest statement**

The author declares no financial or other conflicts of interest in the writing of this paper.
Fig. 5. Water maze test
In TFAM expression mice, even at age 24 months, increase in the number of test errors in a water maze test is restricted, and learning and memory abilities are maintained.

References