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Separate Sexes and the Mitochondrial Theory of Ageing

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An hypothesis is presented by which gamete specialization resolves a conflict between the function and replication of mitochondria. The function of mitochondria is to synthesize ATP by oxidative phosphorylation, which is coupled to respiratory electron transport. This requires a mitochondrial genetic system. However, "incorrect" electron transfers produce free radicals that cause mutation, and the frequency of these events is increased by mutation. Mitochondrial function is therefore detrimental to the fidelity of mitochondrial replication. Damage to somatic mitochondrial DNA may accumulate within, and indeed determine, the life span of individual organisms. Motility of one gamete is required for fertilization, and requires ATP. It is proposed that male gametes maximize energy production for motility by sacrificing mitochondrial DNA to electron transfer and its mutagenic by-products, while female gametes, which are non-motile, repress mitochondrial oxidative phosphorylation, thus protecting mitochondrial DNA for faithful transmission between generations. Male gametes then make no contribution to the mitochondrial genome of the zygote: mitochondria are maternally inherited. This testable hypothesis may help to explain the evolution of separate sexes and a number of their characteristics. Maternal inheritance of chloroplasts may be explained in a similar way, and contribute to the maintenance of separate sexes in plants.

Introduction: the Problem

Respiratory electron transport in mitochondria is accompanied by the generation of mutagenic free radicals of oxygen (Chance *et al*., 1979). Mitochondrial genomes encode several key components of respiratory electron transport chains (Attardi & Schatz, 1988). ATP synthesis, which is coupled to respiratory electron transport in mitochondrial oxidative phosphorylation, is thus accompanied by damage to the mitochondrial genetic system upon which it depends. Free radical-mediated mutagenesis of mitochondrial DNA may initiate and promote further mutagenesis through its effects on the structure and function of respiratory chain proteins, and through effects on mitochondrial gene expression. These events therefore form a positive feedback loop: incorrect electron transfer causes mutation; mutation causes incorrect electron transfer. This process is depicted, in schematic form, in Fig. 1. The damage that accumulates may cause degeneration and ageing, and there is evidence that both bioenergetic function and genetic integrity of mitochondria decline progressively with age (Ernster, 1994; Ozawa, 1995; Shigenaga *et al*., 1994). This theory of ageing is outlined further in the legend to Fig. 1.

Offspring do not inherit their parents' somatic degeneration. If the mitochondrial theory of ageing (Fig. 1) is correct, how can the mitochondrial germ line be exempt? I suggest that the positive feedback loop is broken in mitochondria of the female germ line, from which all other mitochondria derive. The means of the escape of the female germ line from mitochondrial ageing is central to the hypothesis now described.

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Hypothesis: Division of Labour Between Male and Female Germ-line Mitochondria

It is proposed that mitochondria of the female germ line have a repressed bioenergetic function, that they thereby evade mutagenesis from products of respiratory electron transport, and that their genomes thus survive and replicate with minimal damage. Bioenergetically competent mitochondria of the male germ line and of somatic cells of both sexes will then derive only from female germ-line mitochondria, here termed "promitochondria", which replicate in a self-contained cycle. Differentiation of promitochondria into bioenergetically functional mitochondria, active in mutagenesis, would thus be an irreversible step in development. It is further proposed that male gametes have bioenergetically functional mitochondria which are, in consequence, genetically disabled. Selection should thus be expected to favour the elimination of damaged, paternal mitochondrial DNA at, or before, fertilization. The hypothesis is depicted in Fig. 2, and developed further in the legend to Fig. 2.

"Male" is conventionally defined as that sex which produces a large number of small, mobile gametes, while "female" is defined as that sex which produces a small number of large, immobile gametes. However, from the viewpoint of the hypothesis proposed here, these characteristics may be regarded as secondary. If the hypothesis is correct, the primary difference between the sexes may be defined as follows: "male" is that sex in which germ line mitochondria perform oxidative phosphorylation; "female" is that sex in which germ line mitochondria do not perform oxidative phosphorylation. The solution to the conflict between mitochondrial respiration and genetic integrity is thus separate sexes; anisogamy is a division of labour between male and female with respect to mitochondrial function and replication (Fig. 2).

SPECIFIC PREDICTIONS OF THE HYPOTHESIS

The hypothesis is testable since it makes a number of specific predictions. These are listed as follows. Some specific predictions have ancillary implications,

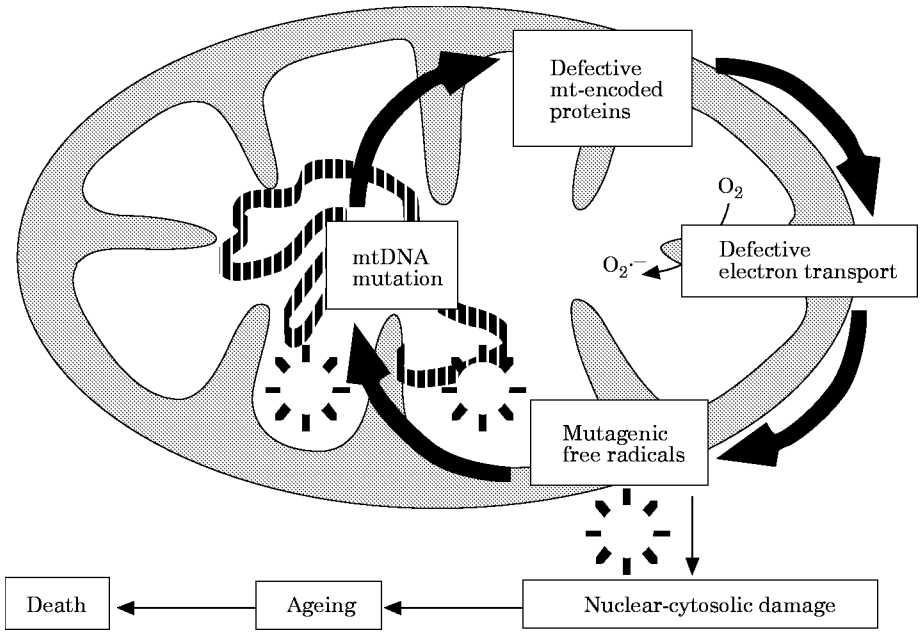

FIG. 1. Why we grow old and die: the mitochondrial theory of ageing. Free radicals (whose reactions are symbolised by a star), including the superoxide anion radical, $O_2\cdot^-$, are produced at a low frequency as by-products of respiratory electron flow in oxidative phosphorylation. Free radical mutagenesis of mitochondrial DNA (mtDNA) then impairs the structure and function of respiratory chain proteins, in turn increasing the frequency of free radical production. Univalent reduction of oxygen by semiquinone anion radicals may be an important initial step (Cadenas *et al*., 1992), since ubisemiquinone is an intermediate in protonmotive Q-cycles in oxidative phosphorylation (Mitchell, 1976), and readily reduces oxygen to the superoxide anion radical, $O_2\cdot^-$ (Cadenas *et al*., 1992). Other oxygen free radicals and sites in the respiratory chain may also be involved. Direct damage to proteins and membranes may accelerate the cycle and initiate somatic degeneration. Mitochondria may minimize, but never eliminate, mutagenic electron transfers. Mitochondrial DNA suffers oxidative damage at about ten times the rate of nuclear DNA (Ames *et al*., 1993). A fixed frequency of electron transfers causing oxidative damage to mitochondrial DNA in different species is also consistent with the inverse correlation between life span and metabolic rate, and hence with the increase of life span with size (Ames *et al*., 1993). In plants, chloroplast genomes may be subject to a similar cycle of redox damage from electron transport in photosynthesis (Allen, 1977). For animal cells, a similar positive feedback loop (Ozawa, 1995; Shigenaga *et al*., 1994), or "vicious circle" (Ernster, L., personal communication), has been proposed as an explanation of ageing.

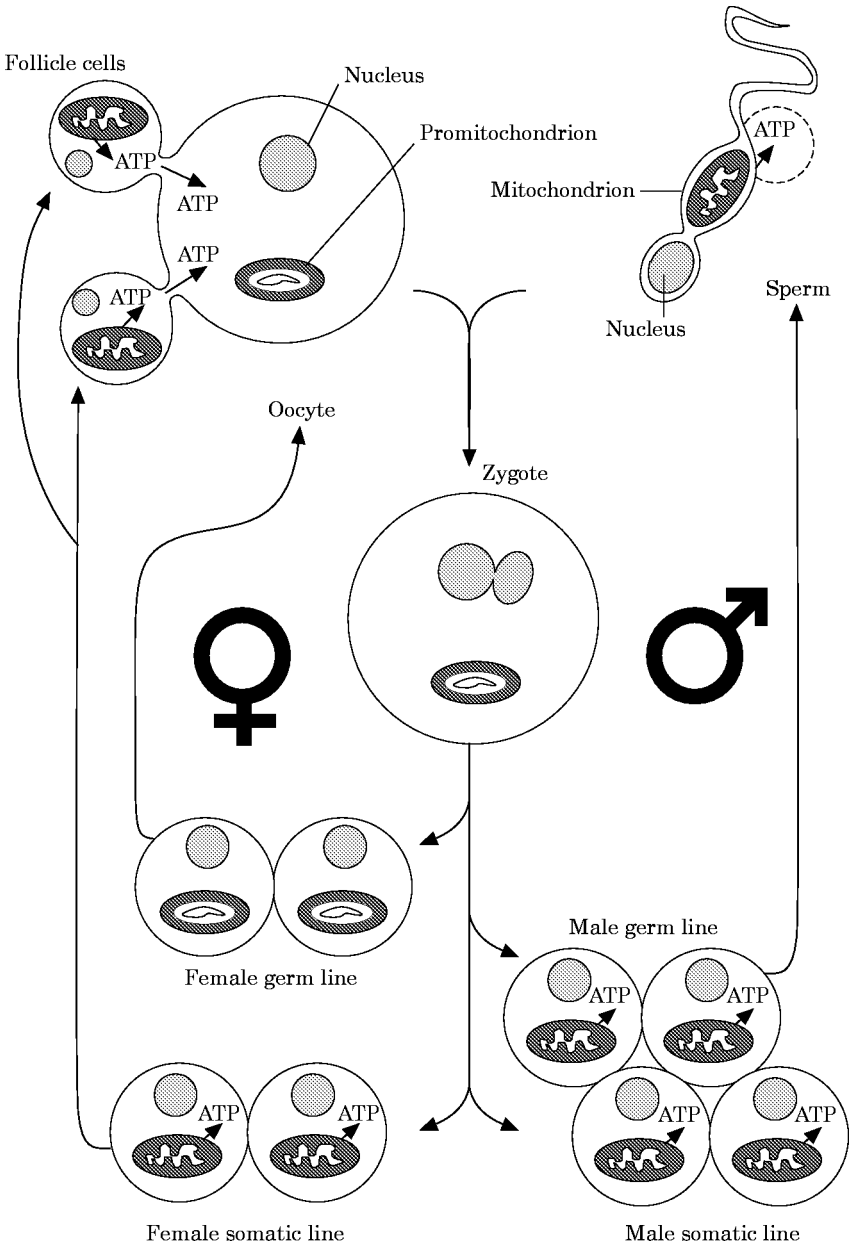

FIG. 2. Why our genes do not die with us: differentiation of male and female gametes for motility and for fidelity of mitochondrial genome replication. The probability of encounters of two gametes is ideally the same whether one or both is motile. Therefore one sex (male) may produce gametes that sacrifice the mitochondrial genome in favour of oxidative phosphorylation. The other sex (female) is then free to produce immobile gametes in which oxidative phosphorylation is repressed in promitochondria, and through which the mitochondrial genome is thus transmitted with increased fidelity. Promitochondria are sequestered early in development in the female germ line. Female oocytes obtain ATP from oxidative phosphorylation in the differentiated mitochondria of ancillary somatic cells (follicle cells in animals). Promitochondria persist in plants in meristematic cells, prior to differentiation of somatic and germ cells. In contrast, any ancillary germ cells (nurse cells in invertebrates) will also require imported ATP, since they share the oocyte's cytoplasm. Gamete differentiation may likewise rescue the chloroplast genomes of plants. Mitochondria and chloroplasts are thus maternally inherited.

which, if false, do not necessarily refute the hypothesis. Where appropriate, these implications are added as additional sentences that comment on the relevant specific, potentially falsifying prediction, which is given in italics. The specific predictions are enumerated. Further, general implications are described in the discussion section.

1. *Female germ line mitochondria have a repressed bioenergetic function*

This repression should take the form of absence of cytochromes and other electron transport complexes. This prediction is consistent with observed morphological characteristics and segregation of two

populations of mitochondria on *Xenopus laevis* oocytes (Tourte *et al*., 1984), only one of which contributes to the germ plasm of the cell (Mignotte *et al*., 1987). Repression of bioenergetic function may occur primarily at the level of transcription of the thirteen mitochondrially-encoded proteins of oxidative phosphorylation. In the mouse, the mitochondrial genome is largely inactive in the egg and two-cell embryo, but transcription is initiated during cleavage, when structural and functional differentiation of mitochondria also occurs (Piko & Taylor, 1987). In *Xenopus laevis*, specific DNA-binding proteins are known to inhibit oocyte mitochondrial transcription (Barat-Gueride *et al*., 1989; Ghrir *et al*., 1991). Nuclear genes for mitochondrial components may be repressed as a consequence of repression of mitochondrial transcription.

2. *Cells of the female germ line are sequestered from somatic cell lines at an early stage in development, prior to differentiation of promitochondria*

Early segregation of the female germ line is necessary for the maintenance of promitochondria, since irreversible differentiation of the remaining promitochondria into somatic mitochondria is required for ATP synthesis by oxidative phosphorylation for cell division and other developmental events.

3. *Female gametes are relatively long-lived, having the lowest metabolic rate consistent with viability, and usually few in number*

Oocytes may have a long but pre-programmed lifetime, since mitochondrial genetic damage may eventually accumulate, despite protective mechanisms, to a point where natural selection will favour the elimination of individual females that are still capable of passing on their mitochondria. This may set a limit to the reproductive phase of females alone in species with an extended life span.

4. *Female gametes are unable to provide their own ATP by oxidative phosphorylation, and depend for their energy supply upon substrate-level phosphorylation and upon import of ATP from oxidative phosphorylation in the mitochondria of ancillary somatic cells*

Follicle cells, for example, may thus supply oocytes with ATP, via gap junctions (Fig. 2).

5. *Further mechanisms exist for the protection of oocytes from free radicals and from requirements for ATP*

Formation of polar bodies at the first and second divisions of meiosis may be involved in such processes. Cell division itself requires ATP, and may be incompatible with maintenance of gap junctions (see prediction 4). Polar bodies may sequester oocyte mitochondria released from repression of oxidative phosphorylation, leaving only promitochondria in the mature ovum.

6. *Male germ-line mitochondria are a genetic dead-end, and male gamete mitochondria are committed to short-term energy production*

Male gametes are therefore short-lived and may be produced at any stage in the lifetime of the individual male. Sperm mitochondria should have even less use for a genetic system than somatic cell mitochondria, since the latter must replicate during mitosis.

7. *Active exclusion of genetically damaged sperm mitochondria from the zygote is selectively advantageous*

This may be one function of the acrosomal reaction of the sperm acrosomal vesicle with the zona pellucida of the ovum. In plants, cells of the gametophytic and sporophytic generations are always separated by thickened boundaries of cell wall material, with no plasmodesmata, which act as barriers to transfer of macromolecules, including nucleic acids (Bell, 1995). Such "molecular filters" may serve to eliminate damaged mitochondrial and chloroplast DNA from the zygote.

EXCEPTIONS AND EVOLUTIONARY IMPLICATIONS

Not all eukaryotes exhibit purely maternal inheritance of mitochondrial DNA. In the mussel, *Mytilus*, for example, paternal mitochondria survive in the zygote of the male line, but not in the female line, where only maternal mitochondria are maintained (Hurst & Hoekstra, 1994; Skibinski *et al*., 1994; Zouros *et al*., 1994). In addition, some organisms, notably in the plant kingdom, seem to be potentially immortal, and may thus be able to escape the ageing cycle (Fig. 1) by other means. Biparental mitochondrial or plastid inheritance does not falsify the hypothesis in such cases, since the hypothesis does not then apply.

The rate of evolution of mitochondrial DNA seems to be generally slower in plants than in animals (Gray, 1989), but it is not known whether this results from a greater stabilizing selection, or from a lower mutation frequency. The hypothesis proposed here suggests a general correlation between mortality and sexual division of labour at the cellular level.

Many plants have an extended haploid stage, and in these cases the hypothesis (Fig. 2) predicts that female gametophytes carry a continuous line of

promitochondria adapted to replication, just as in the diploid sporophyte. In seed plants, the male gametophyte is reduced in size, and usually non-motile (discussed by Bell, 1995). Pollen may exclude mitochondria and chloroplasts with the cytoplasm, increasing its longevity.

The hypothesis may also apply to plastid inheritance. Gamete motility probably relies rarely on direct energy production by photosynthesis in chloroplasts. Photosynthetic electron transport nevertheless generates oxygen free radicals (Allen, 1977). Repression of photosynthetic function in a continuous proplastid line may therefore promote fidelity in transmission of the plastid genome. Thus the hypothesis may equally apply to maternal inheritance of chloroplast DNA.

Sexual reproduction offers many advantages over asexual reproduction, at least for long-term evolution of populations (Maynard Smith, 1978). Sexual recombination of nuclear genes may, for example, increase the extent to which mutations are able to survive as recessive alleles in diploid generations. Sexual reproduction may thus permit phenotypic expression of alleles in a wider range of environments and in combination with a wider range of other genotypes than would be possible by asexual means, allowing greater opportunity for selection in favour of characters that might otherwise be immediately disadvantageous and therefore lost.

The hypothesis proposed here clearly does not explain all the selective advantages of sexual reproduction. However, it does suggest a reason for the prevalence of anisogametic sex. The hypothesis also provides a plausible example of a selective advantage of separate sexes that acts at the level of the individual organism, a simpler and more feasible mechanism than group selection. The evolution of separate sexes by virtue of separation between male and female germ lines of mitochondrial function and replication also fits clearly into the category of a major evolutionary transition as described by Maynard Smith & Szathmáry (1995): division of labour (mitochondrial specialisation) produces increased complexity (anisogametic sex) in a system whose individual components (the two sexes) thereby lose autonomy (in reproduction), become mutually inter-dependent, and gain new evolutionary possibilities (combination of mitochondrial continuity with gamete motility).

Maintenance of Cytoplasmic Genomes: Discussion

Why then do mitochondria and chloroplasts maintain extra-nuclear genomes? Raven *et al*. (1994) propose that the selective advantage of removing genes to the nucleus is to decrease their free radical-induced mutation frequency. One might imagine that this would be universally advantageous, since it will tend to suppress the positive feedback cycle depicted in Fig. 1. However, the retention of some mitochondrial and chloroplast genes may be necessary to permit direct redox control of gene expression (Allen, 1993a,b), and this may decrease free radical production to an even greater extent than if mitochondrial and chloroplast genes were transferred to the nucleus, where redox control by bioenergetic electron transport chains may occur only by indirect means.

An alternative hypothesis for the retention of mitochondrial genomes is that their hydrophobic gene products became unimportable after the emergence of protein secretion from eukaryotic cells (von Heijne, 1986). This and related proposals are discussed elsewhere (Allen, 1993a).

Mitochondrial and chloroplast gene products include key redox proteins of respiration and photosynthesis that may be directly involved in free radical production (Allen, 1993a,b). Free radical production may therefore be minimized by the operation of a negative feedback loop involving redox state of electron transport components and gene expression, maintaining an optimal stoichiometry of electron transport components despite environmental changes that tend to perturb redox homeostasis. Mitochondrial and chloroplast genes encoding components of their own genetic systems may serve a secondary function in permitting the maintenance and replication of the extra-nuclear genetic systems whose primary function is maintenance of correct redox balance in eukaryotic cells. Redox control of chloroplast and mitochondrial gene expression (Allen *et al*., 1995a,b; Håkansson & Allen, 1995) may therefore minimize free radical damage to the cell as a whole, and loss of such genes to the nucleus would entail loss of this control. Rather than being the price we pay for the maintenance of mitochondrial genomes, mortality (Fig. 1) may actually be thus postponed.

From this viewpoint, sex is not a device for preventing the proliferation of ''selfish'' mitochondrial or symbiont DNA, as in alternative hypotheses (Hurst & Hamilton, 1992; Hurst & Hoekstra, 1994; Law & Hutson, 1992). Eukaryotes, and their nuclear genes, rather owe a debt, in a sense, to the apparently altruistic genomes of mitochondria and chloroplasts, which occupy the eukaryotic cell's most hostile internal compartments. I suggest that anisogametic sex is a mechanism for ensuring that at least some mitochondrial and chloroplast genomes survive intact across the generations. It will then serve the

mitochondrion's and chloroplast's primary functions of energy transduction, both in somatic cells and in male gametes whose motility is required for recombination of nuclear genes. Intracellular sequestration of oxidative and photosynthetic phosphorylation may, for these reasons, be inextricably interlinked with cell division. Bioenergetic organelles may thus have been a precondition for the evolutionary development of the eukaryotic separation of nucleus and cytoplasm.

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