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Hypothesis Paper

A Mitochondrial Model for Premature Ageing of Somatically Cloned Mammals

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Summary

Cloned sheep have recently been discovered to have an unexpectedly advanced biological age. We propose that the explanation is a simple consequence of inheritance of acquired, free radical–induced cellular damage with somatic mitochondria that contribute to the mitochondrial population of cloned cells but not to zygotes produced by fertilization in normal sexual reproduction. Each increment of ageing in cloning experiments is therefore predicted to be maternally inherited. The hypothesis suggests practical ways of decreasing the effect. The hypothesis is itself a prediction of the recent proposal that mitochondria of the female germ line function primarily as genetic templates.

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INTRODUCTION

Telomere length suggests that "Dolly," the cloned sheep, is biologically older than a normal sheep of her chronological age (*1*). Dolly's chromosomes have telomeres as short as those of from somatic cells of a 6-year-old ewe (*1*), suggesting that Dolly began life at the biological age of the mammary epithelial cells that were fused with an enucleated oocyte to provide the nucleus of the single progenitor cell (*2*). Here we describe how this unexpected finding is one of a number of simple predictions of a mitochondrial model of ageing.

HYPOTHESIS

There is increasing support for the theory that ageing is initiated by errors in mitochondrial electron transport and is promoted by their accumulation (*3–5*). An accurate template of mitochondrial DNA must then somehow be maintained: One suggestion is that promitochondria, which never carry out their primary bioenergetic function, are sequestered in the female germ line (*6*). If the 6-year-old ovine somatic cell donated 6-year-old, mature mitochondria to the hybrid cell that gave rise to Dolly, then we have an explanation of Dolly's premature ageing and can make a number of further predictions. Fig. 1 is a mitochondrial model for the premature ageing of Dolly and cloned mammals generally. Fig. 2 shows the predicted outcome of genetic crosses according to the mitochondrial model and to a general model based on the assumption that a nuclear, chromosomal factor is involved instead.

Fig. 1 rests on an explicit hypothesis concerning division of labour between female germ line mitochondria and all others. This hypothesis was proposed (*6*) as a solution to the incompatibility between the function of mitochondria and the fidelity with which their genetic material may replicate and be passed on to successive generations (*7*). Mitochondria are primarily energy-transducing organelles, in which synthesis of ATP in oxidative phosphorylation is coupled to respiratory electron transport (*8*). Electron transport inevitably produces oxygen free radicals that react indiscriminately with many cellular components, including DNA. Mutation in mitochondrial DNA (mtDNA) may produce altered respiratory chain proteins, which are encoded there. Such mutations increase the frequency of mutagenic free radical production, so that impaired respiratory electron transport is both a cause and a consequence of mitochondrial mutation. If this "vicious circle" holds, then there is no going back from a mitochondrion that has begun the ageing process to one for which the DNA template is an accurate and faithful copy of the original. The solution may be the persistence of a closed, replicating cycle of promitochondria that never carry out electron transport: the mitochondria of the female germ line. In the case of mammalian somatic cloning, the fusion of a somatic nuclear donor cell with an oocyte may simply have produced a heteroplasmic cell in place of the homoplasmic oocyte, giving bioenergetically

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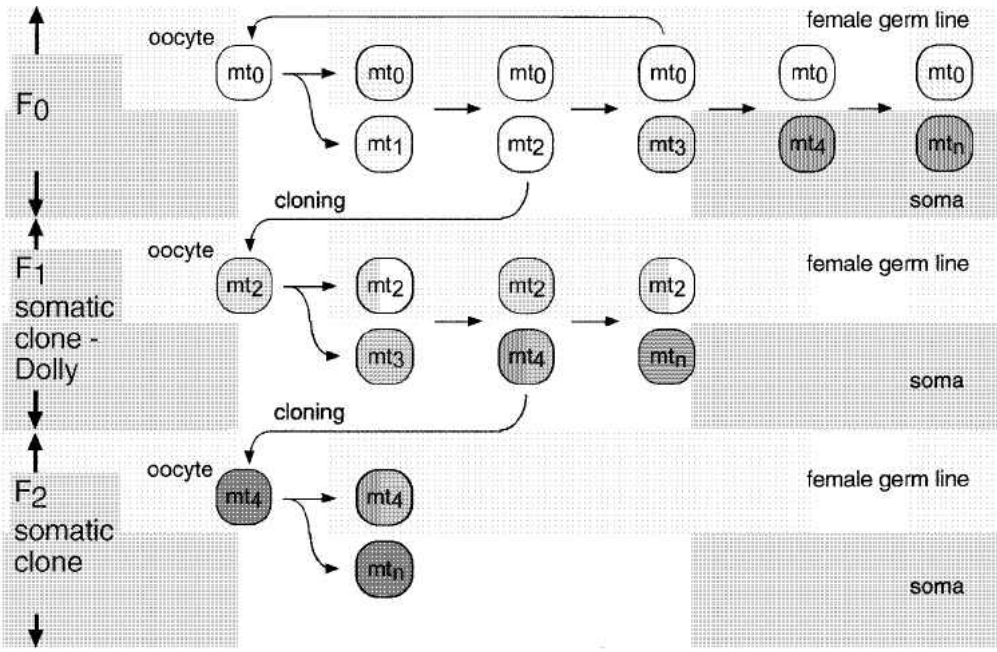

Figure 1. Predicted inheritance of premature ageing after cloning of somatic cells. Mitochondria are maternally inherited and are assumed to be maintained in the female germ line in a nonageing form (*6*). mt_0 represents such "promitochondria"—mitochondria that have never differentiated to perform oxidative phosphorylation. mt_1, mt_2, . . . , mt_n represent differentiated mitochondria in successive, arbitrarily defined stages of ageing in somatic cells or in the male germ line. mt_n represents the final stage of mitochondrial ageing that can support life. Somatic cloning starts the ageing cycle at a stage that is advanced by the increment corresponding to the mitochondrial age of the donor cell, here designated mt_2 for the F_1 somatic clone "Dolly." Dolly's normal sexual progeny will inherit the mitochondrial mt_2 state and begin ageing from mt_2 instead of from mt_0. Somatic donor cells from Dolly will create an F_2 clone that begins at an even more advanced stage, designated mt_4. The F_2 clone should show an even earlier onset of ageing and have a correspondingly shorter life span.

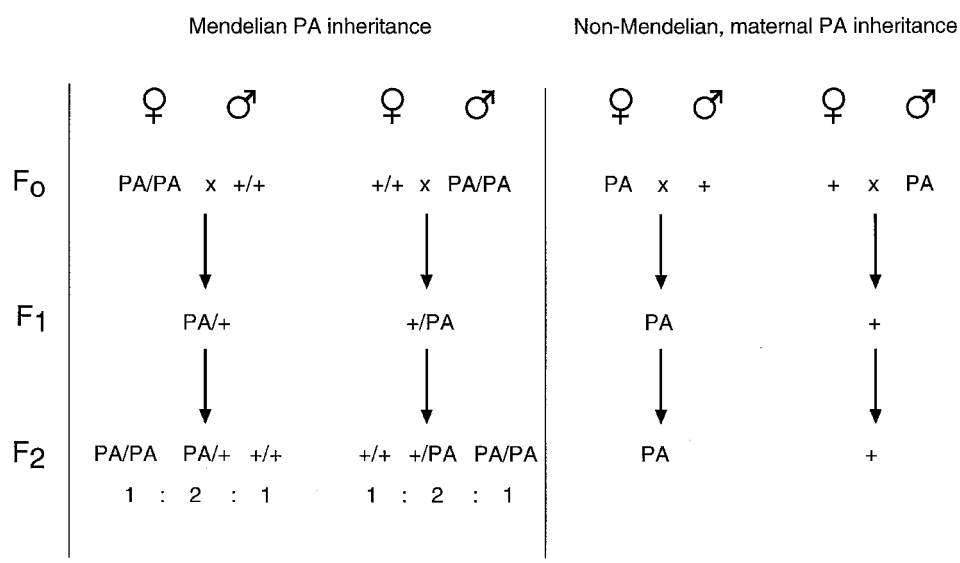

Figure 2. Any "premature ageing" phenotype will exhibit Mendelian inheritance if it is the result of expression of a nuclear chromosomal factor, here depicted for simplicity as a single allele, PA, which may be either dominant or recessive to the wild-type allele, + . In this case (left-hand diagram), there will be no differences in ageing between the progeny of reciprocal crosses. However, if the model proposed in Fig. 1 is correct, PA is not a nuclear factor but an advanced mitochondrial stage (e.g., mt_2 or mt_4), which is inherited only through the female germ line (right-hand diagram). PA will then be inherited in a non-Mendelian, maternal fashion, and reciprocal crosses will give different progeny: PA will be eliminated from sexual progeny of a male somatic clone but fixed in the those of a female one.

functional mitochondria in place of promitochondria: Dolly was thus born with mitochondria well advanced into the ageing cycle (Fig. 1). According to this model, any offspring she bears must be at least equally advanced in biological age: Her own oocytes can have only the impaired mitochondrial DNA of the somatic cell that also provided her nucleus.

PREDICTIONS OF THE HYPOTHESIS

The model in Fig. 1 is testable since it makes a number of further predictions one can make about ageing in cloned animals. The effect of import of aged mitochondria into the oocyte is, first, to advance the baseline of the biological age of the recipient oocyte by the age of the somatic tissue with which it is fused. This effect should be cumulative. Thus, if Dolly's somatic cells are used to donate nuclei and, inadvertently, mitochondria, into a subsequent oocyte, then the developmental age of the "F_2" progeny should be equal to the sum of the ages of the previous parents. In normal sexual reproduction, the advance in age is always zero for each sexual generation, because the female germ line maintains promitochondria as a genetic template. It follows that Dolly's natural offspring will be born at her own biological age at the time of her own "conception" (6 years in this case), whereas any clones made from Dolly's somatic cells will be, biologically, $6 + n$ years old, where n is Dolly's age when her somatic cells are used for cloning. A male Dolly, in contrast, will not supply mitochondria from sperm cells to the oocyte, and the natural offspring of such a cross should therefore revert to ageing normally, starting from the developmental age of the recipient oocyte (zero for noncloned animals) at fertilization. A difference between the progeny from such reciprocal crosses (Fig. 2) is a clear prediction of the mitochondrial hypothesis: Each advance in biological age upon cloning somatic cells is maternally inherited. In contrast, ageing by means of nuclear effects such as telomere shortening (*1*) does not predict a difference between maternal and paternal inheritance of increased age (Fig. 2). Although there may be exceptions with important evolutionary consequences (*9*), maternal inheritance of mitochondria is likely to predominate in breeding experiments and programmes. Moreover, inheritance of somatic mitochondria from the oocyte donor would also operate by a mechanism identical to that depicted in Fig. 1.

FURTHER IMPLICATIONS

If the premature ageing of cloned mammals arises from fixation of the irreversibly aged, somatic mitochondria of a donor cell, what could be done to prevent it? One solution would be to eliminate completely the mitochondria of the somatic nuclear donor from the oocyte. This would be the equivalent of excluding sperm mitochondria in normal fertilization. Shiels et al. (*1*) suggest that the ageing may be a consequence of the time spent by donor cells in culture, and that the effect might therefore be minimized by shortening the culture time and carefully selecting the donor cells. In contrast, according to the explanation we propose here, the single causal factor is the presence of somatic mitochondria, and this view predicts that the age advance will not occur if care is taken to donate only the nucleus of the somatic cell. Injection of somatic nuclei into enucleated mouse oocytes can give rise to healthy, full-term mice (*10*). Dolly and similar sheep clones were obtained by electroporetic cell fusion rather than by nuclear injection (*2*). If mitochondria are donated to the oocyte along with the nucleus, the ageing effect of cloning could be minimised by choosing the youngest possible somatic donor cells rather than by shortening their time in culture. The sheep clone 6LL7 was derived from somatic, fetal tissue and for this reason may have aged less than the other clones (*1*). A further possibility for the exclusion of aged and age-advancing mitochondria might be to use somatic cells from male animals. Elimination of incoming mitochondria at, or soon after, fertilization is the basis of maternal mitochondrial inheritance. It would be important to know whether incoming male somatic mitochondria are eliminated in the same way.

The scheme in Fig. 1 is also testable by monitoring markers for mitochondrial DNA damage such as the proportion of 8-OH-D-guanidine (*11*). Other markers can be correlated with mitochondrial ageing in mice (*12*) and lend support to the view that mitochondrial breakdown is a component in normal ageing as well as in degenerative diseases associated with ageing, which may be enhanced by mutations, whether nuclear or mitochondrial, that cause their early onset (*3*, *12*). Apart from its potential applications, somatic cloning of mammals may thus present new possibilities for testing theories of ageing and the evolutionary significance of separate sexes.

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