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Development

Ubiquitin tag for sperm mitochondria

Like other mammals, humans inherit mitochondria from the mother only, even though the sperm contributes nearly one hundred mitochondria to the fertilized egg. In support of the idea that this strictly maternal inheritance of mitochondrial DNA arises from the selective destruction of sperm mitochondria[1,2], we show here that sperm mitochondria inside fertilized cow and monkey eggs are tagged by the recycling marker protein ubiquitin[3]. This imprint is a death sentence that is written during spermatogenesis and executed after the sperm mitochondria encounter the egg's cytoplasmic destruction machinery.

Figure 1 shows the ubiquitination of sperm mitochondria in fertilized eggs from rhesus monkeys (Fig. 1a; 18 hours post-insemination) and cows (Fig. 1b; 16 hours). At first mitosis, the mitochondrial sheath of the ubiquitinated sperm is evident at one spindle pole (Fig. 1c; 24 hours). The signal typically disappears between the four-cell and the eight-cell stage of development.

The ubiquitination of sperm mitochondria also occurs in the male reproductive tract. The ubiquitinated sites appear to be masked by disulphide bonds during passage through the epididymis. Spermatocyte mitochondria are already ubiquitinated, and most mitochondria are lost to the discarded residual body during spermiation. Mature sperm found on the egg surface and just inside the egg cytoplasm after incorporation are not detectable by ubiquitin antibodies, although several ubiquitinated proteins are found in sperm extracts following the reduction of disulphide bonds. After incorporation and decondensation of sperm within the egg[4], ubiquitinated sperm mitochondria are again apparent. Sperm mitochondria are lost before, or during, the third embryonic cleavage in mammals[5–7].

Remarkably, sperm mitochondria persist in a bovine interspecies cross between the domestic cow and the Asian wild gaur, as they do in murine hybrids[8,9]. Sperm mitochondria from wild gaurs (gift from N. Loskutoff) persist beyond the third embryonic division (Fig. 1d) after incorporation into cow eggs and are not ubiquitinated, as no antibody binding is detectable. The inheritance of sperm and egg mitochondrial DNA is thought to be solely sex specific, but if it is also species specific, then evolutionary clocks based on mitochondrial DNA may need to be recalibrated.

We propose that the uniparental ubiquitination of mitochondria that occurs during spermatogenesis selectively earmarks sperm mitochondria for degradation by the embryo's proteasomes and lysosomes, for example, during the preimplantation stage. In yeast, ubiquitin mutations interfere with mitochondrial segregation[10], and prohibitin[11] (a mitochondrial inner-membrane protein) is one of the likely ubiquitin substrates in mammalian sperm.

Learning more about this destruction pathway is important for understanding inherited mitochondrial diseases, and for evaluating the safety and efficacy of cloning, oocyte cytoplasm 'donation therapy', and fertilization assisted by spermatid or sperm microinjection. The high death rate observed in cloned offspring[12,13] could be due to heteroplasmy, the condition of mismatched mitochondria.

A report of dissimilar mitochondrial DNA in the first babies born after egg cytoplasmic donation[14] is alarming. Because mitochondrial pathways are disrupted during cloning and some infertility treatments, foreign mitochondria introduced into the egg may reproduce semiautonomously unless they are properly recognized and processed. The implications of paternal mitochondria being selectively tagged for extinction may force a re-evaluation of procreative strategies.

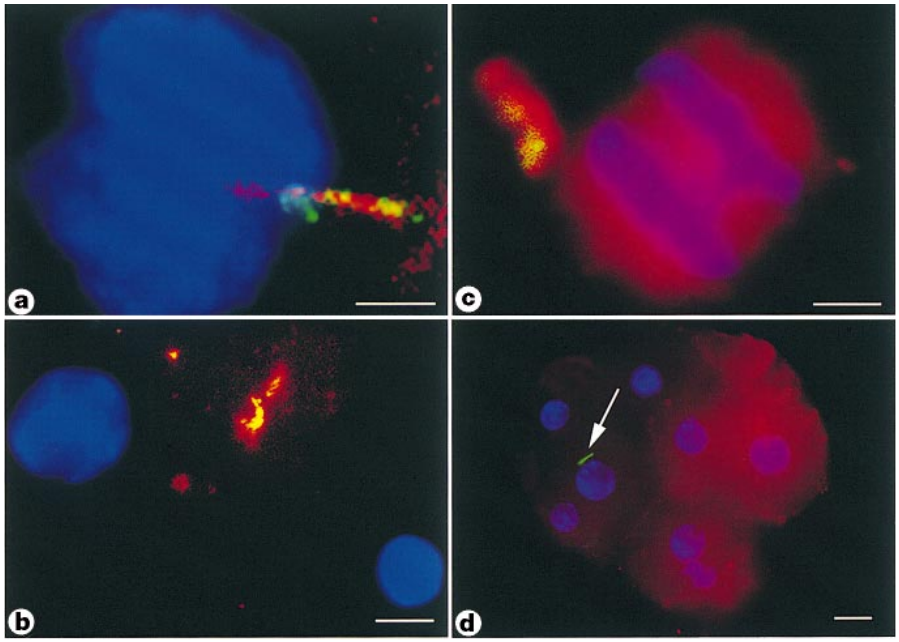

Figure 1 Ubiquitination of sperm mitochondria in mammalian zygotes and embryos. **a–c,** Binding of ubiquitin (red, visualized by anti-ubiquitin antibodies) to the sperm mitochondria (green/yellow, vital dye MitoTracker®) within a pronucleate-stage rhesus-monkey zygote (**a**), a bovine pronuclear zygote (**b**) and an intraspecific (domestic cow × domestic bull) embryo during its first mitotic division (**c**). Sperm mitochondria are not found in intraspecific bovine embryos at the eight-cell stage. **d,** In contrast, the intact mitochondrial sheath (green, white arrow) is found in the hybrid eight-cell embryo from domestic cow oocytes fertilized by the sperm of a wild gaur bull. DNA is visualized in blue within the male and female pronuclei (sperm and egg nuclei, respectively, in **a** and **b**), and as the mitotic chromosomes are aligned and separating during first anaphase (**c**) and eight-cell stage embryonic nuclei (**d**). Scale bars: **a–c,** 5 μm; **d,** 20 μm.

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Oceanography

Iron, nitrogen and phosphorus in the ocean

It has been proposed that widespread deficits of nitrate in the ocean, like those observed today, are caused by iron limitation of marine nitrogen fixation[1]. That is, only when iron is sufficiently abundant to satiate nitrogen fixers will the ratio of nitrate to phosphate in the ocean increase to 16, the average for phytoplankton. Tyrrell[2] developed a simple two-box model of oceanic nitrogen and phosphorus cycles to describe the regulation of both nitrate and phosphate concentrations in the global ocean. His criterion for nitrate deficit in the ocean, a molar ratio of N:P in surface waters (R_s) of less than 16, is satisfied without recourse to iron limitation, calling into question Falkowski's proposal[1] about the biogeochemical significance of iron limitation as it relates to nitrogen fixation and oceanic levels of nitrogen and phosphorus. Here I show that small changes in the assumptions of Tyrrell's model, well within acknowledged uncertainty, can lead to values of R_s greater than 16. Consequently, the consistency of the model with the observed distributions of nutrients in the ocean is uncertain, and the influence of iron may still be considered important.

In Tyrrell's model[2], competition between nitrogen-fixing and other phytoplankton controls the level of nitrate, continually pushing molar concentrations to slightly less than 16 times those of phosphate. Results of the model show that phosphate is the ultimate limiting nutrient because extra phosphate in the system supports the proliferation of nitrogen fixers that can add new nitrogen to the ocean. Even when subjected to extensive sensitivity analysis[2], the model consistently predicts a deficit of nitrate in the surface layer, defined by Tyrrell as $R_s < 16$.

Further calculations (Fig. 1) indicate that the model's prediction of a nitrate deficit in surface waters of the ocean is uncertain. R_s is very sensitive to chosen values for the Michaelis–Menten half-saturation constants for the growth of phytoplankton on nitrate ($K_S[NO_3]$) and phosphate ($K_S[PO_4]$) (N_H and P_H, respectively, in Tyrrell's notation). A 20% increase of $K_S[NO_3]$ to 0.6 mmol m^{-3} from the assumed 0.5 mmol m^{-3} obliterates the predicted nitrate deficit, bringing R_S to 16. The reported[2] uncertainty in $K_S[NO_3]$ is 0.1 to 4.2 mmol m^{-3} (ref. 3), corresponding to an R_S value between 2.7 and 112 mol mol^{-1}.

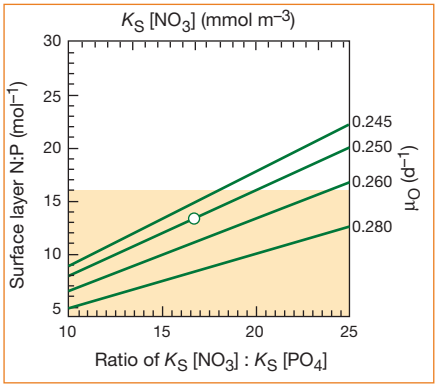

Figure 1 Influence of assumed Michaelis–Menten half-saturation constants and phytoplankton growth rates on steady-state solutions for R_S, the molar ratio of nitrate to phosphate in the surface layer of the ocean (model of ref. 2). Nitrate deficit at the surface is indicated by $R_S < 16$ (shaded). The half-saturation constant for growth versus nitrate, $K_S[NO_3]$, was varied from the specified 0.5 mmol N m^{-3} to obtain a range of $K_S[NO_3]$:$K_S[PO_4]$, keeping $K_S[PO_4]$ constant at the specified 0.03 mmol P m^{-3}. The solution for R_S against $K_S[NO_3]$:$K_S[PO_4]$ is independent of $K_S[PO_4]$ (equation 13 in ref. 2). The maximum growth rate of nitrogen-fixing phytoplankton, μ'_{NF}, was maintained at 0.24 d^{-1}, as specified in the model. The maximum growth rate of other phytoplankton, μ'_O, was varied from the original 0.25 d^{-1}, as indicated. The original model solution is identified with the open circle.

Small changes in the maximum growth rate for other phytoplankton, μ'_O (d^{-1}), compared with 0.24 d^{-1} for nitrogen fixers, also strongly influence R_S (Fig. 1). There is little experimental basis for excluding assumed growth rates that lead to an R_S value of 16.

This simple analysis, based directly on Tyrrell's model, suggests that regulation of oceanic nitrogen fixation by iron cannot be excluded as a potentially important influence on cycles of nutrients and primary productivity in the ocean.

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Tyrrell replies — Cullen shows that changing the values of P_H and N_H ($K_S[PO_4]$ and $K_S[NO_3]$ in his notation) in my model[2] can give rise to a steady-state $[NO_3]$:$[PO_4]$ ratio in surface waters that is greater than or equal to 16. Because of this, and because P_H and N_H are not well known, he questions some of the implications of my model.

Although his analysis is correct, my model must converge to a $[NO_3]$:$[PO_4]$ ratio that is slightly less than the ratio at which NO_3 and PO_4 are equally limiting to growth, regardless of whether the latter ratio is 16 or not. There must therefore be convergence to proximate nitrate (reactive nitrogen) limitation of surface waters.

Equations (1) and (2) of my model[2] can be rewritten as

$$d(NF)/dt = (\mu'_{NF}\,LP - M)NF$$

and

$$d(O)/dt = (\mu'_O \min\{LN, LP\} - M)O$$

where NF and O are the populations of nitrogen-fixing and other phytoplankton, t is time, M is mortality, and LN and LP represent the growth limitations (range, 0 to 1) caused by $[NO_3]$ and $[PO_4]$ shortages, respectively, which were expressed as Michaelis–Menten functions in ref. 2 but have been left unspecified here. For steady state, $d(NF)/dt$ and $d(O)/dt$ must both equal zero (both populations are stable in size), and therefore

$$\mu'_{NF}\,LP = M = \mu'_O \min\{LN, LP\}$$

or

$$\mu'_{NF}/\mu'_O = \min\{LN, LP\}/LP.$$

Because μ'_{NF} is less than μ'_O (ref. 1), this equation cannot be satisfied (equilibrium cannot occur) unless LN is less than LP; that is, the proximate limiting nutrient is reactive nitrogen.

This proof makes no assumptions about the values of N_H and P_H. As Cullen rightly argues, raising N_H allows convergence to a surface N:P > 16, but then the surface ocean is still most strongly limited by reactive nitrogen, precisely because N_H has been raised. But if the simplifying assumption is made that the Michaelis–Menten half-saturation constant for a nutrient is more or less proportional to the rate at which it needs to be taken up to fuel new growth (in which case, N_H/P_H is about 16), then LN < LP also implies that $[NO_3]$:$[PO_4] < 16$ in the surface ocean steady state. Cullen's analysis illuminates the point that a surface ocean could still have nitrogen as the proximate limiting nutrient even if the surface N:P ratio were 100, for instance. It all depends on the values of P_H and N_H, which need to be better constrained.

This analysis confirms my original point: observations from nutrient-enrichment experiments that reactive nitrogen is more limiting to growth than phosphorus in the surface ocean can be reconciled with phosphorus limitation without recourse to the effects of trace metals. If shortages of iron, for instance, further depress nitrogen fixation and reactive nitrogen concentrations in the open ocean, then my model predicts that adding extra iron could cause only a reduction, not full removal, of the proximate nitrogen limitation.

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