In reply—

In their letter, Attardi *et al.* claim that the observation of inter-mitochondrial complementation by Ono *et al.*[1] is a rare phenomenon and cannot be generalized, particularly to an *in vivo* system.

However, using mito-mice carrying exogenously-introduced mutant mtDNA with a deletion of 4,696 bp (ΔmtDNA4696)[2], Nakada *et al.*[3] recently reported unambiguous evidence for the presence of extensive *in vivo* inter-mitochondrial complementation in all tissues examined: all mitochondria in tissues with ΔmtDNA4696 showed normal COX activity until it accumulated, preventing those mice from expressing disease phenotypes. Moreover, coexistence of COX-positive and -negative mitochondria within single cells was not observed. Therefore, these results suggest the occurrence of *in vivo* inter-mitochondrial complementation by the exchange of mitochondrial contents between exogenously introduced mitochondria with ΔmtDNA4696 and host mitochondria with normal mtDNA.

They also claim that the observations of Ono *et al.*[1] are in striking contrast with their previous observations[4,5], which indicated absence of inter-mitochondrial complementation, and suggest that nuclear background would be responsible for these discrepancies.

Their previous observations, however, do not necessarily prove the absence of inter-mitochondrial complementation. In the paper by Enriquez *et al.*[4], for example, the authors fused respiration-deficient cells carrying a mutation in *MTTK* (also known as *MERRF*) and respiration-deficient cells carrying a mutation in *NDUFA4* (also known as *ND4*), and showed that very small numbers of colonies with restored respiratory function grew in a medium that selects for respiration competence. To prove that complementation is a rare event, however, one must show that there is no increase in the frequency of transcomplementing clones even after 10–14 days of growth in nonselective medium. This evidence must be obtained before suggesting the involvement of nuclear factors.

On the other hand, the paper by Yoneda *et al.*[5] showed that completely respiration-deficient cells without mitochondrial translation activity were obtained by the fusion of parent cells carrying a mutation in *MELAS* (with 60% mitochondrial translation activity) and parent cells carrying an *MTTK* mutation (with about 5% mitochondrial translation activity). However, if there was no interaction between these mitochondria, fused cells with both parental mutant mtDNAs should have 5–60% of normal mitochondrial translation activity, but they did not. These observations should therefore be interpreted as showing that parental mitochondria with 60% activity and those with 5% activity fused to produce no activity, suggesting interaction between mitochondria that resulted in complete inhibition of mitochondrial translation activity.

Attardi *et al.* also claim that the small fraction of transcomplementing clones would not increase in number, even by extending the period of growth in non-selective medium to 10–14 days after fusion, as they could not observe an increase after 6 days of growth in this medium. This is not correct. As we outlined in Table 2 of our paper[1], no transcomplementation was observed for 7 days after fusion, with an additional 4–7 days being critical for the restoration of respiratory function.

Based on the *in vitro*[1] and *in vivo*[3] transcomplementation of mitochondria, we recently proposed a new hypothesis, the interaction theory of mammalian mitochondria[6]. In this hypothesis, we suggest that the presence of intermitochondrial cooperation would rescue aged tissues, with various kinds of somatic mutant mtDNAs, from age-associated mitochondrial dysfunction.

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