

Cellular Star Trek: A laser-based shuttle transfers mitochondria into cells

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If a genie were to offer us a single wish, with no second thought, we would ask for tools to manipulate mtDNA as we do with nuclear DNA and bacterial plasmids.

Numerous intriguing theories relating mitochondrial DNA (mtDNA) mutations to aging and diseases have been around for decades, yet most of them cannot be directly tested for lack of a competent technology. More importantly, efficient therapy for treating mitochondrial genetic diseases is sadly lacking. Yet, constant improvements are being made bringing us closer to the holy grail of mtDNA manipulation. This is evident in a recent publication paper by Wu and Sagullo et al. which features a new technique for transferring mitochondria [1].

Mitochondria's double membrane is impermeable to DNA as well as to mRNA and, most importantly, to a few highly hydrophobic proteins encoded by it. Hence, strategies to introducing foreign mtDNA rely not on DNA transduction but on transferring whole mitochondria into cells, which through mitochondrial dynamics spread their content to other mitochondria. The strategies for transferring mitochondria, however, suffer from various limitations. The commonly employed fusion of cells to generate cybrids is a traumatic process for the cells and is un-specific as it consists of not only mitochondrial delivery but also mixing the entire cytoplasmic content of the cells. Microinjection of mitochondria into cells is a more specific approach, albeit quite an inefficient one. To introduce mitochondria into cells in a controlled fashion and at higher throughput Wu and Sagullo and colleagues have adapted a tool they had previously developed for the introduction of bacteria into host cells [2]. They used a technique named photothermal nanoblade. This technique consists of positioning a micropipette tip containing mitochondria on the plasma membrane of the acceptor cell, followed by induction of a laser-pulse to form a bubble in the culture media that punctures the membrane, thus creating a passageway large enough for mitochondria to enter. The membrane perforation is transient and localized, making this technique less stressful and more specific than most alternatives. This technology is unique in its ability to control parameters that could not be controlled before, including dosage, content, and timing. As such, it enables the study of parameters that modify the successful incorporation into the cells.

In their study, Wu and Sagullo et al. used photothermal nanoblade to rescue Rho0 cells (cells depleted of their mtDNA) [1]. The efficiency of mtDNA delivery using this approach was found to be 2%, which, albeit still low, is 10 times that reported for microinjection. To select for cells that have successfully imported mitochondria, the researchers cultured the cells in a uridine-free-medium. Rho0 cells are pyrimidine auxotrophs, therefore only cells that have incorporated mitochondrial DNA could replicate. At the end of a 4 week selection, 3 uridine-independent clones enriched with mtDNA were obtained.

Interestingly, in spite of the replenishment of mtDNA, the bioenergetic profiles of the clones were distinct. Two clones remarkably reestablished a nucleus-encoded TCA cycle related gene expression profile and had metabolite profiles similar to the parent cells from which the Rho0 recipient cells were derived. The third clone, however, showed weak metabolic recovery with a reduced growth rate on galactose (which favors mitochondrial respiration), a reduced ATP content as well as a reduced activity in complex I, II and IV as compared to other clones and to the parent donor cells (though higher than Rho0 cells). The metabolite profile and the metabolism-related gene profile were more similar to that of Rho0 cells than to other clones or to the parent.

The cause of the heterogeneity in cells subjected to mitochondrial transfer remains to be investigated. It is possibly the result of a selection that occurred along the transfer process. More interesting, however, is the possibility that transferred mitochondria are able to reprogram the recipient cells, but some cells may lose their ability to make use of their nuclear encoded mitochondrial genes while adapting to the lack of functional mtDNA. Such a phenomenon may represent a case in which patients with a high rate of heteroplasmy of mutant mtDNA with impaired respiratory function cannot benefit from mtDNA transfer and heteroplasmic shift as correcting mtDNA alone is not sufficient to restore their respiratory capacity. It also is reminiscent of the situation in which ketogenic diets are detrimental to patients with near homoplasmic states [2]. If nuclear gene expression happens to be irreversibly altered during mitochondrial diseases, therapy might prove to be a more challenging task than previously thought, requiring an intervention to restore nuclear DNA expression as well.

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Commentary

To conclude, photothermal nanoblade appears to be a valuable technology for transferring mitochondria in a controlled and specific manner. Wu and Sagullo et al. have used it to recover Rho0 cells [1]. It remains to be used in cells that have mtDNA in which the donor mitochondria will have to compete with the host mitochondria and where their capability to change the host metabolic profile and nutrient preferences may prove useful.

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