Mitochondrial gene therapy by allotopic expression is gaining momentum

Sir: Imitation is undoubtedly the sincerest form of flattery, so I am of course most gratified that Drs. Owen and Flotte (7) have reproduced so faithfully—albeit without citation—my proposal (2-4) for using inteins to overcome the hydrophobicity barrier to allotopic expression of normally mtDNA-encoded proteins. This idea, originally considered wildly ambitious, has also been considered favourably in another recent review (11); let us hope that it is attempted soon.

However, Owen and Flotte’s treatment of this approach contains a number of important inaccuracies, as well as omissions of recent developments, which I would like to take this opportunity to summarise. Firstly, I should save your readers the labour of wading through the 766 PubMed entries matching the word “allotypic”, since doing so will not enlighten them on the subject of Owen and Flotte’s review. Second, the complementation of Complex I defects by the yeast NDI1 gene (10) is potentially a vital component of gene therapy for mtDNA mutations as well as for those in nuclear-coded subunits, because four of the six human mtDNA-encoded proteins never yet found nuclear-coded in any species are Complex I subunits. We must hope that efforts to construct NDI1 transgenic mice will soon succeed, allowing the testing of its in vivo ability to complement such mutations. Thirdly, Owen and Flotte’s objection to import using split inteins (namely, that the N-extein’s import would not be facilitated) relies on the curious assumption that the intein must be located C-terminal of the first unimportable domain of the mature protein. This is avoided by my proposal (2-4) to engineer inteins within such domains, since their bifurcation should robustly prevent tight folding.

In reporting their failure to import even small segments of ATPase 6 and NAD6, Owen and Flotte are guilty of overinterpreting a negative result. The most important recent development in this area is unequivocally the report (12,11) of robust phenotypic rescue of an inactive ATPase subunit 6 by a nuclear transgene in a mammalian cell culture system. Zullo et al. did not follow Owen and Flotte’s strategy—which, incidentally, has also failed in others’ hands (5)—of using the leader sequence of a hydrophobic, nuclear-coded mitochondrial protein; they instead used that of ornithine transcarbamylase. By performing their experiment in CHO cells, in which a mutation in ATPase 6 has been isolated that confers resistance to oligomycin (1), they were able to demonstrate unambiguous phenotypic rescue by including this mutation in their transgene and observing only transient inhibition of growth by an oligomycin concentration that rapidly terminated the proliferation of untransformed controls.

Another key advance has been made by Pérez-Martínez et al. (8,9): the cloning of COX (cytochrome c oxidase) subunits 2 and 3 from Chlamydomonas reinhardtii. The chlamydomonas are apparently unique among plants in having experienced just as strong evolutionary pressure as animals to move mitochondrial genes to the nucleus; since plants lack the evolutionarily insurmountable obstacle of a variant mitochondrial genetic code, this has resulted in six of our 13 protein-coding mitochondrial genes being successfully transferred. The sequences of these genes, now that they are at last becoming known, may well lead rapidly to more rational design of transgenes for allotopic expression. Particularly remarkable is that the C. reinhardtii COX 2 “subunit” is encoded by two genes, one for its N-terminus and one for the C-terminus, with extensions that are absent in other species and are hypothesised to promote binding of the two proteins into broadly the standard COX2 tertiary structure (9). This bears an astonishing
resemblance to the split-intein proposal (2-4); moreover, it may in some cases be easier to engineer than a system involving rearrangement of peptide bonds.

Taken together, these developments call for an even greater degree of optimism with regard to the medium-term potential of allotopic expression than I (2-4) or Drs. Owen and Flotte (7) have previously expressed. Though first suggested as a therapeutic strategy as long ago as 1990 (6), it has been sorely under-funded hitherto, presumably on account of its perceived infeasibility. That argument is crumbling; the floodgates of intensive work on this technology are opening. Allotopic expression is a therapy whose time for development, albeit not yet application, has finally come.


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