Response to A. de Grey

Dear Editor:

We appreciate the comments by Dr. de Grey with regard to our recent review article, and we regret the omission of a citation to his own recent review on the topic in which he speculates about the possibility of using inteins to circumvent difficulties with import of hydrophobic mitochondrial enzyme subunits. Although we attempted to be as inclusive as possible of relevant original research articles and some reviews, we unfortunately omitted his article, which we agree is very relevant. However, we are puzzled by Dr. de Grey's implication that our review was written in imitation of his own, because we have cited others in the field whom he did not cite, including individuals who have generated original data relevant to these points. It is also difficult to understand how we could be co-opting his ideas and simultaneously disagreeing with him as to the likelihood of success of intein-mediated correction.

As we pointed out in our article, the intein approach has been described by several researchers in the field, and was experimentally tested by Dr. Williams' group in an article, which we did cite (1). As we also noted, that study showed less than optimal results. In the absence of additional experimental data, it is difficult to determine whether or not Dr. de Grey's optimistic view of intein-mediated correction is justified. Our more conservative view was based both on Dr. Williams' data and on our own data suggesting that import of even relatively short hydrophobic domains of mtDNA-encoded proteins was inefficient. Although Dr. de Grey may be right in postulating that short hydrophobic stretches could be imported more efficiently if separated by inteins, there are potential problems with such an approach. One potential problem for the N-terminal extein would be that if the presequence alone were present upstream of even a shortened hydrophobic segment, it might still be trapped while transiting the membrane. This problem might not particularly be dependent upon whether the intein were split or intact, nor upon whether the intein followed the hydrophobic segment or occurred within the segment. Once again, in the absence of data, it is impossible to assert with certainty that this approach would be successful. We share Dr. de Grey's hope that it would be.

Dr. de Grey also takes exception to our use of the term "allotypic" as opposed to his use of the term "allotopic." His term implies simply a different *location* for transcription of the genetic material; our term implies a different *type* of transcription, namely one that employs the universal genetic code and mammalian codon usage. We would assert that either descriptive term could apply, but we acknowledge that allotopic is the favored term in the field.

Finally, we recognize that our review was not all-inclusive and that Dr. de Grey's letter has included a number of references that are worthy of recognition. We extend our regrets to those authors as well.

REFERENCE

1. Williams LR, Ellis SR, Hopper AK, Davis EO, and Martin NC. Splicing before import—an intein in a mitochondrially targeted preprotein folds and is catalytically active in the cytoplasm in vivo. *FEBS Lett* 476: 301–305, 2000.

Address reprint requests to:
Terence R. Flotte
Renius Owen, IV
University of Florida

E-mail: flotttr@gtc.ufl.edu

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