TAT Opens the Door

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Mitochondria are complex organelles that perform many key activities in the cell. They contain multiple copies of their own genome, which encodes 13 proteins in humans and the transfer RNAs and ribosomal RNAs needed to translate them.[1] Thus, almost all of the hundreds of proteins needed for their function must be encoded in the nucleus and imported across the mitochondrial membranes. Within the mitochondria they undergo proteolytic processing and targeting to multiple compartments and enzyme complexes.[2] There are numerous diseases arising from mutations or defects in either the mitochondrial genome or those nuclear encoded and targeted proteins. These disorders typically have serious health consequences, and there are currently no cures for these mitochondrial disorders.

Repair of mitochondrial disorders is necessarily more complex than replacement of a cytosolic gene product and must take into account not only the need to target and cross multiple membranes in mitochondria but also the fact that many enzymes in mitochondria are hydrophobic and not readily soluble. Additionally, many of the mitochondrial gene defects cause severe neurologic symptoms as the primary, or most prominent, phenotype, and drug delivery across the blood–brain barrier is notoriously difficult.[3] As a result, current therapies for mitochondrial defects focus primarily on the use of small molecules to enhance flux through the electron transport chain, such as with coenzyme Q for OXPHOS diseases,[4] or altering the precursor pool of substrate to avoid the defective metabolic pathway, such as changing dietary intake of fatty acids to avoid medium-chain lipids in medium-chain acyl-dehydrogenase deficiency.[5] Thus far, neither gene therapy nor enzyme-replacement therapy (ERT) has been accomplished for mitochondrial defects, much less to correct a defect across the blood–brain barrier. However, in this issue of *Molecular Therapy*, Rapoport *et al.* seem to have taken an important step toward this difficult goal.[6]

In their report the authors use the protein transactivator of transcription (TAT) domain to deliver lipoamide dehydrogenase (LAD) to mitochondria in fibroblasts from patients suffering from LAD deficiency. Rapoport and coworkers convincingly demonstrated that TAT could transduce the LAD protein across the cell and mitochondrial membranes. Furthermore, this transduced enzyme appears to be able to replace the defective enzyme in a large multisubunit complex to restore near-normal enzymatic function. LAD is the third catalytic subunit (E3) of three multicomponent enzymatic complexes (α-ketoacid dehydrogenase complexes) in the mitochondrial matrix that are crucial for the metabolism of carbohydrates and amino acids.[7] The α-ketoacid dehydrogenase complexes consist of pyruvate dehydrogenase complex, α-ketoglutarate dehydrogenase complex, and the branched-chain ketoacid dehydrogenase complex.[7,8] A mutation of the LAD gene leads to alteration of the normal activity of α-ketoacid dehydrogenase complexes, resulting in lactic acidemia, dysfunction of the Krebs cycle, and impaired branched-chain amino acid degradation. These are serious metabolic disruptions, and patients with this disease can present with severe neurological symptoms in infancy, or recurrent episodes of liver failure or myoglobinuria that can be fatal.[9,10] Thus, there is a significant human health impact to developing ERT for this disease, as well as establishing a model for other mitochondrial disease therapies.

TAT is a short cationic peptide derived from the larger TAT protein of HIV that has cell-penetrating properties.[11–13] Cell-penetrating peptides are typically small cationic peptides that can transport a cargo of molecules, such as proteins, peptides, or oligonucleotides, into cells that otherwise could not absorb large-molecular-weight compounds. Various transduction peptides such as polyarginine, synB5, antennapedia, and herpes simplex virus type I protein have been used to transport complex proteins into cells and have thereby shown promise as therapeutic strategies for several disease conditions.[14–17] This is important because delivery of drugs and therapeutic compounds is primarily limited by their ability to penetrate the cell membrane. The bioavailability of compounds targeted to intracellular sites depends on the conflicting requirements of being sufficiently polar for administration and distribution, yet nonpolar enough to diffuse through the nonpolar lipid bilayer of the cell.[3] In addition, the molecular weight of most drugs that can easily traverse the lipid membrane is approximately 500 Da.[18] Thus, most successful compounds have narrow physical characteristics. Many promising drugs fail because they fall outside this range, and efforts to make them available may be toxic. In addition to this, many sites of action for presumed therapeutic compounds, such as enzymes or regulatory proteins, require processing and targeting of the compound once inside the cell. This step alone represents a significant hurdle to the development of many strategies to repair defects within a cell. Cell-penetrating peptides offer exciting potential to overcome many of these problems for ERT.

Rapoport *et al.* took advantage of the native mitochondrial targeting sequence for LAD (TAT-LAD)[7] and showed that it was necessary for maximal restoration of LAD enzymatic function. Deleting the mitochondrial targeting sequence (TAT-Δ-LAD) restored a significantly smaller amount of LAD activity within the mitochondria. This is a subtle but important point to consider: TAT can move both ways across a membrane and thus pull the therapeutic cargo out of the mitochondria. With the mitochondrial targeting sequence included,

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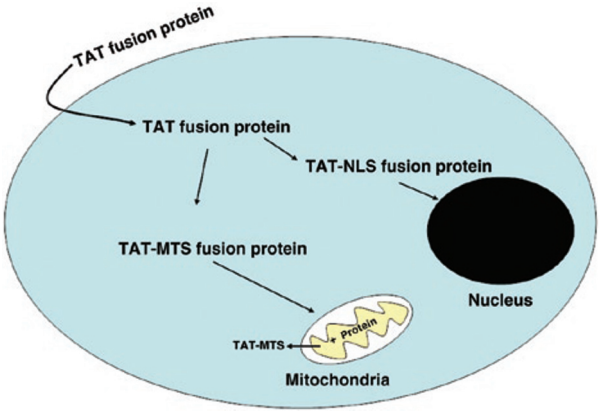

Figure 1 Site-specific delivery of TAT-conjugated proteins. A TAT fusion protein containing either a mitochondrial targeting sequence (TAT-MTS fusion protein) or a nuclear localization signal (TAT-NLS fusion protein) can transduce into a cell. If the protein transduces into the mitochondria and contains a MTS, the mitochondrial processing peptidases will recognize the MTS and clip it, leaving the protein in the mitochondrial matrix. The TAT-MTS peptide will transduce out of the mitochondria. Although the nucleus does not have processing peptidases that recognize the NLS sequence, the presence of this sequence should increase the frequency of transduction of the TAT fusion protein into the nucleus.

the matrix processing peptidases recognize the sequence and clip it, and the cargo (mature LAD) is left in the matrix while the TAT peptide can transduce out of the mitochondrion (Figure 1). Repeated dosing should therefore result in accumulating amounts of cargo in the mitochondria over time. A similar strategy might be used for other organelles, such as the nucleus, where incorporation of a nuclear localization signal (NLS) would increase transduction of a TAT fusion protein into the nucleus.

TAT is capable of transducing single components of very large enzyme complexes to restore activity of the native complex. The authors clearly demonstrate that TAT-LAD can substitute for the mutated LAD enzyme within the pyruvate dehydrogenase complex and restore its activity to almost normal levels. Proteins are normally imported into mitochondria in denatured form,[19] and mitochondria are thus well equipped to refold proteins into an active configuration for incorporation with larger enzyme complexes. Because cell-penetrating peptides depend on the fusion protein's being sufficiently denatured to make the penetrating peptide available to initiate transduction, protein-transduction technology is perfectly suited for ERT in mitochondria.

The authors also found that it was not necessary to restore all the missing protein to restore adequate amounts of enzymatic activity in mitochondria. This is important for point mutations that have a dominant-negative impact on a larger enzyme complex. Small amounts of normal protein may be adequate to repair the defect. This concept is also important for organs in which it is typically difficult to deliver drugs, such as the brain or placenta. TAT has been shown to cross the blood–brain barrier and placenta quite well.[11,20,21] This opens the possibility of initiating ERT for mitochondrial defects before birth, or for neurological disorders that manifest well after birth.

ERT had not been accomplished for a mitochondrial defect before this new report, although it is an established therapy for other metabolic disorders, such as the lysosomal diseases of Hurler's syndrome and Pompe's disease. TAT has been used to replace missing enzymes in animal models of disease, such as purine nucleoside phosphorylase,[15] and has delivered a variety of exogenous proteins to cytosolic and mitochondrial locations. Thus, it was a logical extension of these earlier findings that TAT can deliver a mitochondrial therapeutic cargo. Rapoport and colleagues are to be acknowledged for opening the door even further for the use of TAT as a potential treatment strategy for a difficult set of mitochondrial diseases. Although protein-transduction technology is still a basic tool at this time, it is virtually certain to advance rapidly in the future as better and more efficient transduction domains are discovered and the mechanism(s) for protein transduction are better understood.

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