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Energetically Hybridized Mitochondria in a Mammal (E-HMM)

Background

Human biology is limited by our absolute dependency on oxygen. Here we propose a program to generate the first ever mammalian tissues that are able to harness an energy source that is independent of oxygen. Successful implementation of the program will improve biomedical procedures (organ transplant, surgeries, stroke recovery) and slow down the progression of aging.

One of the most conserved aspects of our physiology is how central metabolic activity supports our cellular energy demand. In each of our cells, there are small engine-like organelles called the mitochondrion. The mitochondria are fundamental to the metabolism and have shaped the evolution of eukaryotes by, at least partially, increasing the overall capacity of synthesizing ATP by utilizing oxygen. ATP is the 'energy currency of life'.

While bioenergetics sustains us, it also constrains us. The constant need for oxygen limits tissue recovery from stroke or heart failure and the ability to sustain an organ for transplantation for longer period, and it extends the duration of surgical procedures by requiring for tissue re-oxygenation pauses. The dependency on oxygen also poses meaningful logistical considerations in terms of presence and travel in space. By enabling our mitochondria to tap into a second, non-oxygen-coupled energy source, such as light, we will advance several areas of biomedicine.

Additionally, our current bioenergetics is likely limiting our health and maximal lifespan. That is because what sustains our life, comes along the production and irreversible accumulation of toxic byproducts in our cells. This accumulation, much like an engine wear and tear, leads to eventual aged and dysfunctional mitochondria. Mitochondrial dysfunction is a central hallmark of aging and chronic disease, yet most current interventions for this hallmark, ranging from caloric restriction to repurposed drugs like metformin, only marginally delay decline without reversing the fundamental bioenergetic decay that drives it. While these interventions are to be seen as 'low-hanging fruits', they have a limited potential to increase human life span, let alone human maximal lifespan. In order to meaningfully strive to extend human life span, we should explore new approaches that would alter the basic aspects of our physiology. Despite significant investment in longevity biotech, the field remains constrained by incrementalism.

Why now?

The convergence of novel technologies makes organelle-level metabolic engineering feasible for the first time. Among them are synthetic biology, mitochondrial genome editing, AAV-mediated in vivo transfection, scalable CAR-T manufacturing and ATP-independent nano-lantern bioluminescence which is paving the way to introduce energy source such as light in vivo. Additionally, preliminary scientific work has demonstrated that synthesizing ATP in mitochondria via light can increase the lifespan of worms and benefit the health of flies, thus providing a rationale to translate these advances to mammals.

Need for Investment

No commercial entity will bridge these disparate technical domains into integrated systems biology without de-risking the fundamental feasibility question first. Academia often lacks the funding or risk tolerance to pursue radical physiological re-engineering, while startups and large companies tend to prioritize low risk returns over foundational breakthroughs. Consequently, there is a critical innovation gap: no program has successfully reimagined energy metabolism at the organelle level to fundamentally upgrade mammalian bioenergetics.

The program and milestones

To establish a strong scientific basis for potential human experiments, the program will focus on developing the first mammal tissues that are capable of harnessing a secondary energy source in its mitochondria.

This will be achieved via two parallel technical areas aimed to design and deliver of synthetic mitochondria and secondary power source into a mouse respectively. The following goal is to integrate the two technical areas into a functional in vivo model.

1. **Design of synthetic mitochondria and its delivery into a >25% of the cells mouse or tissue: Successful integration of synthetic upgrade into the mitochondria, coupled with the** development of an efficient whole body delivery of such mitochondria that harbor functional ability to absorb alternative power source to produce ATP independently of oxygen **(18 months, €14mil)**.
2. **Introduction of the power source inside a 3D body:** Establishing a method to distribute the activating energy in vivo for a period >7 days **(18 months €5mil).**
3. Demonstrating that the power source can be energetically **transduced into synthetic mitochondria** for continuous >30 days with 10% synthetic ATP generation **(18 months, €12mil)**.
4. **Exploring the health benefits** of activating synthetic mitochondria in a mouse **(6-18 months €6mil)**.

1. **Design and delivery of synthetic hybrid mitochondria into a mammalian tissue**

The program will develop a strategy to introduce a novel function into mammalian mitochondria that enables the harness of alternative (non-oxygen dependent) power source to synthesize ATP. This effort will combine the expertise of genetically engineering the mitochondrial DNA and the optimization of a whole body or tissue mito-transplant of such mitochondria. Overall, our first goal will be focused on establishing a pipeline to quality control the transformation of a mammalian mitochondria into a synthetic one, prior to in vivo mito-transplantation. The following step is to successfully transplant the synthetic mitochondria into as many mouse tissues or organs are possible with a minimum threshold of 25% of the targeted cells.

2. **Introduction of secondary power source inside a 3D body**

Here the program will focus on the molecular design that will carry a secondary power source such as light (for example nano lantern) or other. Such power source should be sustained for a period of no shorter than one weeks in a measurable manner. It is expected to establish a protocol that would be ultimately feasible for long chronic activation.

3. **Inner body activation of photosynthetic mitochondria**

Combining the advances made possible by Task Areas I and II, the program will test the successful and continuous flow of energy transduction in vivo from a power source. The activated power source should be able to generate at least 10% of the ATP needed in the target tissue. This step will serve as proof of principle that synthetic mitochondria can harness the energy in the inner body tissues.

4. Health benefits of E-HMM

As part of the potential transition of the program, the performers will assess the benefits of using hybrid mitochondria in further sustaining and prolonging the preservation of organs in que for transplantation. Similarly, the program will explore the potential of such mitochondria for the optimization of surgeries where reoxygenation is a limiting factor and recovery from stroke or heart attack. In parallel, the program will aim to test whether the hybrid mitochondria can be a potential therapy for treating metabolic diseases of patients.

As part of its vision, this program will assess whether synthetic mitochondria can provide health benefits to a mammal. While a life span improvement would indicate a strong benefit, the program will first assess, within the proposed timeframe, potential improvements of frailty or attenuating age-associated phenotypic alterations. For example, regrowth of hair loss or improved frailty in aged mice can provide evidence for improved metabolic activity.

Impact

Overall, with the emergence of new synthetic technologies, it is the right time to bring together various concepts from different fields and combine them into altering and upgrading the manner that our central metabolism provides the energy for our cells for the last hundreds of millions of years. The program will bring to life the first semi-photosynthetic human organ and mouse. The resulting impact will serve as a basis to the much-awaited next generation human therapy capacities such as improved surgical procedures and longer organ transplantation capacities – which is predicted to being implemented three years following to start of the program. Importantly, the implementation of the program's milestones will meet overreaching medical needs to treat metabolic maladies, some which currently have no viable cure, as well in basic research in enabling the activation of optogenetic proteins in inner tissues of mammals.

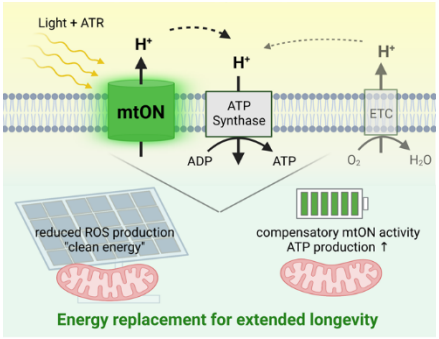
Of interest, successful implantation of the program may even impact our resilience to survive in space and open new avenues for future endeavors such as travel to Mars. Finally, as breaking the human longevity barrier of 12 decades is likely to require the fundamental alteration of basic mammalian biology, the E-HMM program will offer a new concept to extend human life span in a manner that was never test previously.

Key risks and de-risking strategy

Mitochondrial Engraftment Failure — Transplanted mitochondria rejected by cellular quality control (mitophagy), achieving <25% stable integration	Pursue parallel delivery methods (direct organelle transfer, AAV-mediated in situ modification, nanoparticle carriers) in Stage 1.
Insufficient power Flux — Internal power source generates orders of magnitude below threshold for meaningful ATP synthesis	**Power Budget Engineering**: Stage 1 establishes minimum energy requirements via titration experiments in cell culture. For example, in regard to light, parallel approaches include higher-brightness nano-lanterns, increased cell loading, and photon-concentrating structures.
Insufficient ATP Contribution — Even with functional system, photosynthetic contribution remains <10% of total cellular ATP, below threshold for phenotypic effects	**Metabolic Stress Testing**: Evaluate system under high-demand conditions where native ATP synthesis is stressed. Design experiments to detect benefits in energy-limited scenarios rather than homeostatic baseline.

Pathway Example: Using light as a power source

1) Generation and Delivery in vivo of synthetic (s-)mitochondria into a mouse: Performers should show a successful insertion of light activated proton pump (LAPP) into the inner membrane of the mammalian mitochondria along with its functionality. As part of this TA, performers are expected to show in vitro and in vivo that LAPP successfully contribute to the synthesis of ATP production and PMF and assess in various metrics what is the contribution. This TA could be explored in the following three method of delivery.



A) Introduction of LAPP gene into the genome of mammalian mitochondrion.

B) Transfection of LAPP via AVV in vitro.

C) AVV transfection of LAPP in vivo

For 1A, the performers are expected to demonstrate the integration of the LAPP gene into the mitochondrial DNA, along with detecting its expression.

For 1B, the performers will design a LAPP-AAV and transfect several mammalian cells lines.

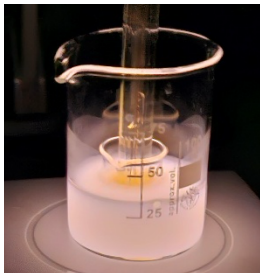
In parallel and in relation to 1A and B, mitochondrial transplant technique will be developed with the goal of transplanting s-mitochondria. Performers will refine the technical path to achieve successful transient or persistent mitochondrial transplantation in an adult mouse. It is expected that a pipeline for s-mitochondria quality control would be generated prior to any in vivo transplant.

For 1C, other teams will generate a library of AAV harboring LAPP. They will then transfect the mice or organ with these AAV and then extract the transfected tissues for in vitro testing. This TA also requires an early evaluation of the AAV transfection the LAPP activation in the eye.

In all of the approaches we expected the performers to achieve the following milestones:

i) Integration of LAPP protein in the to the inner membrane with the current orientation
ii) Light mediated ATP synthesis in vitro
iii) Expression of LAPP protein in vitro with largest tissue distribution

Additionally, other performers should seek an alternative ATP synthesis method by using magnetic, sound or other energy sources, including synthetic organelles. We expect the demonstration of proof of principle that such energy sources can be used to either facilitate the activation of LAPP or the rotation of ATP synthase directly. Successful implementation of such path will be followed by similar streamline of integration into mitochondria and proof of principle promotion of ATP synthesis.



2) Introduction of light inside a mouse: in order to optogenetically activate proteins in vivo, performers will develop new method that will result in delivering continuous light throughout the entire body of a mouse. This can be achieved for example, but not limited, via CAR-T therapy where the t-cells are harboring nano-lantern. Alternative methods are welcomed to be suggested. Overall, we expect the performers to achieve the following milestones:

i) Demonstration of generating light inside an adult mouse
ii) Activation of an optogenetic target within a mouse

It is important to also assess the potential impact of in-vivo light generation on the stability of DNA, along with the reaction of the immune system.

3) The completion of TA 1 and 2 are required to move to TA 3. Here, selected performers will integrate the successful delivery of LAPP in mitochondria in vivo and optogenetic activation of the LAPP in vivo. We expected the performers to quantify the conversion of nano lantern activity into ATP synthesis in various tissues. A new and detailed protocol should be established to the frequency and intensity of activation of s-mitochondria via establishment on the precise administration of CAT-T cells along with flurofurimazine injections.

4) Potential health benefits of LAPP in vivo activation: The primary goal of this TA is to discover the potential benefits that light activated mitochondria has on the health of human organs, human surgery optimization and recovery from stroke or heart attack. Furthermore, a secondary goal is to use the synthetic upgrade in adult mice to provide the basis for future longevity experiments. Here performers will follow TA 3 and evaluate various potential benefits of in vivo chronic activation of LAPP. While we are open to approaches, potential examples are improved histology, transcriptome profile, frailty, hair regrowth, ATP levels and other metrics of health.