

#### Pyk2 Expression and Localization in Cardiac Mitochondria and Its Role in Mitochondrial Calcium Regulation

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#### Introduction

TRPM2 is a non-selective cation channel located in the plasma membrane of the cell. Upon activation, the channel opens, allowing calcium to enter into the cytosol of the cell, leading ultimately to the phosphorylation and activation of the enzyme Pyk2 (proline-rich tyrosine kinase 2). Once phosphorylated, Pyk2 translocates from the cytosol to the mitochondria, where it regulates the formation of the pore component of the mitochondrial (MCU) calcium uniporter complex. Consequently, this interaction is a key factor in mitochondrial calcium uptake and therefore mitochondrial bioenergetics.

## 1. High levels of activated Pyk2 are found in the cardiac tissue

Total Pyk2



# 3. Pyk2 localizes to the matrix of cardiac mitochondria

Heart Extraction, Homogenization, and Differential Centrifugation

Hypothesis

We hypothesize that tonic activation of TRPM2 facilitates mitochondrial Ca<sup>2+</sup> uptake through the phosphorylation and translocation of Pyk2 and its interaction with the MCU complex. This pathway ensures the proper bioenergetics. mitochondrial cardiac pathway leads of this Disturbance to dysfunction mitochondrial the due to impairment mitochondrial calcium in dynamics.



**Proteinase K** 

(0.01µg/µL)

Results

Levels of total Pyk2 and P-Pyk2 were compared in the total homogenate fraction of heart and thymus via Western Blot. Despite the low expression of Pyk2 in the heart, a high proportion of Pyk2 is phosphorylated and therefore activated.



### 2. In the heart, activated Pyk2 is preferentially located in the mitochondria





An accessibility assay was performed to determine the specific localization of Pyk2 within the mitochondria. Increasing amounts of digitonin were added to Percoll purified mitochondria along with a fixed amount of proteinase K.

The results were compared to known matrix proteins. The comparison between HSP60 and Pyk2 demonstrates that Pyk2 is located in the matrix of the isolated cardiac mitochondria.

<u>Aim:</u> To determine the role of Pyk2 in regulation of mitochondrial Ca<sup>2+</sup> uptake via MCU complex phosphorylation and oligomerization

Total CardiacCardiacHomogenateMitochondria

Mitochondria were isolated from cardiac tissue via differential centrifugation. Levels of total Pyk2 and phosphorylated (activated) Pyk2 were compared via Western Blot. Data shows a preferential localization of activated Pyk2 in the mitochondria of cardiac tissue. Conclusion: In the heart, Pyk2 is localized to the mitochondrial matrix, where it can consequently regulate mitochondrial calcium uptake.

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