１０６ 年 科 技 部 補 助 大 專 學 生 研 究 計 畫

研究計畫摘要表(C802)

# Information Transduction Capacity of Mitochondrial Retrograde Signaling


邱紹庭

**Supervisor: Dr. 魏安祺**

**National Taiwan University**

## ABSTRACT

Mitochondrion is the powerhouse of the eukaryotic cells, which participates in crucial cellular processes such as ATP production and intermediate metabolism. Though mitochondria possess their own genome, most of the mitochondrial proteins are encoded in nucleus. The mitochondria-nucleus communication network is essential for the mitochondrial quality control which includes removing damaged mitochondria and fission-fusion dynamics. However, molecular noise restricts the ability of the nucleus to decode input signals of different strengths and obtain information about the mitochondrial quality. In the language of information theory, nucleus receives mitochondrial quality information via the noisy channel. An interesting question is how much information can be transferred through mitochondria-nucleus biochemical pathway? To say, how many mitochondrial statuses can be detected by nucleus via the noisy biochemical channel? In order to answer this question, multiple concentrations of mitochondrial uncoupler will be used to make mitochondria dysfunctional by reducing mitochondrial membrane potential ($\Delta\psi m$). The channel capacity, a standard information-theoretic calculation, will be obtained from the input signal (mitochondrial membrane potential) and output signals (expression levels of retrograde-responsive genes). *Saccharomyces cerevisiae* will be used as a tool to study mitochondrial retrograde signaling.



Keyword: retrograde response, mitochondrial membrane potential ($\Delta\psi m$), information theory

- **RESEARCH MOTIVATION**

Mitochondrial defects have been associated with many human diseases, especially in the diseases related to neuron degeneration or aging[1]–[4]. My previous research reveals that the aged mitochondria from replicatively aged cells have destructive impact on the replicative lifespan of cells in *Saccharomyces cerevisiae*. These senescent cells tend to possess dysfunctional mitochondria.

Besides, mitochondrial retrograde response is essential for maintaining mitochondrial function and genome stability [5], [6]. However, most of the mitochondrial genes are encoded in nucleus, the decision related to mitochondrial quality control depends on the transmission of the information about mitochondrial environment via noisy biochemical signaling. The biochemical noise results in the biological variability which constrains the information attained by biochemical networks as well as influences the cell decision[7], [8].

In this project, the main question is about the channel capacity of the RTG pathway (Fig 1.), one of the mitochondrial retrograde pathway[9], [10], in baker's yeast— *S.cerevisiae*. The quantitative measurement and calculation based on information theory will be applied to answer the quantitative amount of information transmitted via RTG pathway per channel use (Fig 2.). Moreover, the details in circuit control characteristics will be explored based on the modified expression levels of proteins related to RTG pathway.

- **INTRODUCTION**

Mitochondria retrograde signaling is a pathway of communication from mitochondria to the nucleus that influences many cellular activities[11]. The most detailed information in the network of mitochondrial retrograde response is RTG pathway (Fig 1.) which is first discovered as a retrograde-responsive network in *S. cerevisiae*[12]. Three positive regulatory factors, Rtg1p, Rtg2p, and Rtg3p, and other four negative regulatory factors included Mks1 participate in RTG pathway[13].

Mitochondrial membrane potential ($\Delta\psi m$) is related to oxidative energy metabolism. It is also a indicator of mitochondrial function, which can be measured by tetramethylrhodamine methyl ester (TMRM) and using fluorescence imaging and voltage clamp[14].

Molecular noise restricts the ability of cell to gather information from input[8], [15], [16]. The channel capacity is a theoretical calculation in information theory, which describes the amount of information can be transmitted per channel use[17]. The mathematical properties of noisy channel

are first applied in communication systems[18], [19]. The information transduction capacity of noisy biochemical signaling networks has been analyzed in TNF—NF-κB pathway[7]. They found that the bottlenecks likewise constrain information attained by networks signaling through multiple genes. Furthermore, the resulting channel capacity is around 1 bit which is theoretically enough for binary decision.

Before going into details, it is important to introduce some definitions and properties related to this project in information theory.[17]:

(1) Shannon's information[18]

$$\mathrm{H(X)} = -\sum_{x \in X} P(x) log P(x)$$

where X is a random variable.

(2) Mutual Information

A. Mutual information is symmetric.

$$\begin{aligned} \mathrm{I(X;Y)} &= \mathrm{H(X)} - \mathrm{H(X|Y)} \\ &= \mathrm{H(Y)} - \mathrm{H(Y|X)} \\ &= \mathrm{I(Y;X)} \end{aligned}$$

where X and Y are random variables

B. $\mathrm{I(X;Y)}$ is a concave function of $P_X$ for fixed $P_{Y|X}$.

(3) Channel Capacity

A. For a discrete memoryless channel (X, $P_{Y|X}, Y$), the maximum rate of information transmitting with vanishing error probability is

$$\mathrm{C} = max_{P_X(.)} I(X;Y).$$

called channel capacity (C)

B. Feedback does not affect the channel capacity.

## ● MATERIALS AND METHODS

*Yeast strains, growth conditions*

*Saccharomyces cerevisiae* W303 (ura3-1 ade2-1 his3-11,15 Leu2-3,112 trp1-1 can1-100 ho::HPH ( hygromycin B )). Cells will be grown at 30 degree Celsius in YPD or CSM. Different concentrations of mitochondrial uncoupler will be added to reduce mitochondrial membrane potential $(\Delta\psi m)$**.** Deletion strains related to RTG pathway, overexpression and reduced expression of Mks1, Rtg3, Rtg1, Rtg2. Fluorescent protein labels of CIT2, DLD3 and other genes related to RTG pathway response.

*Mediums & Buffers*

(1) Yeast Extract Peptone Dextrose Medium (YPD)

(2) Complete Supplement Mixture (CSM)
(3) Phosphate buffered saline (PBS)

*Chemicals*

(1) Potentiometric dye tetramethyl rhodamine methyl ester (TMRM)
(2) Uncoupler SF6847[14]

*Mitochondrial Membrane Potential Measurement*

(1) The $\Delta\psi m$ of cells will be marked by potentiometric dye tetramethyl rhodamine methyl ester (TMRM).
(2) Mitochondrial membrane potential($\Delta\psi m$)—single cell measurement

The distribution of $\Delta\psi m$ in time series will be first measured by confocal microscopic with TMRM marker after adding the mitochondrial uncoupler. This experiment is aimed to solve the main question —are the mitochondria homogeneous under the influence of the mitochondrial uncoupler?

In order to simplify the model (Fig 2.), both response of the RTG pathway and $\Delta\psi m$ will be measured by cell flow cytometry under the conditions of the homogeneous signal inputs. The homogeneity will be confirmed by confocal microscope.

*Numerical Computations of Mutual Information*

The mutual information of two discrete random variables can be computed by using the standard formula[17].

$$I(R;S) = H(R) - H(R|S)$$

where $H(R) = -\sum_R P(r) log P(r)$ and I(R;S) denotes the mutual information. Here, R denotes the probability distribution of RTG pathway response and S denotes the probability distribution of mean $\Delta\psi m$ value in single cell.

For finite data, bias likewise contaminates estimates of the maximum mutual information. Bias can be corrected by applying the following equation.

$$I_{biasd} = I_\infty + \frac{a_1}{N} + \frac{a_2}{N^2} + \ldots$$

where $I_{biased}$ is the biased estimate of the mutual information, $I_\infty$ is the unbiased estimate of the mutual information, N is the total number of samples, and the $a_i$ are coefficients that depend on underlying distribution of the signal and the response. when N is sufficiently large, terms of second order or larger are negligible. The jackknife algorithm will be used for variance and bias estimation.

*Computing the channel capacity*

The capacity C of the discrete channel (S, $P_{R|S}$, R) is given by

$$C = max_{P_S(.)} I(R;S)$$

the maximization over $P_S(.)$ stands for choosing the best possible input distribution so that the amount of information transfer is maximized. The Matlab's function fmincon will be used to calculate the minimum value of –I(R;S) because -I(X;Y) is a convex function of $P_S$ for fixed $P_{R|S}$.

- **EXPECTED RESULTS**

(1) The Distribution of $\Delta\psi m$ in Single Cell (Fig 3.)

The distribution of $\Delta\psi m$ in single cell will be obtained by measuring the fluorescence of TMRM.

Under the normal condition, the distribution is possibly unimodal with small variance which is eligible because of the mitochondrial fision-fusion[20], [21].

After adding the mitochondrial uncoupler which contributes to decreased $\Delta\psi m$, the polarization may occur due to the decreased fusion rate of mitochondria with low $\Delta\psi m$[22] and heterogeneity of uncoupler concentration in cell. Besides, I expect the steady state of $\Delta\psi m$ become homogeneous and unimodal after adding the mitochondrial uncoupler (Fig 3.).

The unimodal distribution of $\Delta\psi m$ can simplify the mathematical model of input signal. It is important to keep mitochondria homogeneous when measuring input signals in the cell flow cytometry.

(2) The Channel Capacity of RTG Pathway

The mean value of $\Delta\psi m$ in single cell is taken as input signal (S) of the network, and the fluorescent intensities of genes related to retrograde response are taken as response (R).

There are two variability in the measurement: biological variability and experimental variability. the experimental variability of the fluorescence is determined by measuring the direct fluorescence and immunofluorescence. If the channel capacity is 1 bit, a probable value, which means the information is enough for binary decision[23]. The qualitative amount of information depends on the input and output measurement.

(3) The Channel Capacity of Modified RTG Pathway

The influence of specific protein related to network on the channel capacity can be measured by the same method as (2) with genomic modified strains. Some negative feedback can reduce the noise and achieve channel

capacity[7]. The deletion of negative feedback may decrease the channel capacity of RTG pathway.

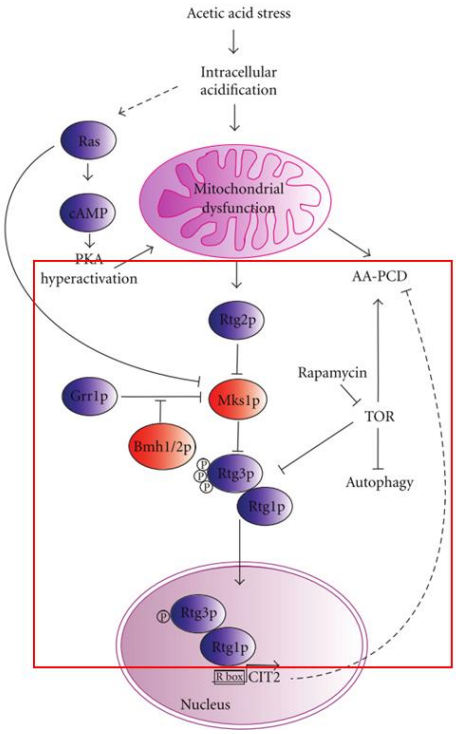


**Fig 1. Diagram of the RTG signaling pathway (Red block) and related pathway represented in biochemical form.(modified figure from L. Laera et al. [25])**

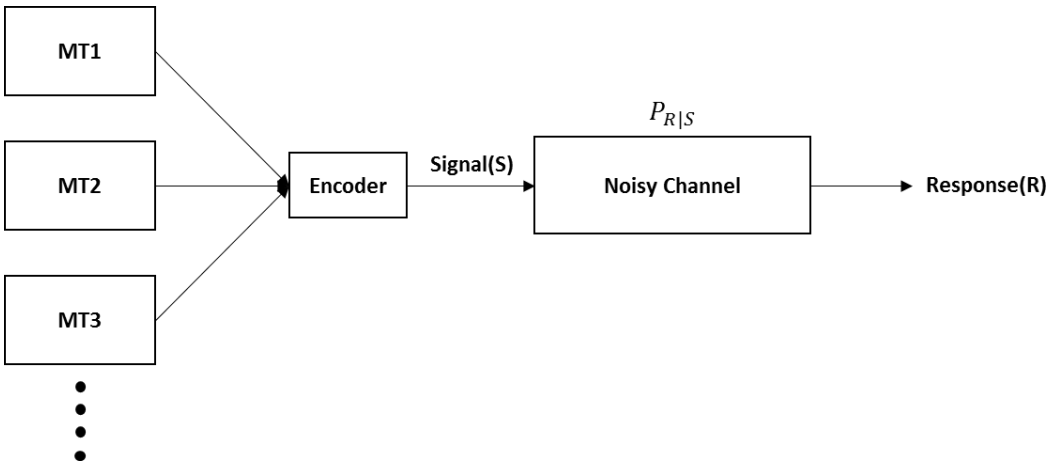


**Fig 2. Diagram of the mitochondrial retrograde signal as a noisy communication channel. (MT denotes mitochondria)**

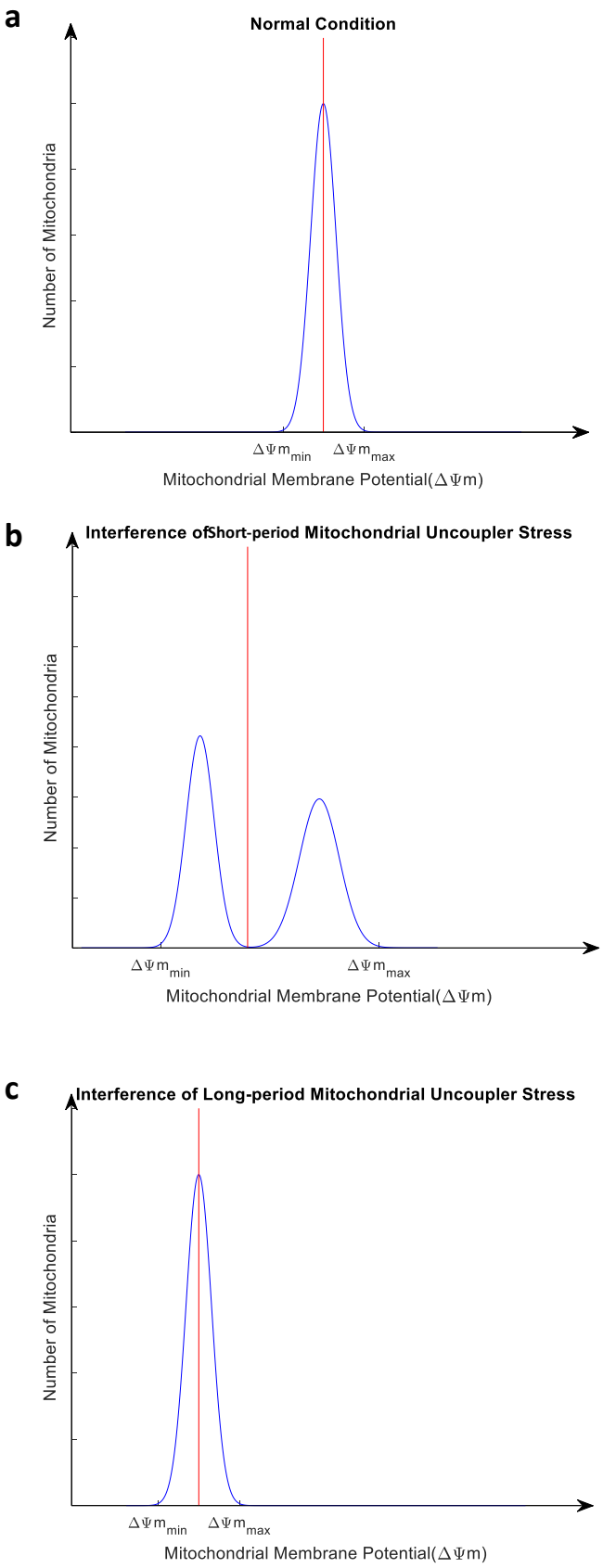


**Figure 3. Expected distribution of mitochondrial membrane potential. (a) Distribution of $\Delta\psi m$ in normal condition where cells grow without mitochondrial uncoupler. The expect distribution is unimodal with small variance due to mitochondrial fission-fusion [20], [24] (Red line marks the mean value) (b) Distribution of mitochondrial uncoupler influenced $\Delta\psi m$ in short period. Here, I expect the polarization will occur and result in not only bimodal distribution but decreased mean value because low-$\Delta\psi m$ decreases the rate of fusion process[22] and the uncoupler is heterogeneous in cellular environment. (c) Distribution of mitochondrial uncoupler influence on $\Delta\psi m$ in long period. The expected variance decreased because of mitochondrial fission-fusion[20].**

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- **需要指導教授指導內容**
  (1) Experimental Design
  (2) Mathematical Modeling