



## Parallel Session - Methodological Developments for Systems Biology

### 1PS2-03 Information-theoretic causality predicts proteomic control of clinical parameters and reveals cancer network biomarkers

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**Keywords** Systems Medicine, Causal Networks, Cancer, Genotype to phenotype, Reverse Phase Protein Array, Tissue Microarray, Multiscale networks, Information theory, Graph theory, Precision medicine

We developed an algorithm for causal network inference (Gabi) to connect genotype with phenotype, tailored for Systems Medicine applications – where available datasets typically contain tens to hundreds of observational variables. Indeed, systematic perturbation approaches are not applicable in a clinical setting. Gabi successfully integrates clinical observations with Reverse Phase Protein Array (RPPA) or Tissue Microarray (TMA) datasets. The algorithm includes a novel relevance thresholding procedure, facility to incorporate prior knowledge, and information-theoretic directionality inference that employs conditional mutual information. Evaluation on blind test data found that Gabi outperformed existing state-of-the-art approaches in terms of both network connectivity and directionality inference. Available as an easy to install R package, Gabi also offers a novel strategy to obtain directed, signed networks for downstream systems biology applications.

We applied Gabi to derive a causal information-flow network for invasive hormone-driven breast tumours ( $n=284$ ) with 137 nodes and 2101 edges; nodes represent eleven clinical parameters, 106 proteins and twenty phospho-forms. Proteomic measurements, including post-translational modifications, are key for molecular dissection of cancer mechanisms. Findings include a switch involving the estrogen receptor (ERalpha) and its phosphorylated form, which had opposing regulatory effects on many common targets. For example, ERalpha drove growth and increased adhesion, whereas phospho-ERalpha activated Epithelial to Mesenchymal Transition. Gabi predicted proteins that control important clinical parameters (e.g. tumour stage). Analysis of our causal network identified patient risk groups that clearly stratified by overall survival. Multivariate modeling controlling for clinical variables demonstrates that the network-based risk groups have significant prognostic value.

### 1PS2-04 Understanding the System Dynamics of Mitochondrial Retrograde Signaling from a Differential Equation-based Framework

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**Keywords** Mitochondrial retrograde signaling, Differential equation-based network, Multiple-input single-output System (MISO)

Mitochondrial quality control is essential to maintain cell viability. Retrograde signaling is the process in which mitochondria send quality information to regulate the nuclear genome. Moreover, mitochondria communicate with nuclear genome via biochemical networks; the nucleus responds properly depending on the signal sent by multiple mitochondrial agents, making retrograde signaling a multiple-input-single-output (MISO) problem in communication theory. However, it is unclear how the cell extracts multidimensional mitochondrial information from dynamical concentration patterns of signaling molecules. To address this issue, we used budding yeast, *Saccharomyces cerevisiae*, as a model organism to investigate the communication between the mitochondrial network and nucleus. Mitochondrial membrane potential and translocation of Rtg3p/Rtg1p are considered to be the input and output of the communication system. The mathematical model of yeast retrograde signaling was constructed by a differential equation-based framework; the parameters of the model were generated by Monte Carlo Method and fitting with the translocation data of Rtg proteins. In this study, we have provided a control theory view of mitochondrial retrograde signaling that may lead to a better understanding of the intracellular communication between the mitochondrial network and the nucleus genome.

# **Understanding the System Dynamics of Mitochondrial Retrograde Signaling from a Differential Equation-based Framework**

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National Taiwan University, Taiwan**

**ICSB 2019, Japan**

# Mitochondrial Retrograde Signaling

- Mitochondrial quality control requires **retrograde signaling** to interact with the nucleus genome.
- Multiple mitochondria send information via the same channel – A case of **multiplexing problem**.

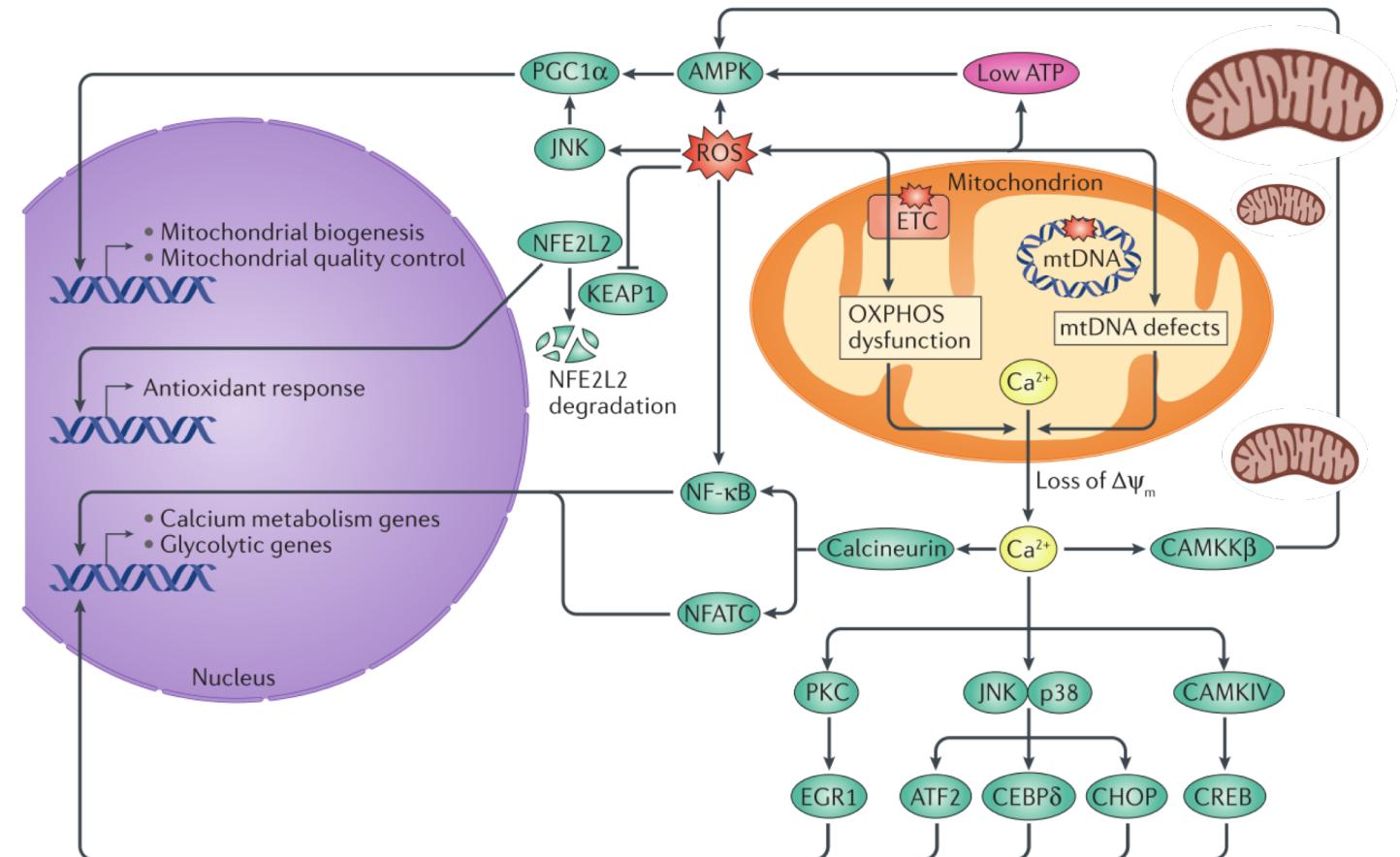


Fig1. Mitochondrial retrograde signaling.\*

\*Figure from Pedro M. Quirós et al., 2016

# Mitochondrial Retrograde Signaling in Yeast- RTG Pathway

- **Yeast** possesses relatively simple mitochondrial retrograde signaling model.
- **Rtg1 and Rtg3**: are transcription factors
- **Bmh/Mks** modulates the signal
- **Rtg2** is the sensor of the mitochondrial quality.

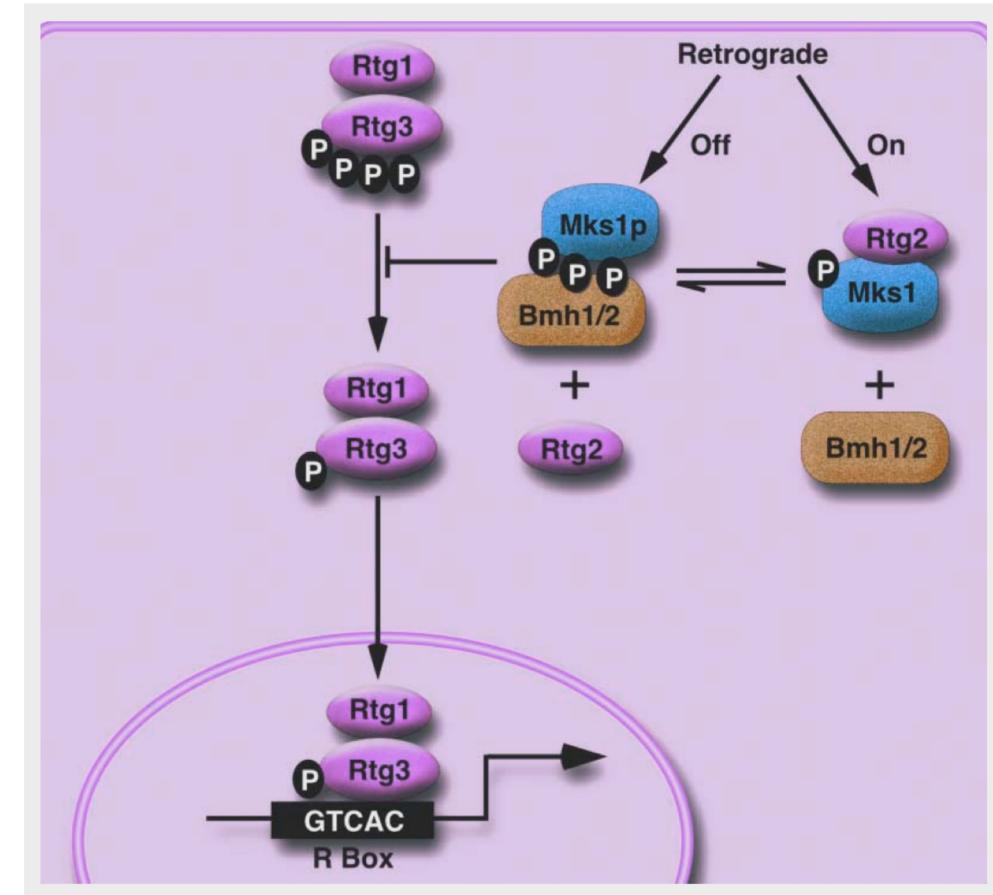


Fig2. Mitochondrial retrograde signaling in yeast\*.

\*Figure from Ronald A. Butow et al. 2004

# Properties of Retrograde Signaling – A Qualitative Approach

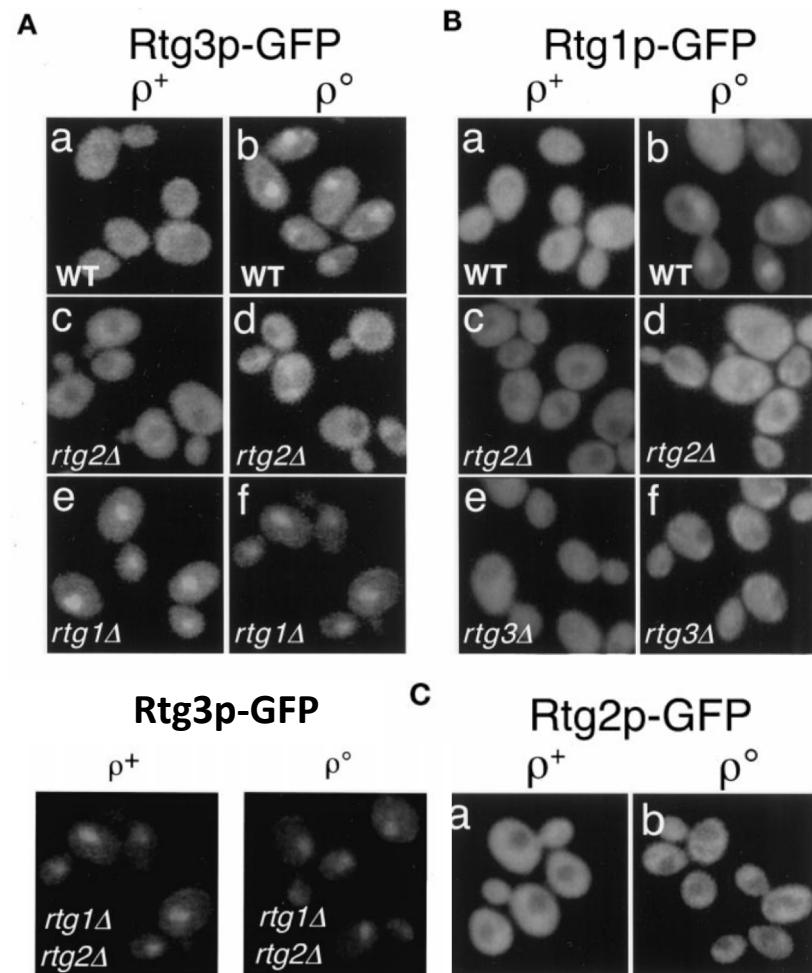
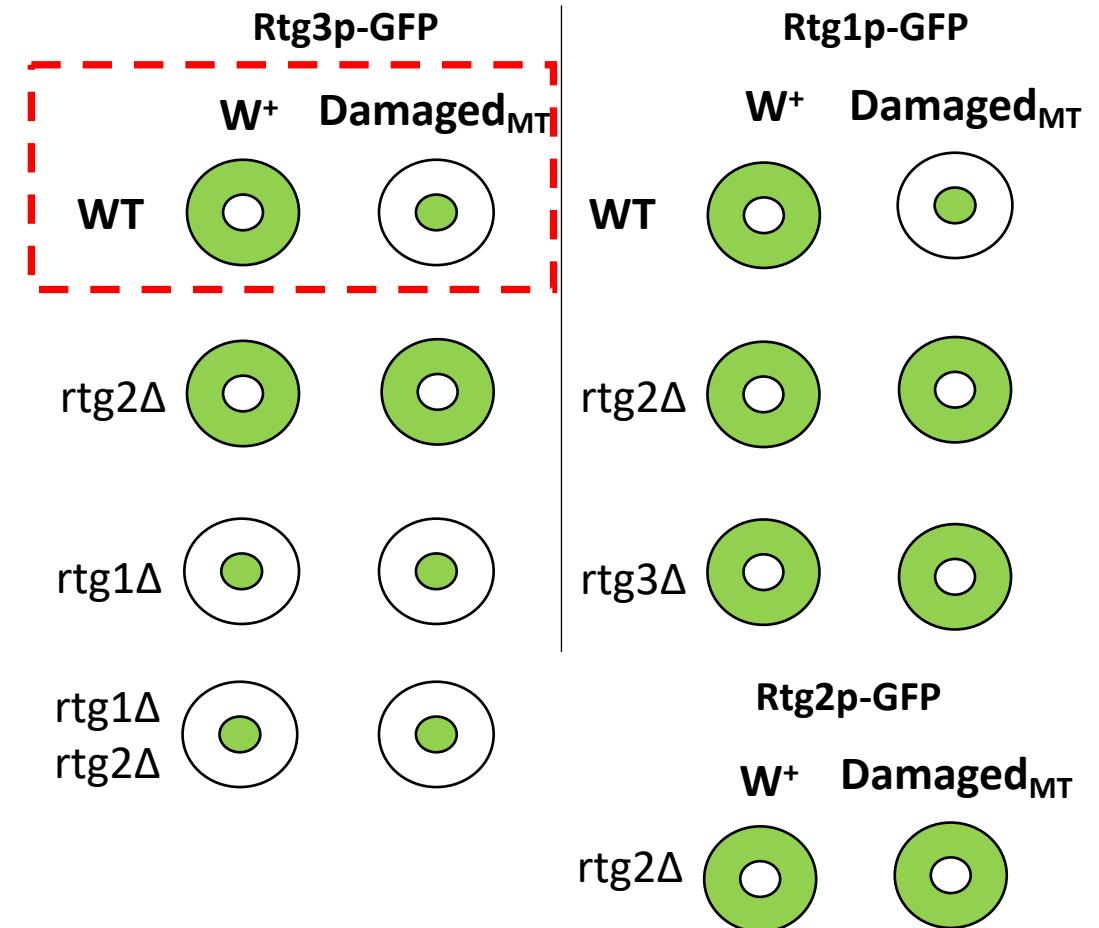


Fig3. Subcellular localization of Rtg1p, Rtg2p and Rtg3p\*

\*Figure (left) from Takayuki Sekito et al., 2000

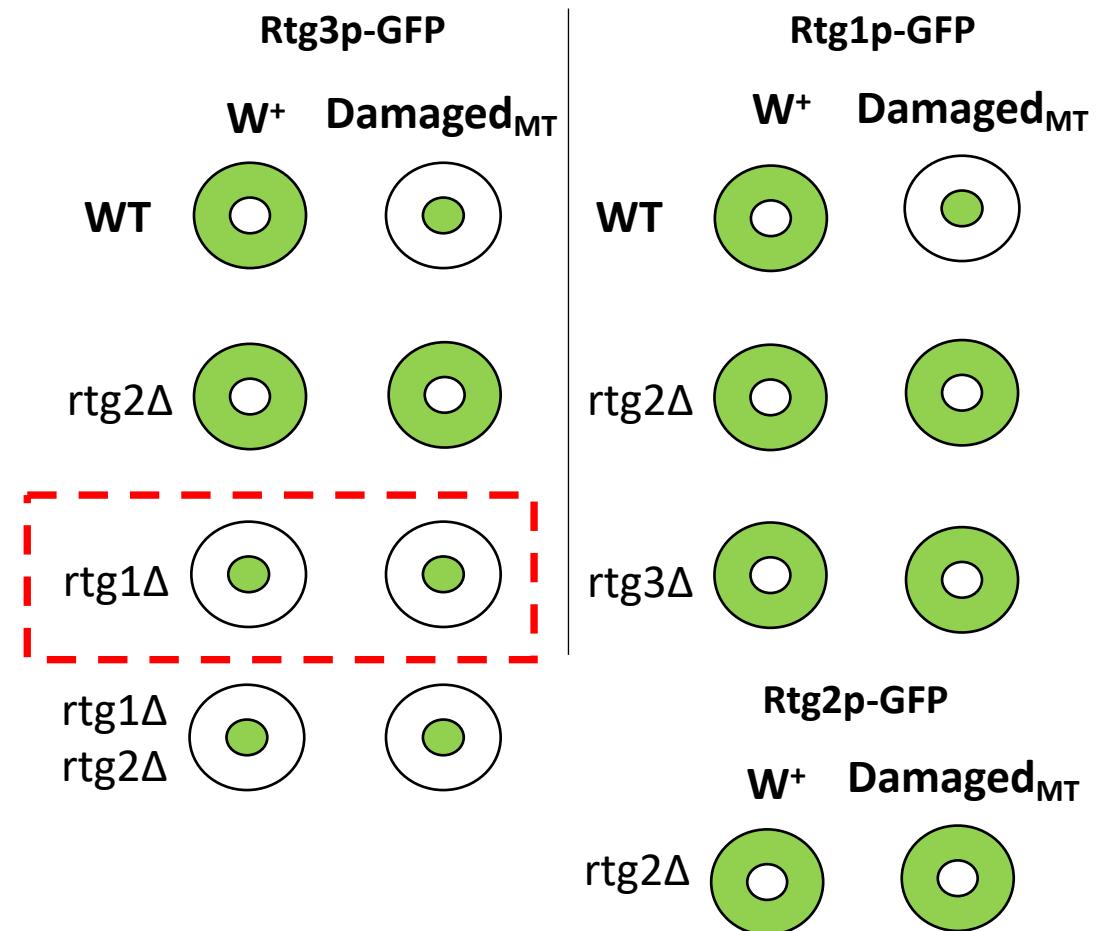


W<sup>+</sup>: Wild type | Damaged<sub>MT</sub>: Deletion of mitochondrial genome

# Properties of Retrograde Signaling – A Qualitative Approach

## Network Characteristics

- Rtg2p is always **cytoplasmic**.
- The success response requires **translocation of both Rtg1p and Rtg3p**
- The nucleus accumulation of Rtg3p-Rtg1p heterodimer **requires Rtg2p**.
- Rtg1p inhibit the translocation of Rtg3p; Rtg1p regulates the pathway both **positively and negatively**.



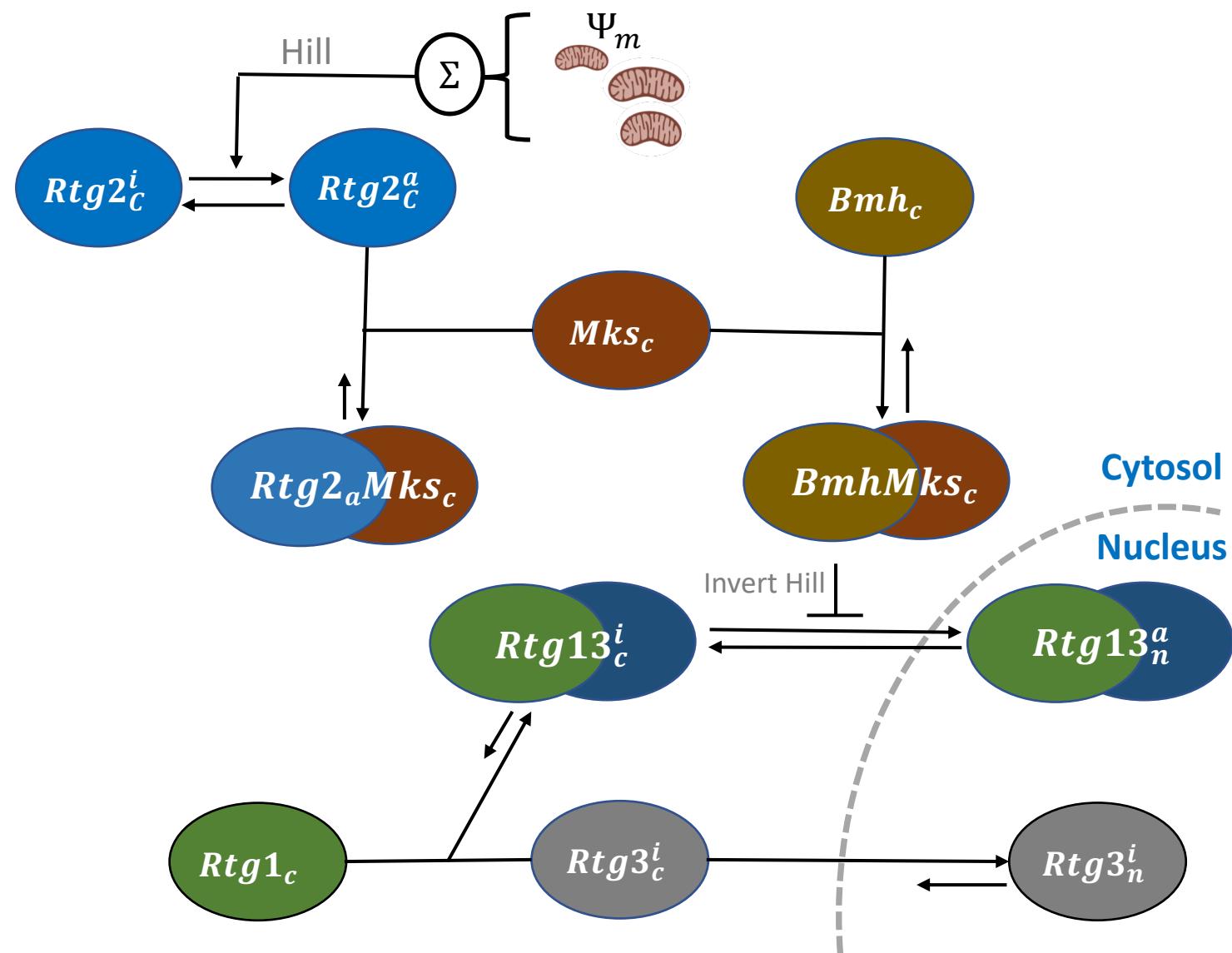
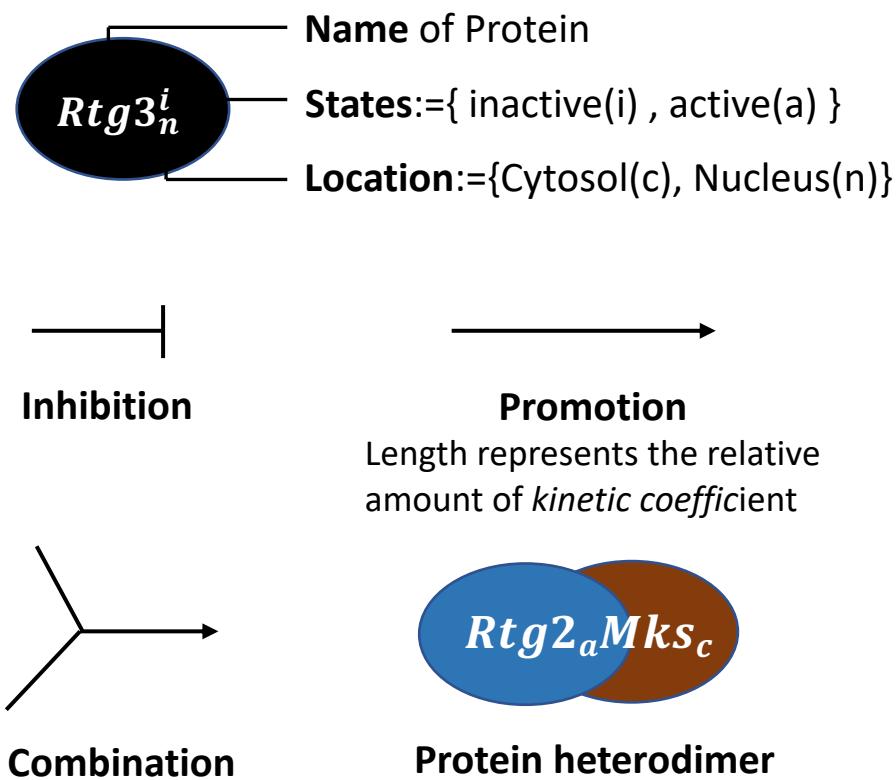
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## Problem Statement

- What is the system dynamics of mitochondrial retrograde signalling?
- How does nucleus sense multiple mitochondria via single channel of protein-protein interaction?

# Model

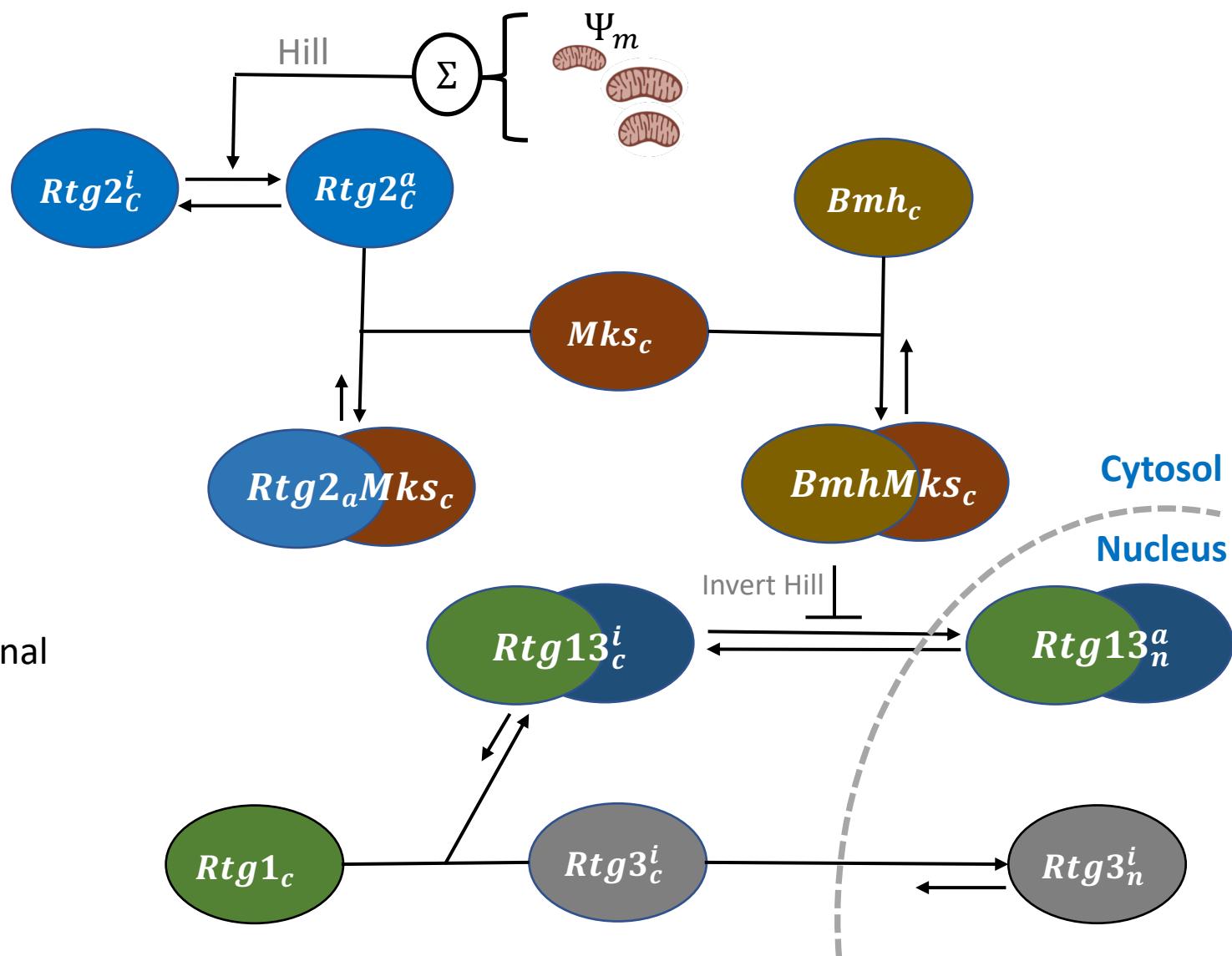
## Notation



# Model

## Assumptions

1. Law of Mass Action
2. Law of Conservation
3. Linearity mitochondrial retrograde signal



# Modulation Layer

## Formulation

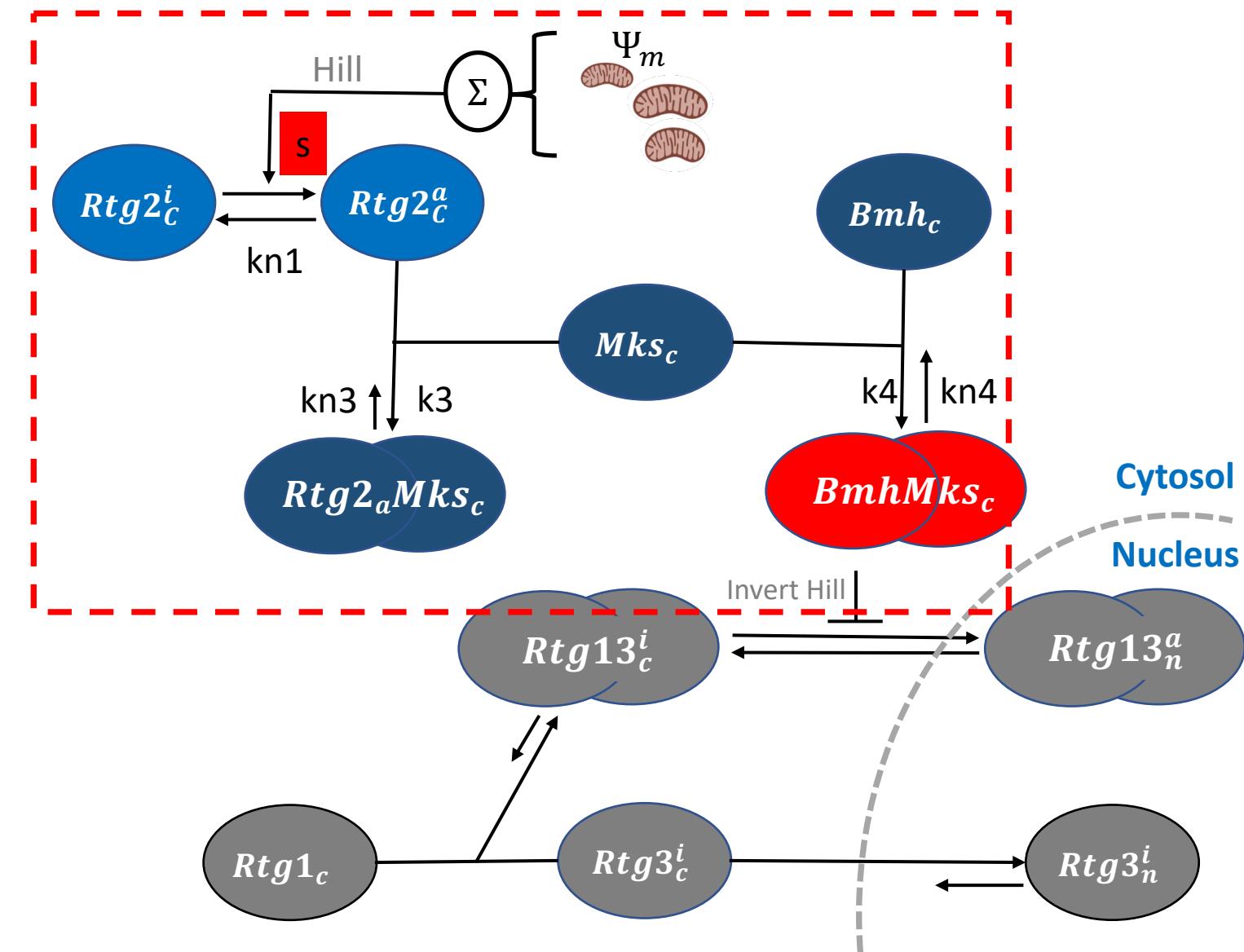
### Rtg2-Bmh-Mks Model

$$Rtg3_n^i \xleftarrow[kn1]{Hill(\overbrace{s}^{Input}, k1_0, k1_a, n1)} Rtg2_c^a$$

$$Rtg2_c^a + Mks_c \xleftarrow[kn3]{k3} Rtg2_a Mks_c$$

$$Bmh_c + Mks_c \xleftarrow[kn4]{k4} \underbrace{BmhMks_c}_{Output}$$

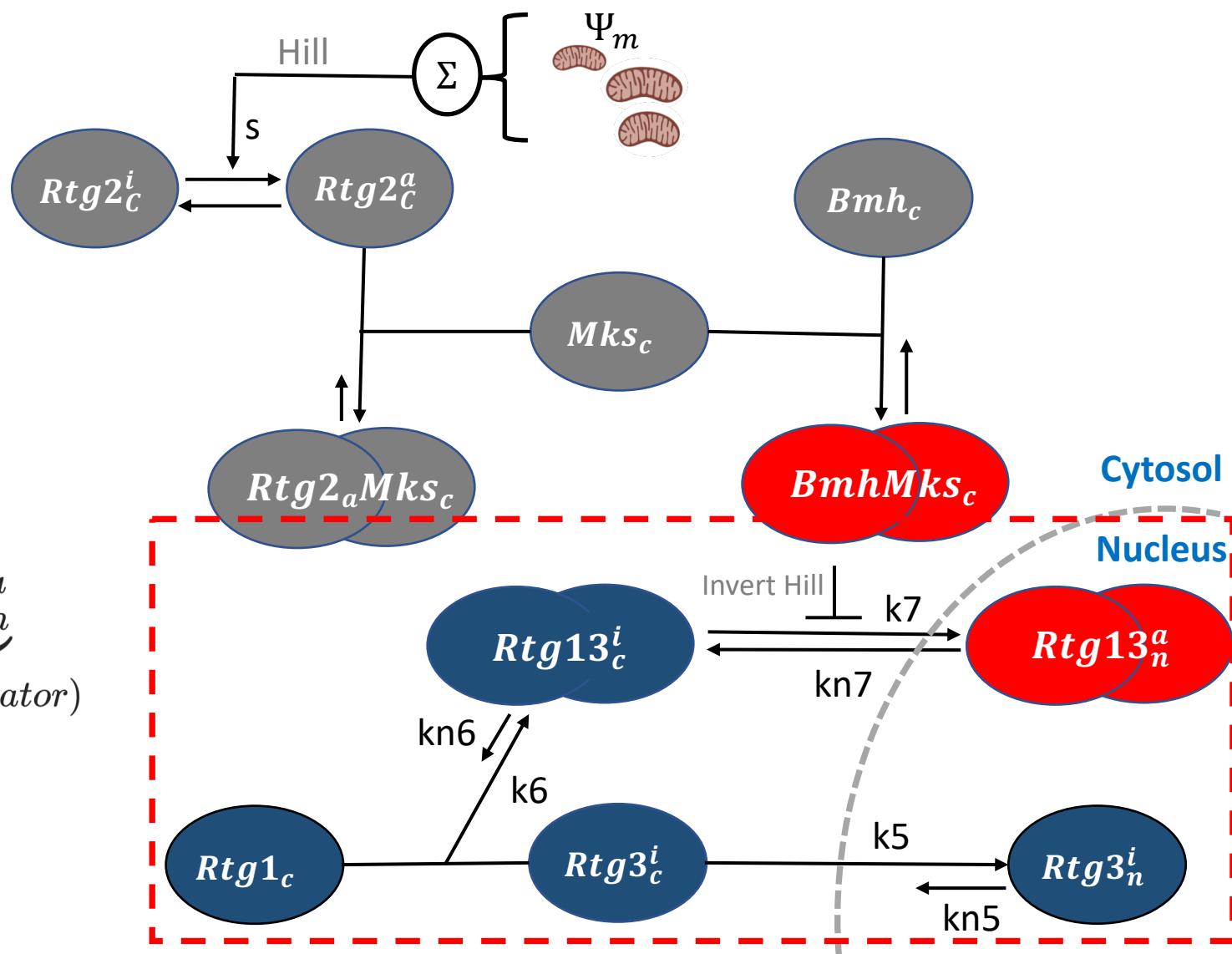
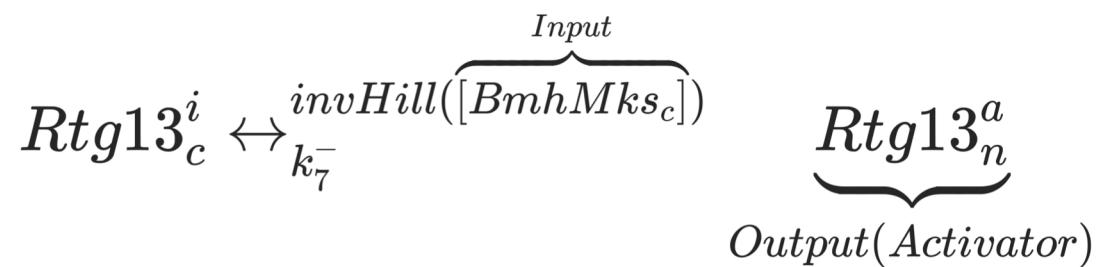
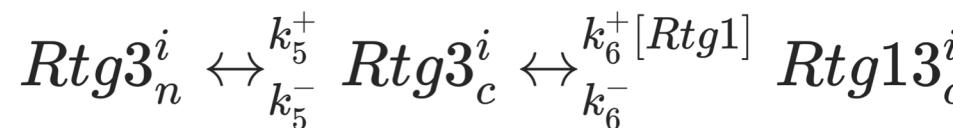
$$Hill(s, k1_0, k1_a, n1) = k1_0 \frac{k1_a^{n1}}{k1_a^{n1} + s^{n1}}$$



# Activation Layer

## Formulation

### Rtg13 Translocation Model



InvHill : Inverted Hill function

# Simulation Methods



- **ReactionNetwork.jl**
  - Building protein interaction network
- **DifferentialEquations.jl**
  - Toolbox for differential equation problems
- **BlackBoxOptim.jl**
  - Toolbox for black-box Optimization
- **Scipy**
  - ODE simulation and optimization
- **Sympy**
  - Deriving analytical solution of steady-states

# How to find the parameters?

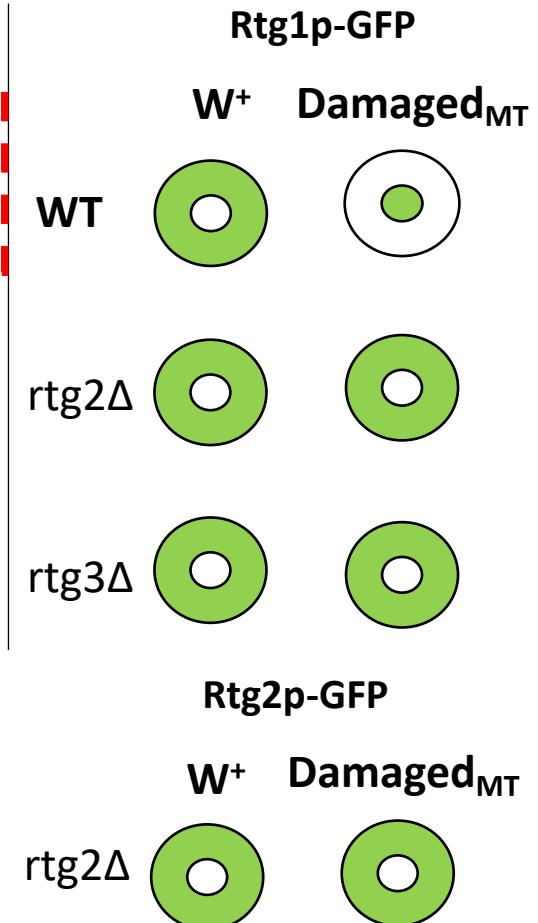
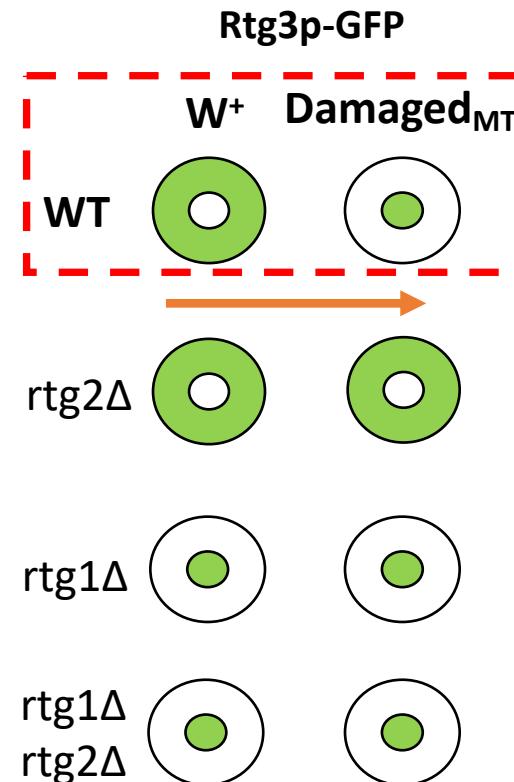
Condition

Action

$Bool([*-Rtg1_*])$	$Bool([Rtg2_C])$	S	$Translocation_{Rtg3}$ (Output)
0	0	0	x
0	0	1	1
0	1	0	1
0	1	1	1
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	1

$Bool([Rtg3^*])$	$Bool([Rtg2_C])$	S	$Translocation_{Rtg1}$ (Output)
0	0	0	x
0	0	1	x
0	1	0	0
0	1	1	0
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	1

X: Don't care term

W<sup>+</sup>: Wild type | Damaged<sub>MT</sub>: Deletion of mitochondrial genome

# How to find the parameters?

## Modulatioin Layer

$$Rtg3_n^i \xleftrightarrow[kn1]{Hill(\widehat{s}, k1_0, k1_a, n1)} Rtg_2^a$$

$$Rtg_2^a + Mks \xleftrightarrow[kn3]{k3} Rtg2^a Mks_c$$

$$Bmh_c + Mks_c \xleftrightarrow[kn4]{k4} \underbrace{BmhMks_c}_{Output}$$

## Activation Layer

$$Rtg3_n^i \xleftrightarrow[k_5^-]{k_5^+} Rtg3_c^i \xleftrightarrow[k_6^-]{k_6^+[Rtg1]} Rtg13_c^i$$

$$Rtg13_c^i \xleftrightarrow[k_7^-]{invHill([\overbrace{BmhMks_c}^{Input}])} \underbrace{Rtg13_n^a}_{Output(Activator)}$$

## Solving the Inequalities

$$(1) [Rtg3_c^i]_{ss} K_2 K_3 * InvHill(s) - 1 < 0$$

$$(2) [Rtg3_c^i]_{ss} K_2 K_3 * InvHill(s) - 1 > 0$$

$$(3) K1 < 1$$

$$(4) [Rtg1_c]_{ss} (K2 K3 - K2) - 1 + K1^{-1} < 0$$

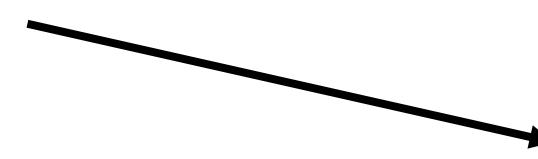
$$(5) [Rtg1_c]_{ss} (K2 K3 * InvHill(s) - K2) - 1 + K1^{-1} > 0$$

**Necessary Conditions** for a valid model

# How to find a valid parameter set?

## Random Search with boundary

Assigning **4** parameters



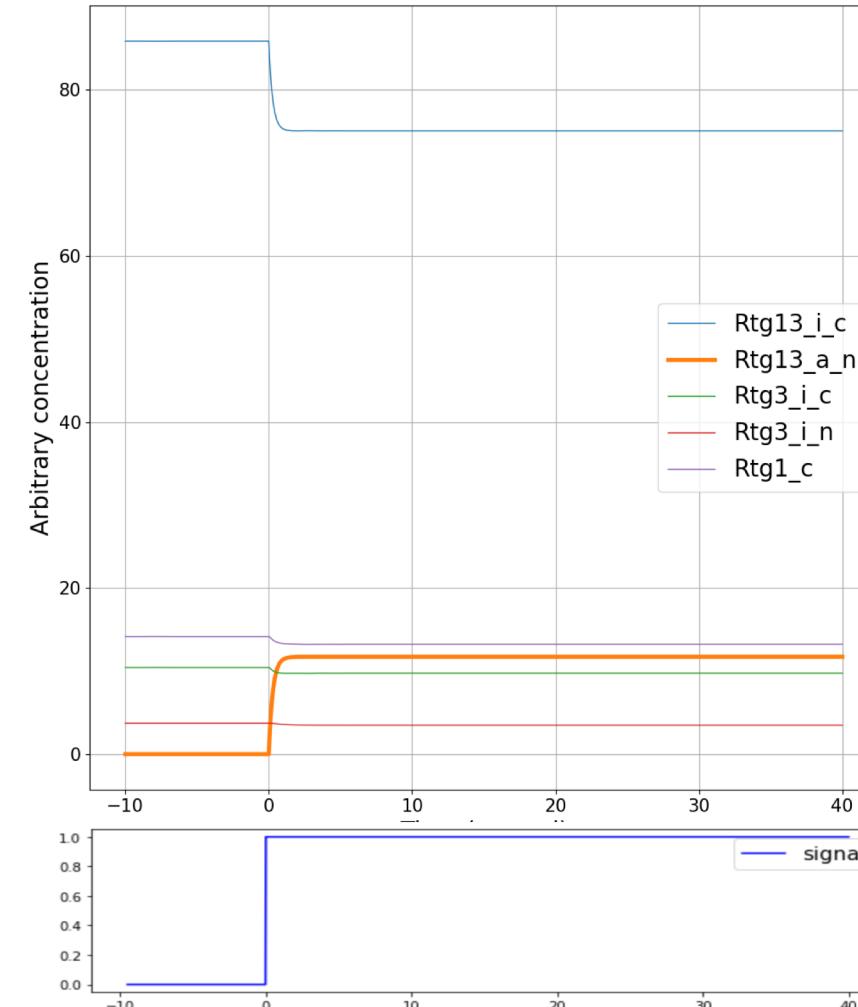
Get the rest of parameters

By random search (approx. 2000 iter.)

Using the **Inequalities**

# How to find a valid parameter set?

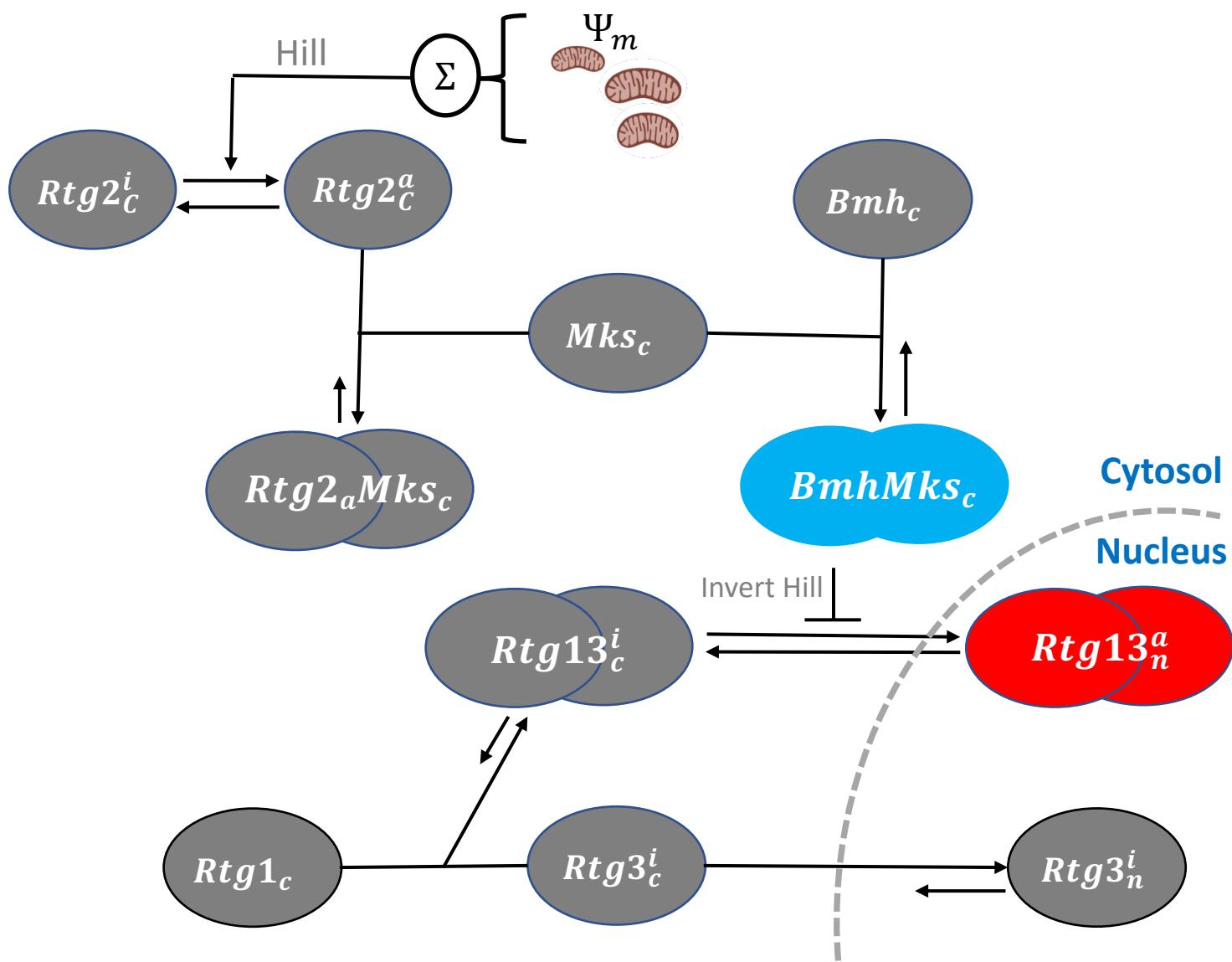
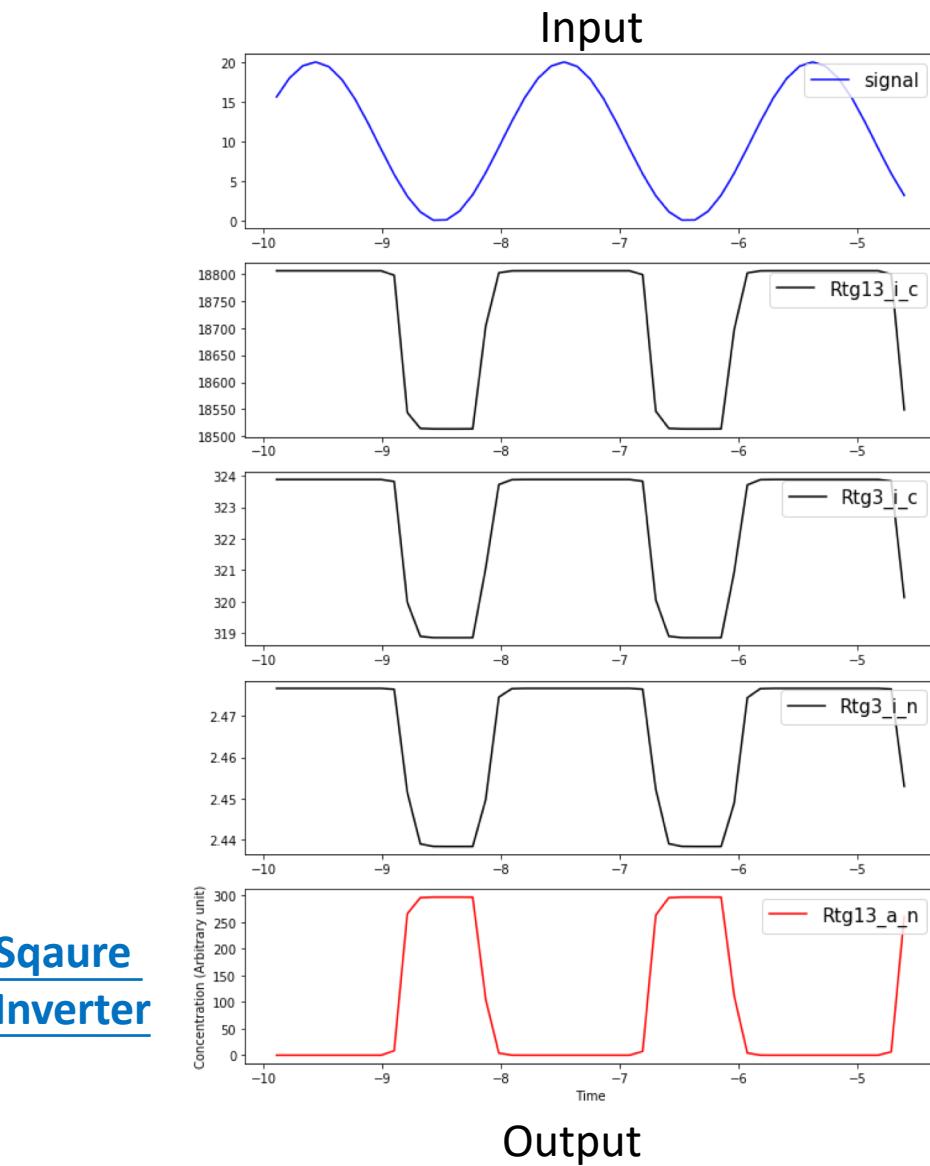
## Step Response



### Validation

1. Solve the differential-equations
2. Compare with truth table

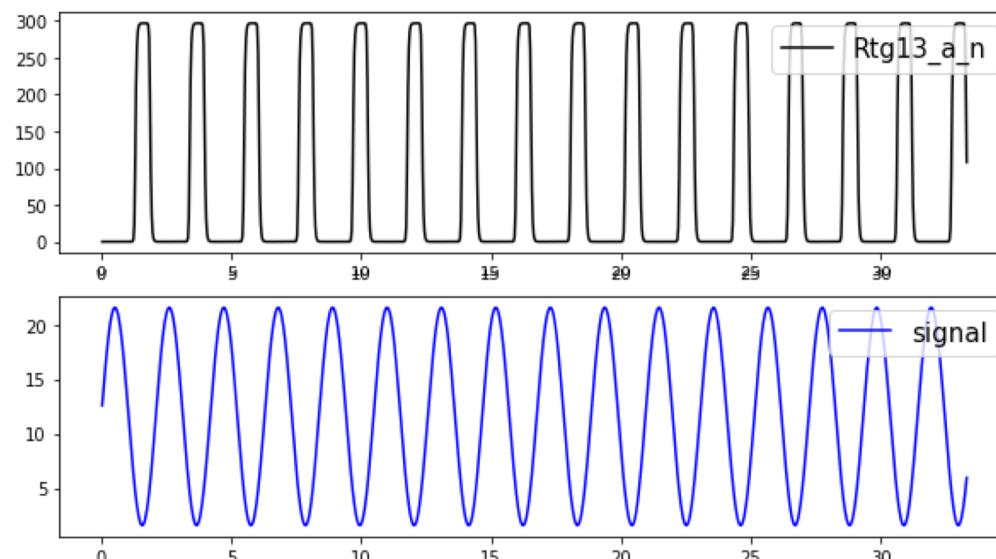
# Systems Dynamics of Mitochondrial Retrograde Signaling



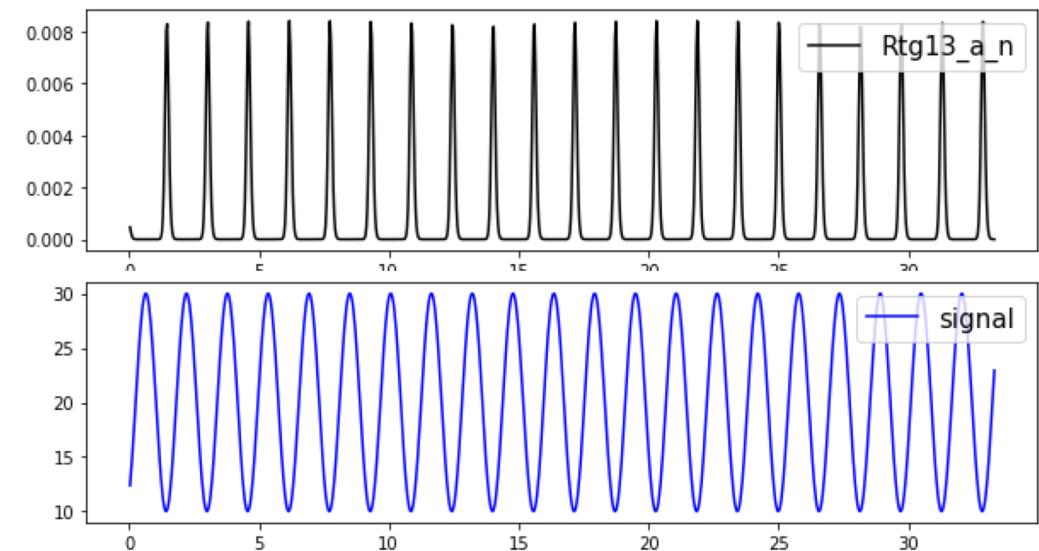
# Systems Dynamics of Mitochondrial Retrograde Signaling

## Frequency Response

Output



Increase  
Frequency



Input

### Signal Deformation

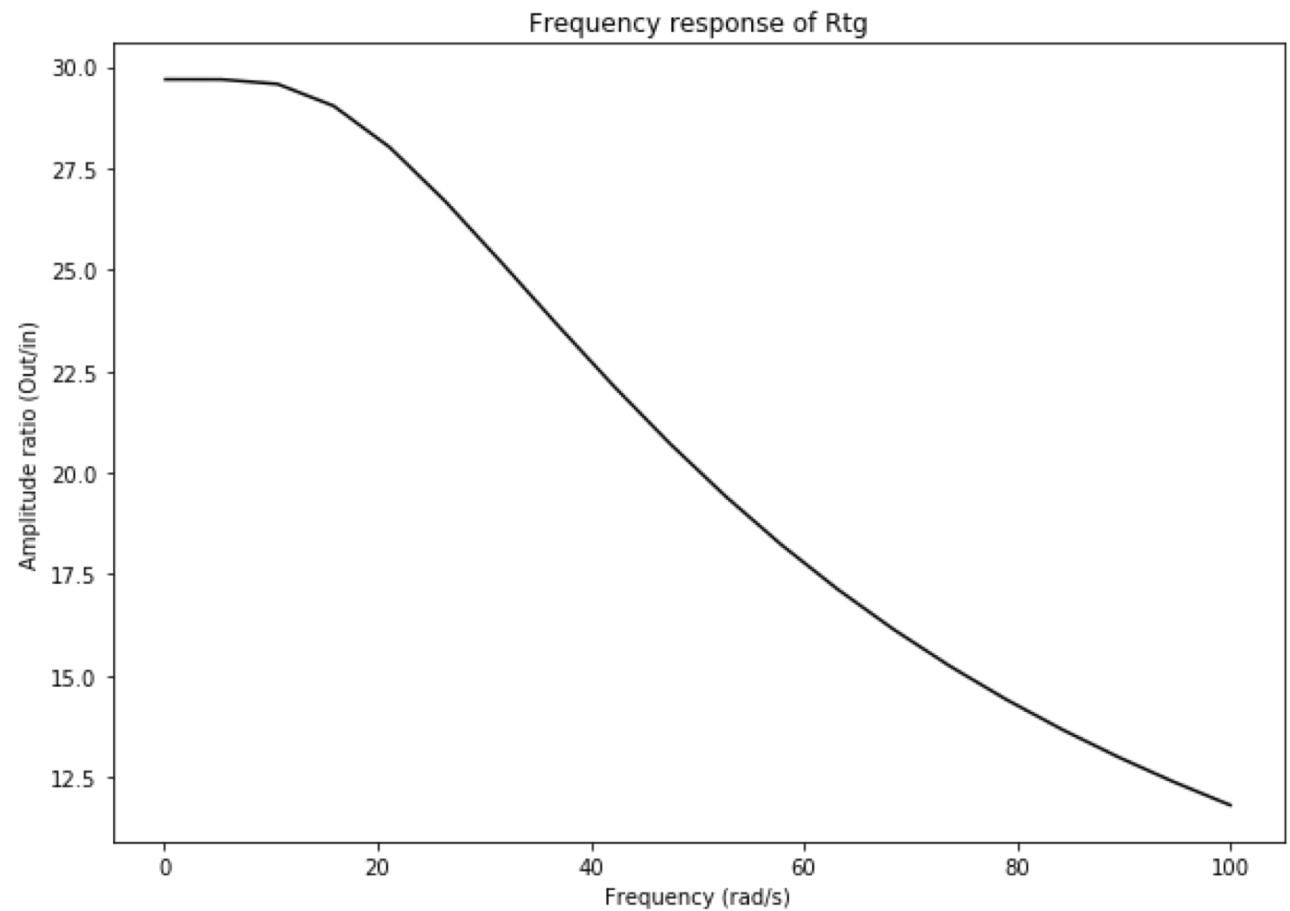
Input : Sin wave  
Output: Square wave

### Frequency Modulation

- Phase shifting
- Amplitude attenuation

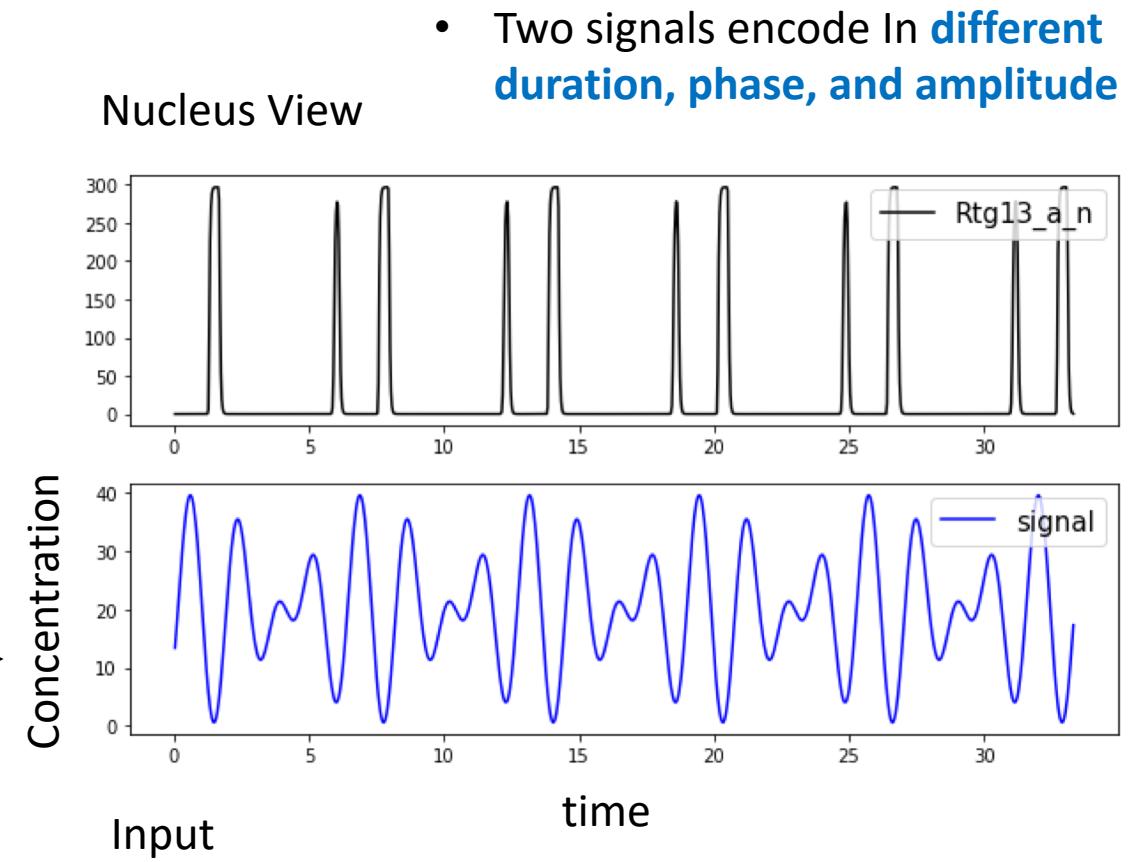
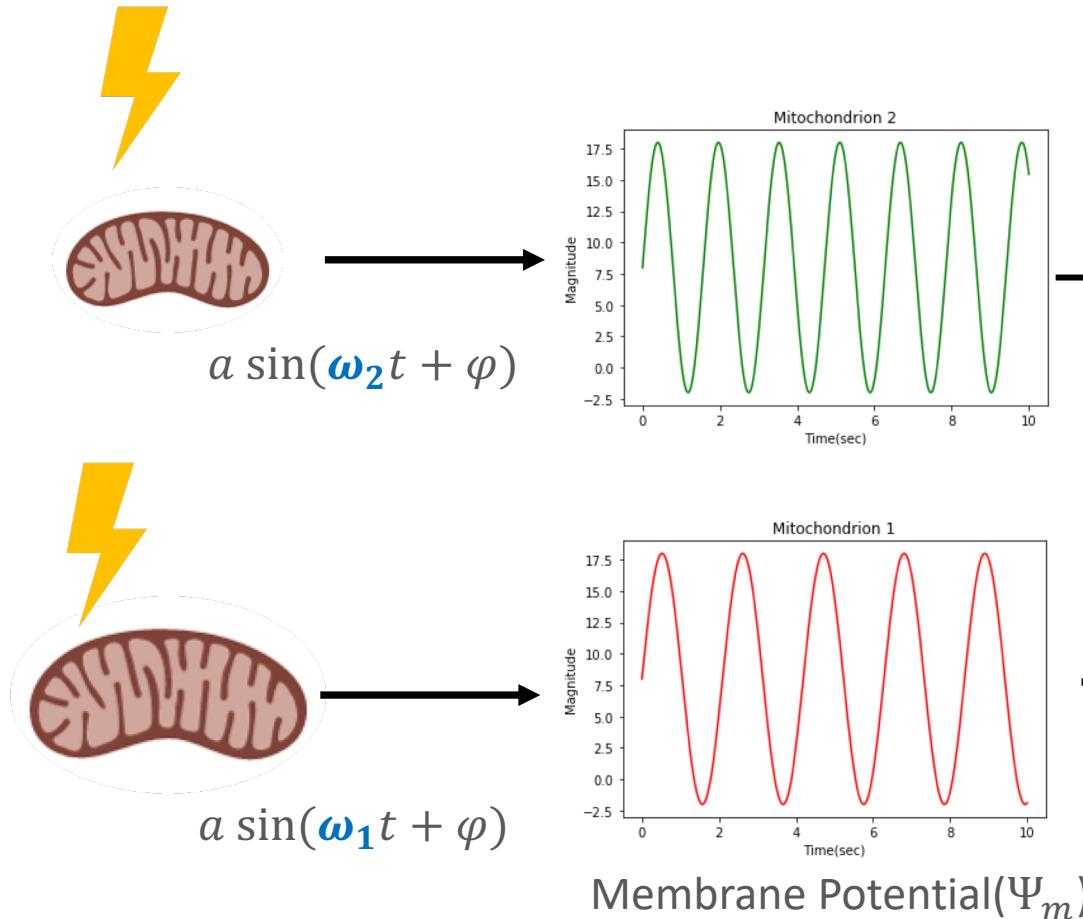
# Frequency Response of RTG Pathway

- RTG pathway as **Low Pass Filter**



# How can nucleus sense multiple mitochondria via single protein channel ?

## Frequency Modulation of RTG Pathway – The Encoding Process



\*Larger mitochondrion processes highter electrical capacity.

## Summary

- The proposed differential-equation model satisfies the microscopic data which provides the logic table of Rtg1/3 translocation.
- The frequency modulation of RTG pathway provides the decipherable message from multiple mitochondria to single nucleus based on protein-protein interaction.

## References

1. Liu, Zhengchang, et al. "RTG-dependent mitochondria to nucleus signaling is negatively regulated by the seven WD-repeat protein Lst8p." *The EMBO journal* 20.24 (2001): 7209-7219.
2. Miceli, Michael V., et al. "Loss of mitochondrial membrane potential triggers the retrograde response extending yeast replicative lifespan." *Frontiers in genetics* 2 (2012): 102.
3. Srinivasan, Visish, et al. "Comparing the yeast retrograde response and NF-κB stress responses: implications for aging." *Aging cell* 9.6 (2010): 933-941.

# Acknowledgements

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  - Dr. Jun-Yi Leu



Biological Systems Lab., National Taiwan University



National  
Taiwan  
University

Thank you.

# Supplemental Materials

# Properties of Retrograde Signaling – A Qualitative Approach

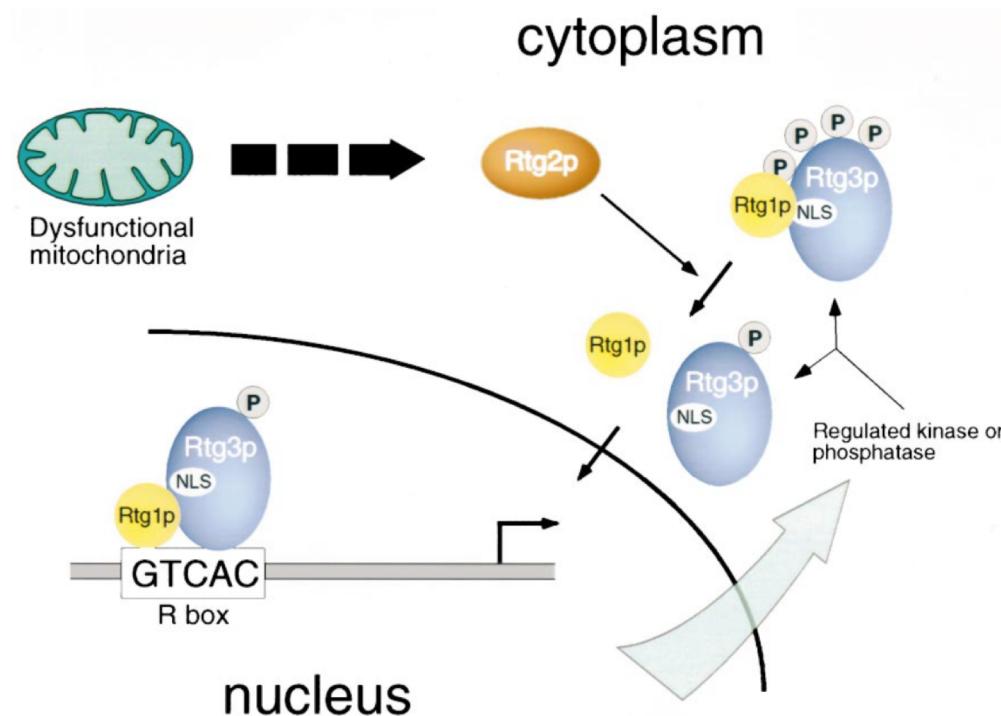


Fig4. Model of mitochondrial retrograde signaling.\*

- Rtg2p is always **cytoplasmic**.
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- Rtg1p inhibit the translocation of Rtg3p; Rtg1p regulates the pathway both **positively and negatively**.
- The nucleus accumulation of Rtg3p-Rtg1p heterodimer **requires Rtg2p**.

\*Figure from Takayuki Sekito et al., 2000

# How to get quantitative data via experiment

## Optogenetics

- locally and reversibly depolarize mitochondria
  - Channel rhodopsin
- Rtg3-GFP and Rtg1-BFP
  - Measure the output

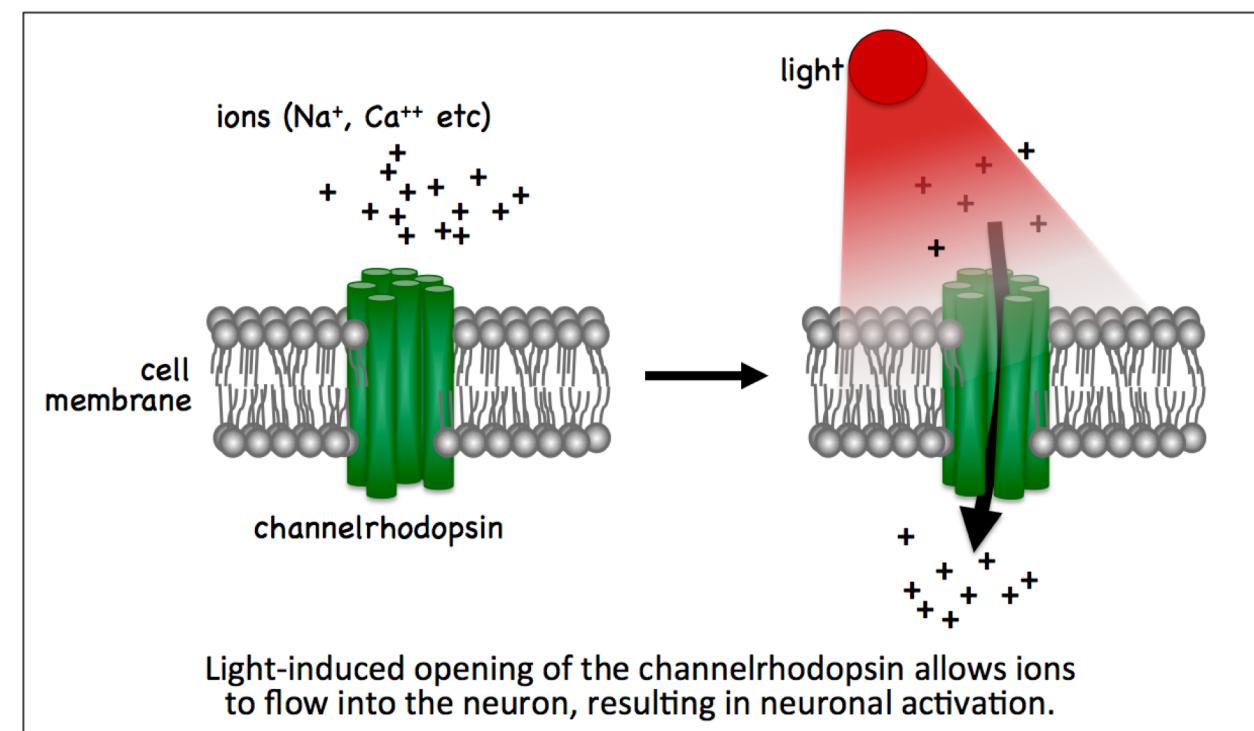
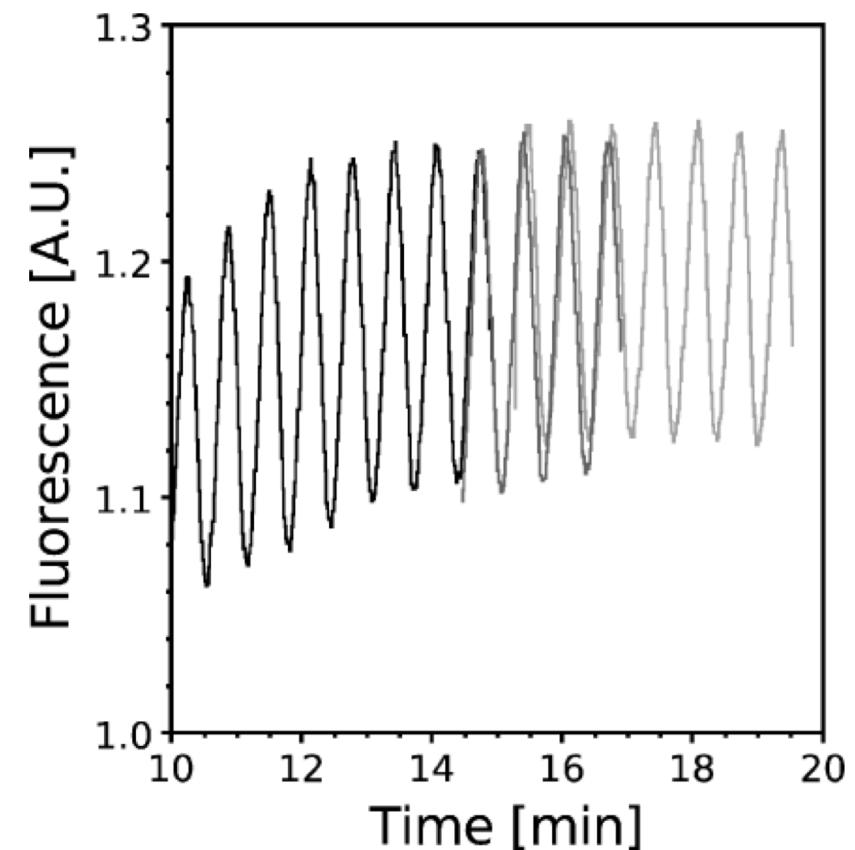


Image from Bloomington Drosophila Stock Center

# Oscillation of Mitochondrial Membrane Potential

- In glucose-fermenting yeast
- nicotinamide adenine dinucleotide (NADH)



Andersen, Ann Zahle, et al. "On-line measurements of oscillating mitochondrial membrane potential in glucose-fermenting *Saccharomyces cerevisiae*." *Yeast* 24.9 (2007): 731-739.

# Optimization for complex model

- Fuzzy logic
- Fitness function: Leaky RELU and RELU
- Optimization algorithm: Stochastic differentiation