

Information Transduction Capacity of Mitochondrial Retrograde Signaling

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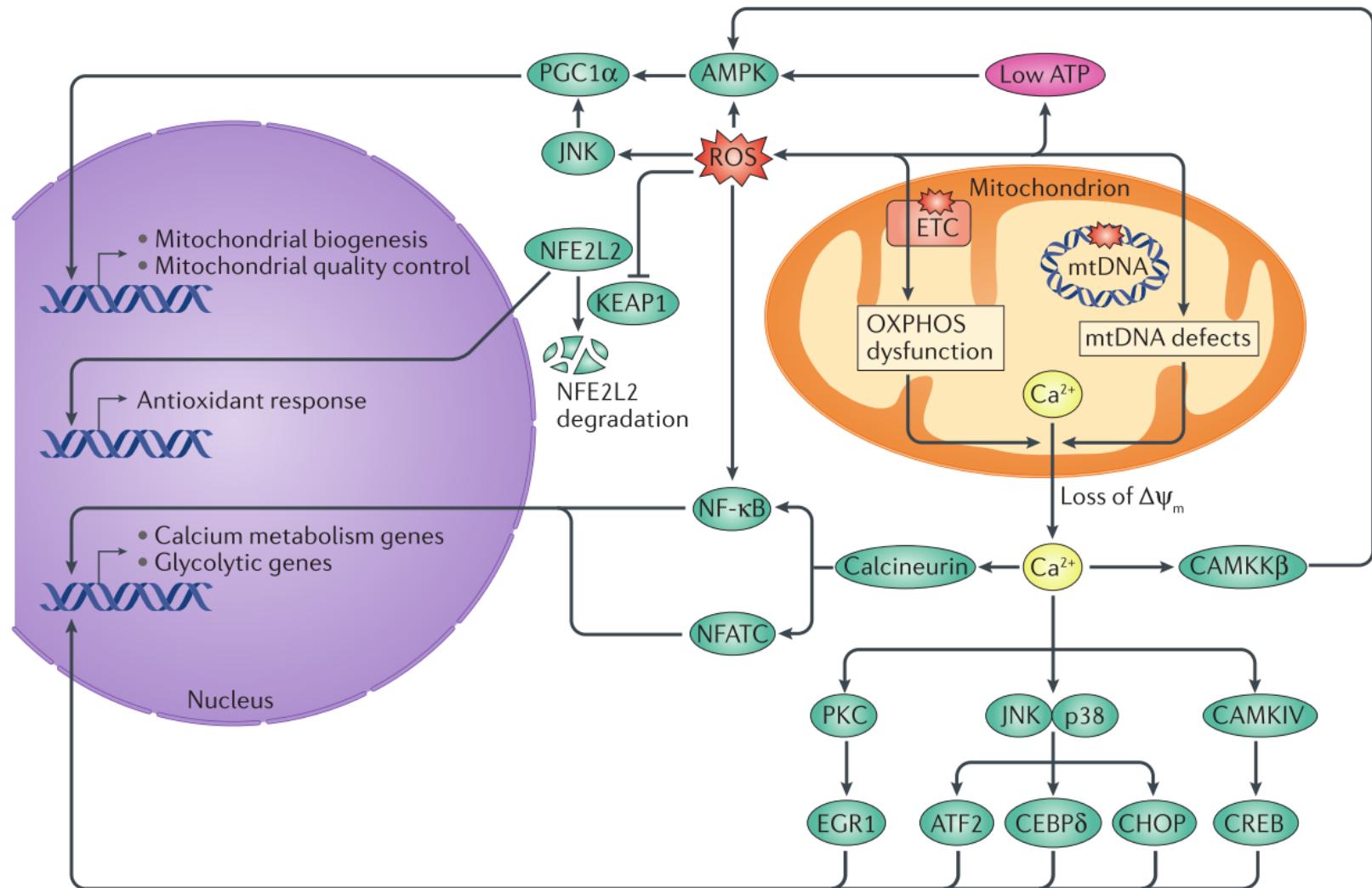
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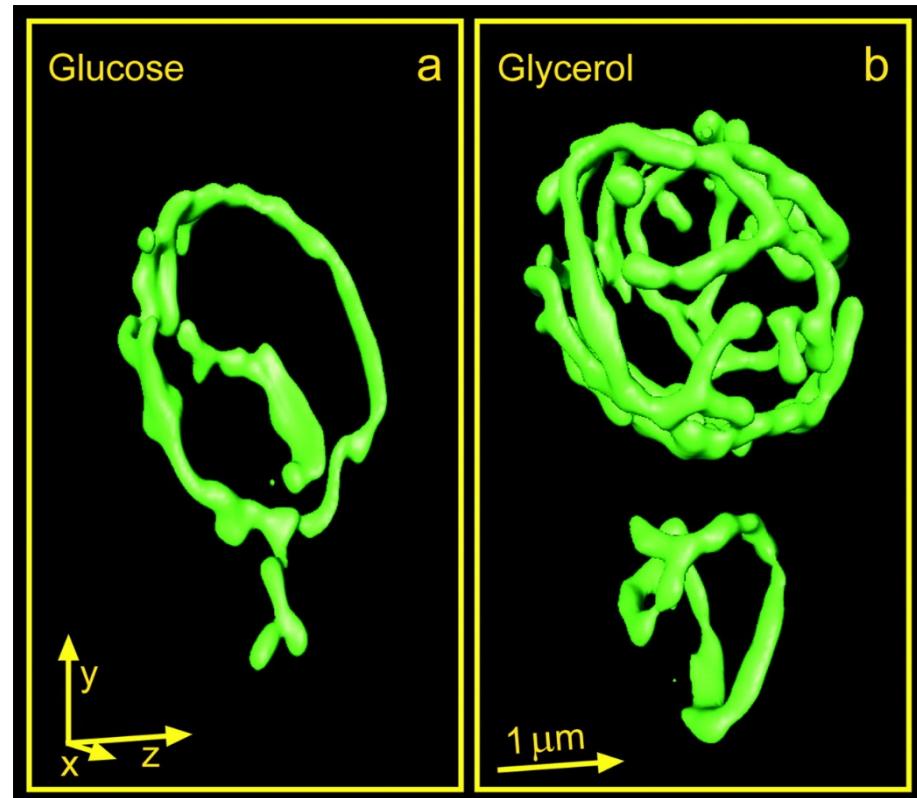
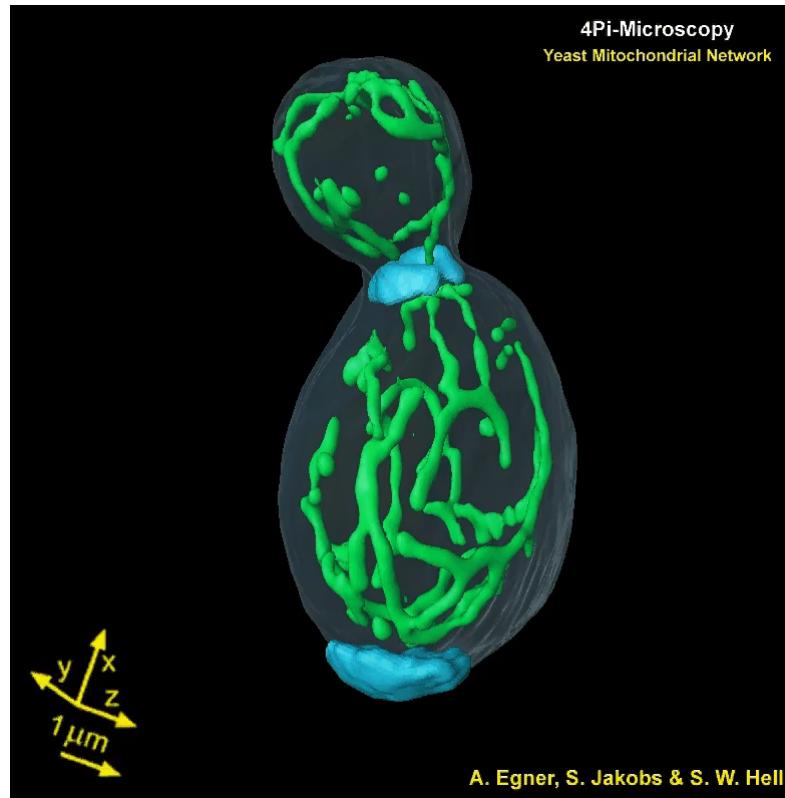
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Keyword: retrograde response, mitochondrial membrane potential ($\Delta\psi m$), information theory

The Communication Between Mitochondria and Nucleus

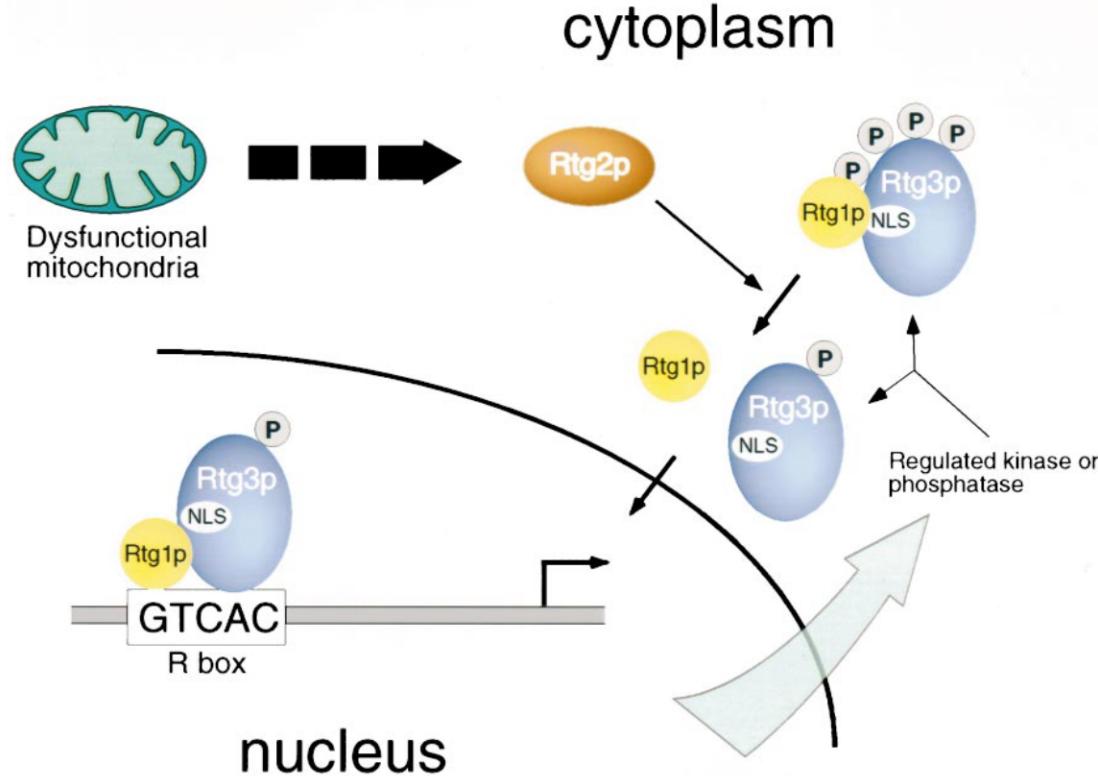


Yeast Mitochondrial Network



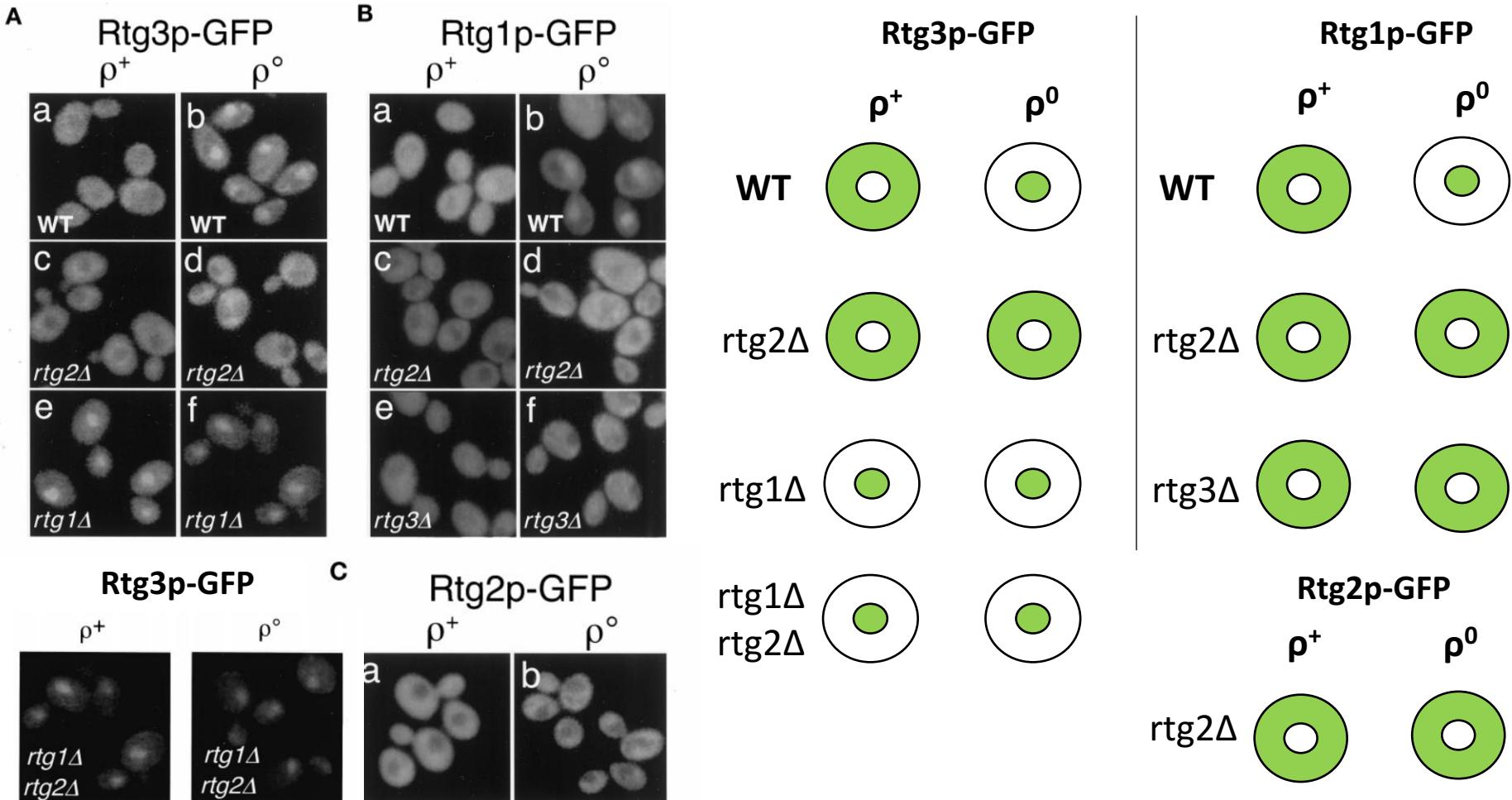
GFP-labeled mitochondrial compartment of live *S. cerevisiae*. Images were taken by fast 100-nm resolution three-dimensional microscope. (A. Egner et al., 2002)

Properties of Retrograde Signaling – a binary prospect



Model of the control of mitochondria-to-nuclear signaling. (Takayuki Sekito et al., 2000)

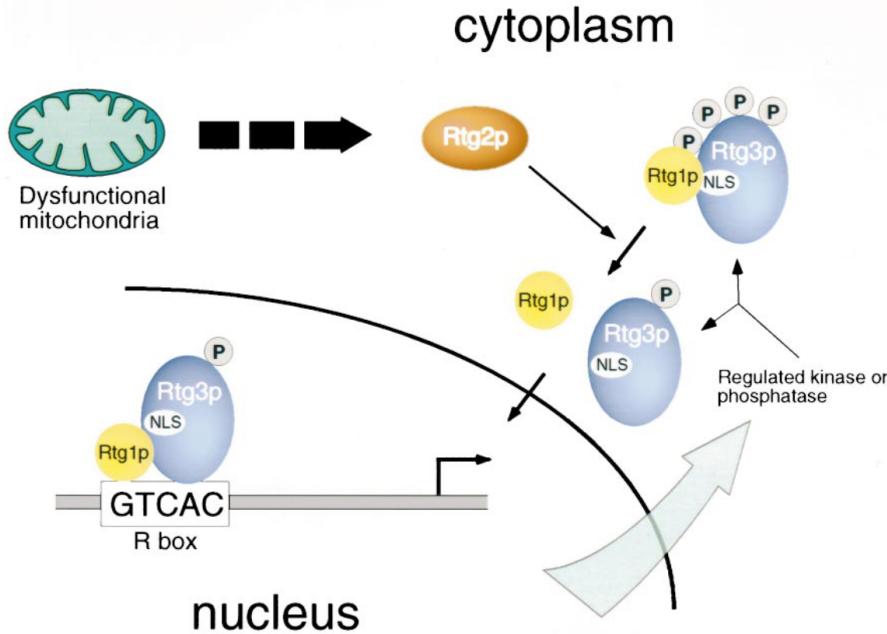
Properties of Retrograde Signaling – a binary prospect



Subcellular localization of Rtg1p, Rtg2p and Rtg3p
 (Takayuki Sekito et al., 2000)

* ρ^+ : Wild type; ρ^0 : Deletion of mitochondrial genome

Properties of Retrograde Signaling based on binary experiments

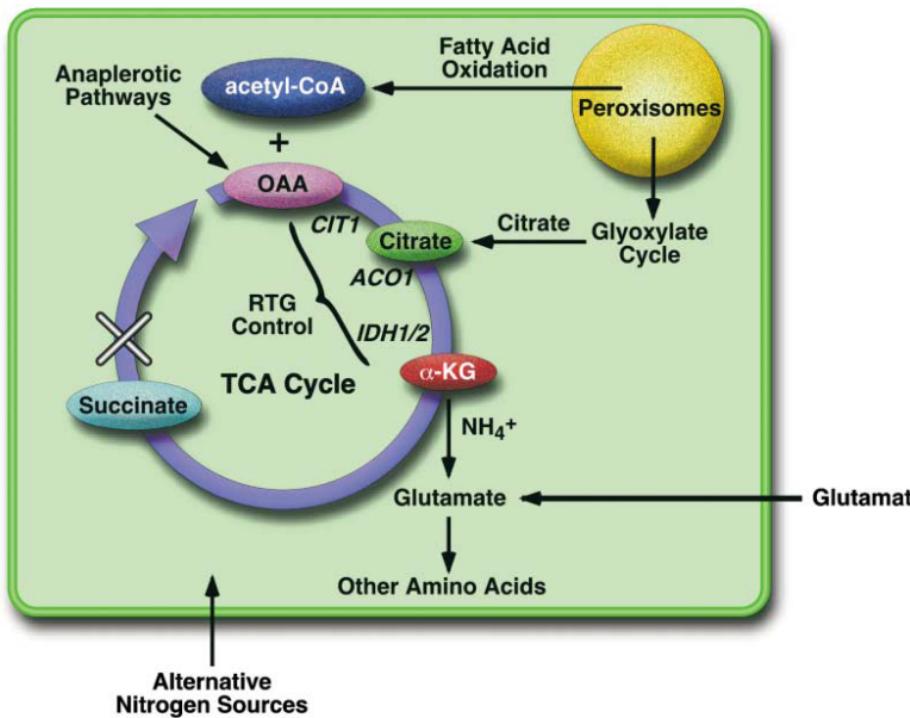


Model of the control of mitochondria-to-nuclear signaling. (Takayuki Sekito et al., 2000)

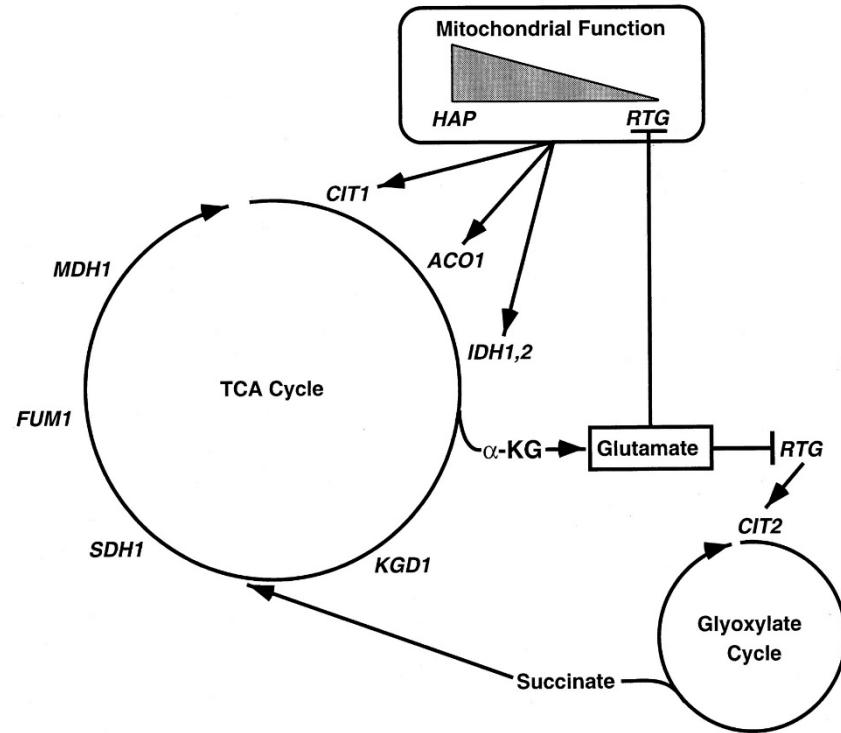
- Rtg2p is always cytoplasmic.
- The expression of RTG regulated genes require translocation of both Rtg1p and Rtg3p
- Rtg1p inhibit the translocation of Rtg3p; Rtg1p regulates the pathway both positively and negatively.
- The nucleus accumulation of Rtg3p-Rtg1p heterodimer requires Rtg2p.

Which genes are regulated by Retrograde Response in Yeast?

- *CIT2 is also regulated by RTG pathway



The retrograde pathway enables respiratory-deficient cells to maintain glutamate supplies by providing substrates for α -ketoglutarate (ETC inhibited)



Possible mitochondrial signals that can trigger retrograde response

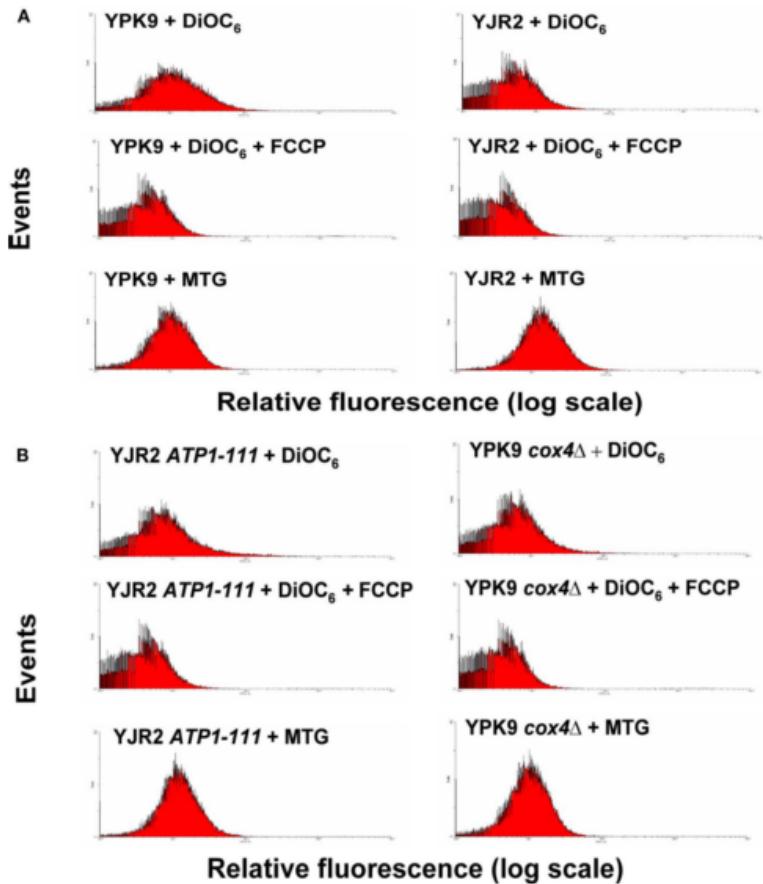
Absence of mitochondrial DNA may cause

- Drop in the concentration of ATP
- ROS production
- Loss of MMP

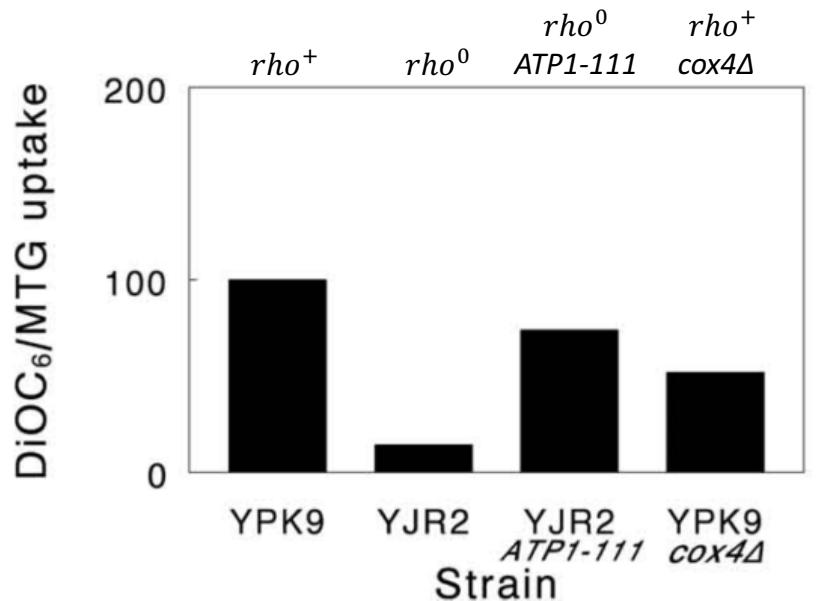
Related phenomenon

- Aging yeast cells exhibit reduced MMP and activate the retrograde response
- Lowering of MMP with the uncoupler dinitrophenol has been shown to increase RLS in yeast
 - MMP($\Delta\psi_m$) manipulation
 - ATP1-111 Mutation
 - Del-Cox4
 - ρ^+, ρ^0
 - Effect of retrograde response
 - Increase expression of CIT2, IDH1, IDH2 and ACO1
 - Rtg3 Translocation to the Nucleus

Properties of Retrograde Signaling – a tertiary prospect



Measuring mitochondrial membrane potential by flow cytometry. (M. V. Miceli et al., 2012)



Genetic manipulation of mitochondrial membrane potential. (M. V. Miceli et al., 2012)

Properties of Retrograde Signaling – a tertiary prospect

- An increase in *CIT2* expression is considered diagnostic for the activation of the retrograde response.

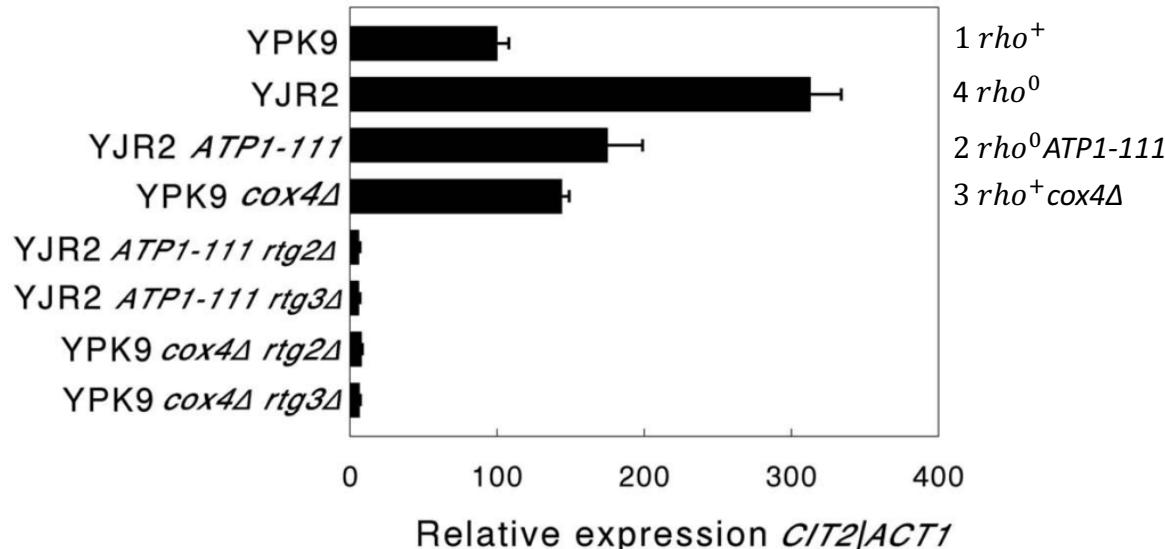


FIGURE 2 | *CIT2* expression. Expression of *CIT2* relative to *ACT1* was determined by qRT-PCR as described in Section “Materials and Methods.” The strains used were as follows: YPK9 (ρ^+); YJR2 (ρ^0); YJR2 *ATP1-111*; YPK9 *cox4Δ*; YJR2 *ATP1-111 rtg2Δ*; YJR2 *ATP1-111 rtg3Δ*; YPK9 *cox4Δ rtg2Δ*; YPK9 *cox4Δ rtg3Δ*. Error bars are \pm SEM for $N = 3$ or 4 determinations. *P*-values adjusted for multiple comparisons are $P < 0.0002$ for YPK9 vs. YJR2; $P < 0.05$ for YJR2 vs. YJR2 *ATP1-111*; $P < 0.02$ for YPK9 vs. YPK9 *cox4Δ*.

MMP May be the trigger of Mitochondrial Retrograde Response

- IDH1, IDH2 and ACO1 are also considered diagnostic for the activation of the retrograde response

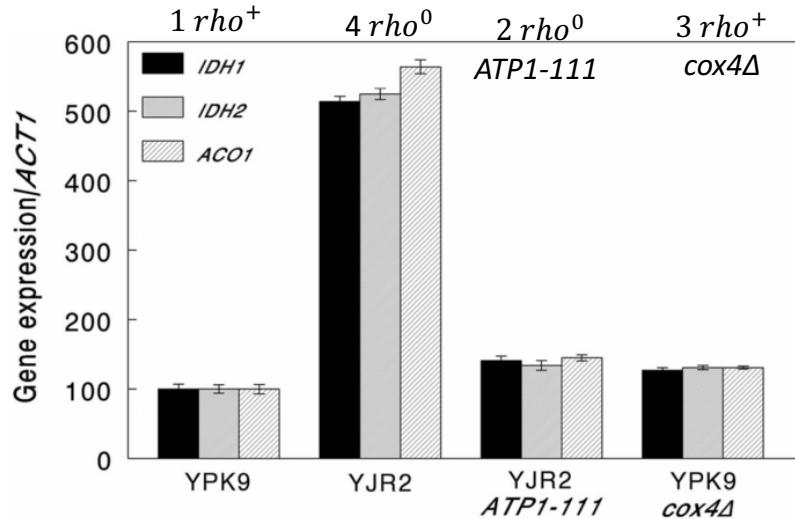


FIGURE A1 | Expression of retrograde response target genes.

Expression of *IDH1*, *IDH2*, and *ACO1* relative to *ACT1* was determined by qRT-PCR as described in Section "Materials and Methods." The strains used were as follows: YPK9 (ρho^+), YJR2 (ρho^0), YJR2 *ATP1-111*, and YPK9 *cox4Δ*. Error bars are SEM for $N=6$ determinations. Expression of *IDH1*, *IDH2*, and *ACO1* increased in YJR2 compared to YPK9 ($P = 3.96 \times 10^{-4}$, 3.06×10^{-4} , and 0.0024 , respectively) and in YPK9 *cox4Δ* compared to YPK9 ($P = 0.0102$, 0.0081 , and 0.0051 , respectively). Expression of *IDH1*, *IDH2*, and *ACO1* decreased in YJR2 *ATP1-111* compared to YJR2 ($P = 1.08 \times 10^{-4}$, 2.73×10^{-4} , and 0.0021 , respectively). P -values are adjusted for multiple comparisons.

MMP May be the trigger of Mitochondrial Retrograde Response

- RTG3-GFP translocation as a indicator of retrograde signaling

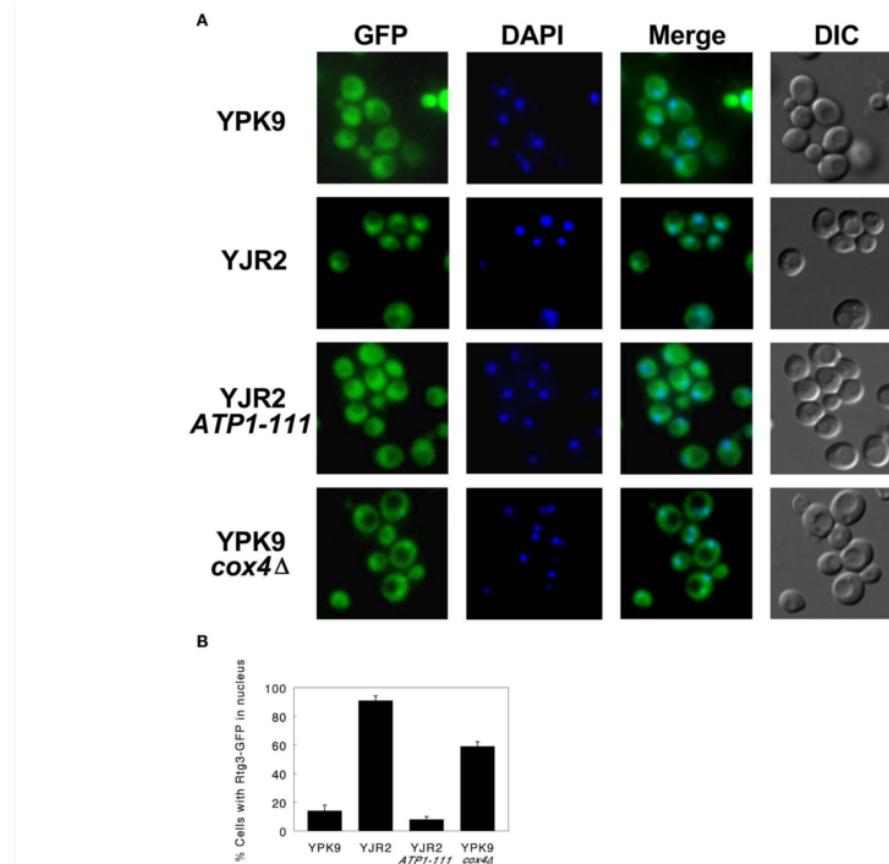


FIGURE 3 | Fluorescence deconvolution micrographs and corresponding DIC images of cells containing an RTG3–GFP fusion construct. Cells were harvested at mid-log growth and imaged as described in Section “Materials and Methods.” **(A)** Fluorescence and DIC micrographs. YPK9: GFP fluorescence is diffuse with only a few cells showing RTg3 translocated to the nucleus. YJR2: The majority of the cells show GFP concentrated in the

nucleus. YJR2 *ATP1-111*: Translocation pattern resembles that seen for YPK9. YPK9 *cox4Δ*: Cells exhibit a fluorescence pattern intermediate to YPK9 and YJR2. DAPI fluorescence indicates the nucleus. **(B)** Quantification of fluorescence micrograph images. Three separate fields of at least 100 cells each were scored for GFP nuclear translocation. Error bars are \pm SEM for $N = 3$ fields.

Functions of RTG-regulated Genes

TCA

- ACO1 :Aconitate hydratase with role in tricarboxylic acid (**TCA**) cycle and mitochondrial genome maintenance; also shows DNA binding activity; localized to mitochondria and cytosol
- IDH1/2: Subunit of mitochondrial NAD(+) -dependent isocitrate dehydrogenase; complex catalyzes the oxidation of isocitrate to alpha-ketoglutarate in the **TCA cycle**.
- CIT1: **Citrate synthase**; catalyzes the condensation of acetyl coenzyme A and oxaloacetate to form citrate; **the rate-limiting enzyme of the TCA cycle**; nuclear encoded mitochondrial protein

Glyoxylate Cycle

- CIT2: **Citrate synthase**, peroxisomal isozyme involved in **glyoxylate cycle**; catalyzes condensation of acetyl coenzyme A and oxaloacetate to form citrate; **expression is controlled by Rtg1p and Rtg2p transcription factors**; SCF-Ucc1 regulates level of Cit2p to maintain citrate homeostasis; oxaloacetate-dependent positive feedback loop inhibits Cit2p ubiquitination; CIT2 has a paralog

e 13.1 Important Parameters of the VTC of the Logic Inverter (Refer to Fig. 13.3)

Output low level

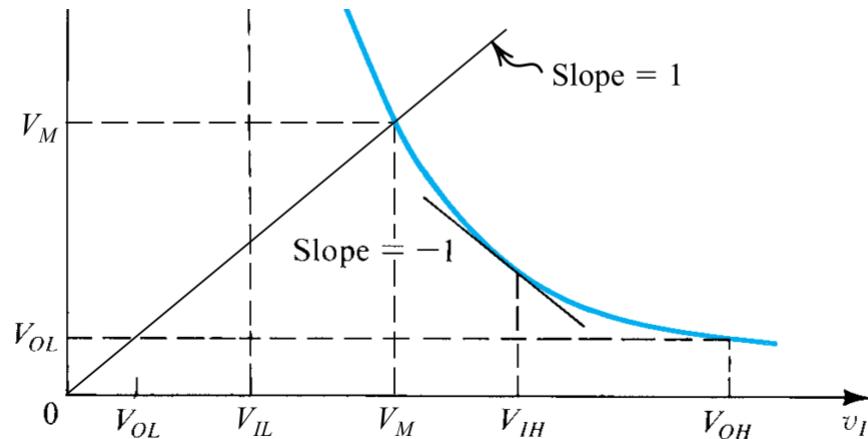
Output high level

Maximum value of input interpreted by the inverter as a logic 0

Minimum value of input interpreted by the inverter as a logic 1

Noise margin for low input = $V_{IL} - V_{OL}$

Noise margin for high input = $V_{OH} - V_{IH}$



Retrograde response may be a inverter-like response

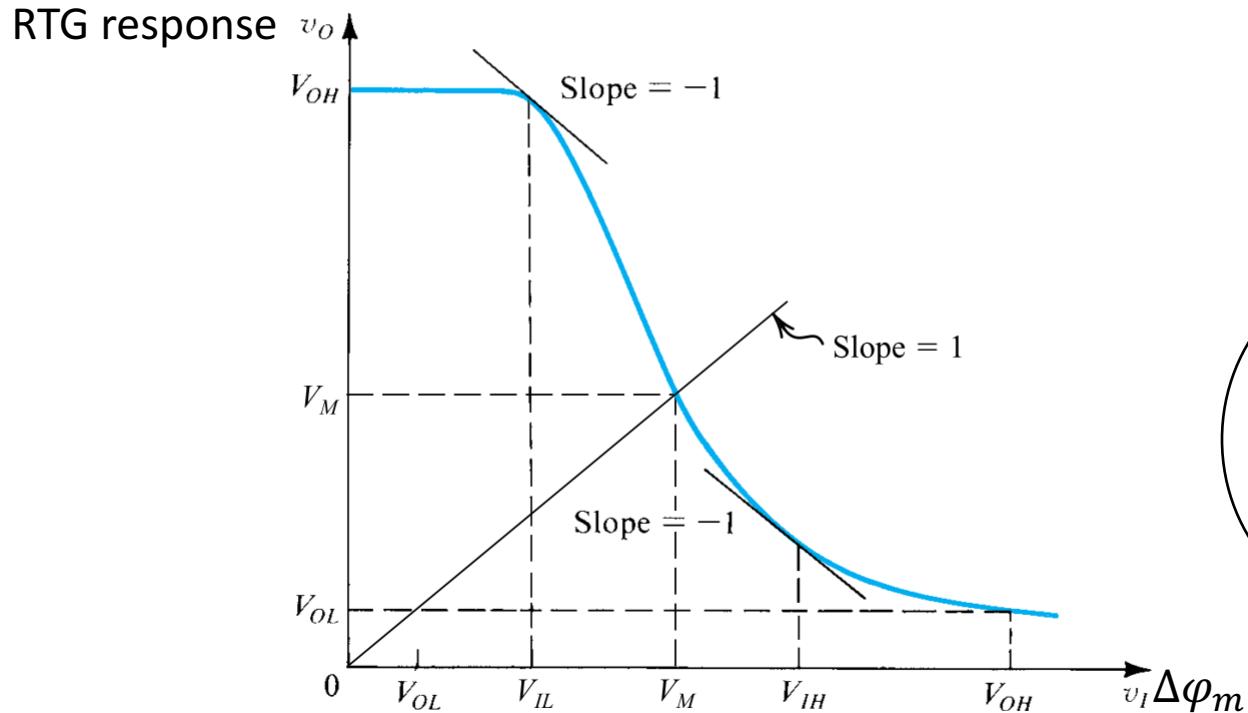
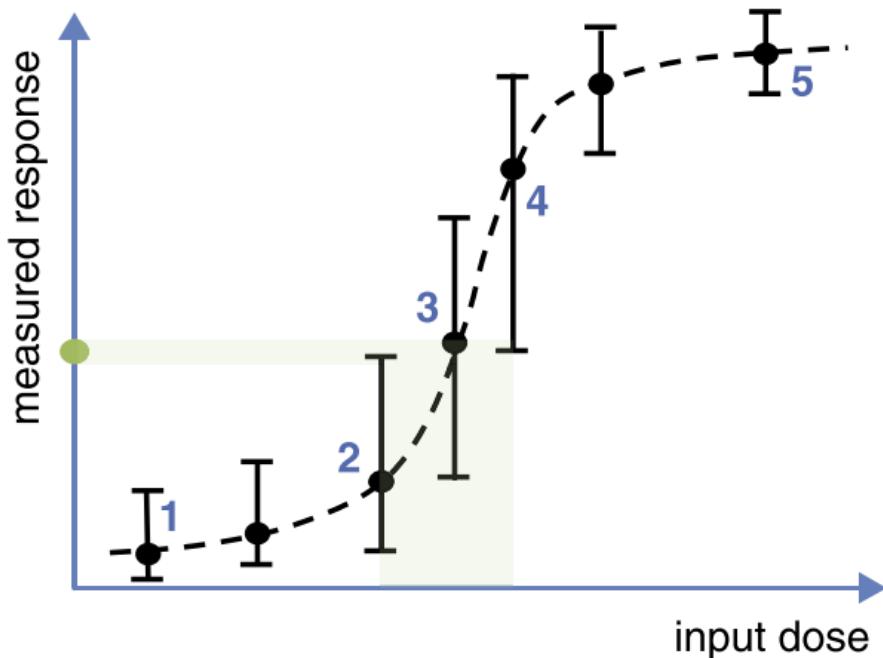


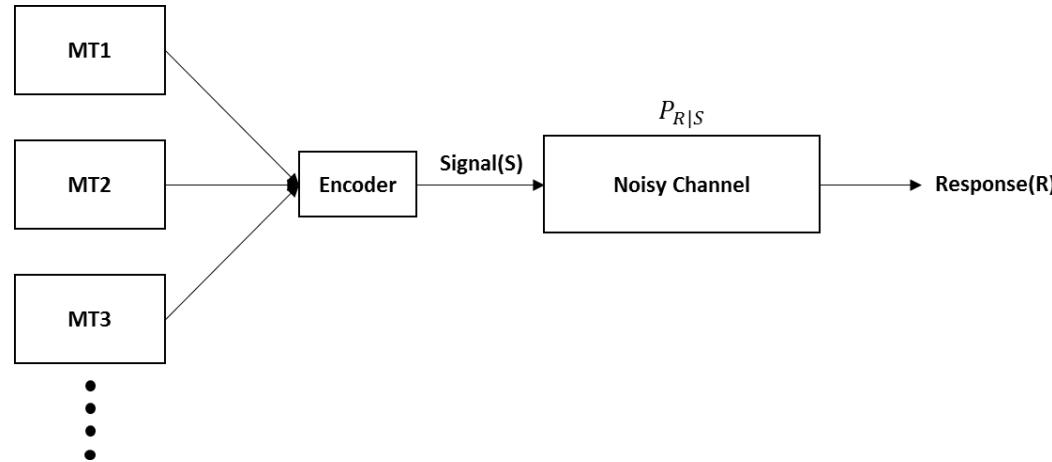
Table 13.1 Important Parameters of the VTC of the Logic Inverter (Refer to Fig. 13.3)

V_{OL} :	Output low level
V_{OH} :	Output high level
V_{IL} :	Maximum value of input interpreted by the inverter as a logic 0
V_{IH} :	Minimum value of input interpreted by the inverter as a logic 1
NM_L :	Noise margin for low input = $V_{IL} - V_{OL}$
NM_H :	Noise margin for high input = $V_{OH} - V_{IH}$

Biochemical Noise Limits the information-transmission rate

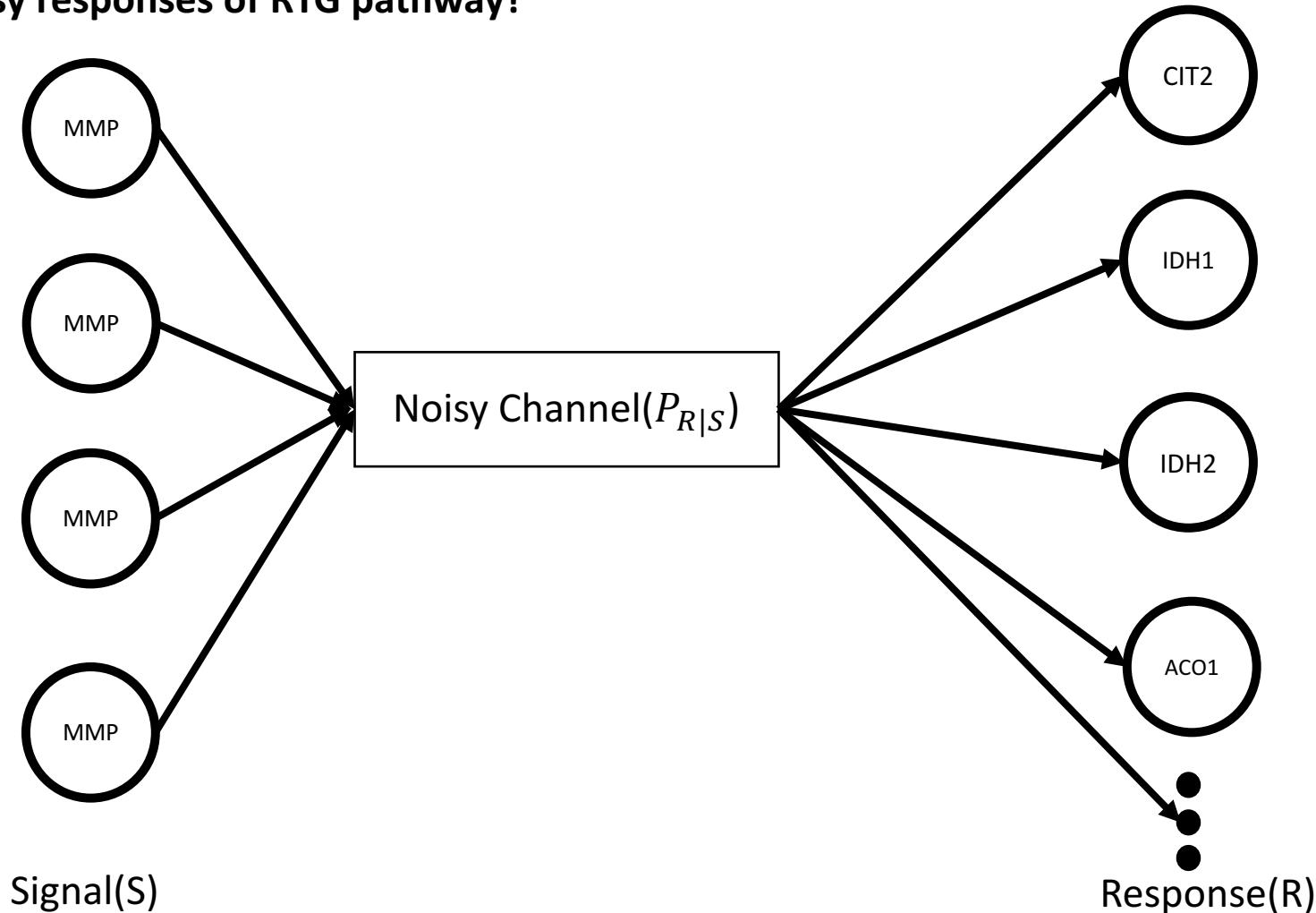


Biochemical Pathway of Retrograde Response



Problem Setup

- What is the maximum number of MMP stimuli that a cell can distinguish based upon the noisy responses of RTG pathway?



Key Points in Information Theory

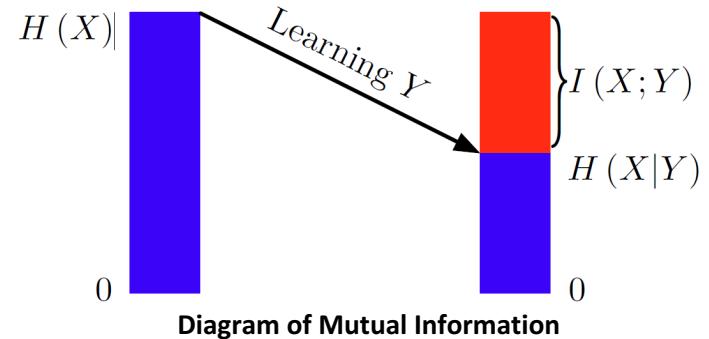
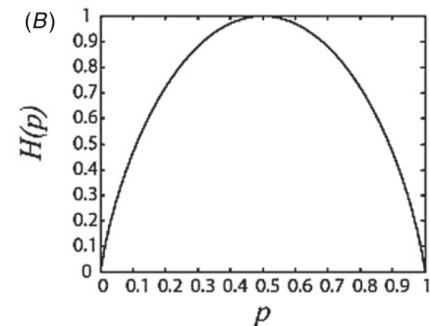
1. Shannon's information

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

where X is a random variable.

2. Mutual Information

- $I(X; Y) = H(X) - H(X|Y)$
- Mutual information is symmetric. $I(X; Y) = I(Y; X)$
- $I(X; Y)$ is a concave function of P_X for fixed $P_{Y|X}$.
- $I(X; Y) = I(X'; Y')$,
for all invertible $X' = X'(X)$, $Y' = Y'(Y)$



3. Channel Capacity

- For a discrete memoryless channel $(X, P_{Y|X}, Y)$, the maximum rate of information transmitting with vanishing error probability is
- $C = \sup_{P_X(\cdot)} I(X; Y)$ called channel capacity (C)
- Feedback does not affect the channel capacity.

Response and Mutual Information

$$I(X; Y) = H(X) - H(X|Y)$$

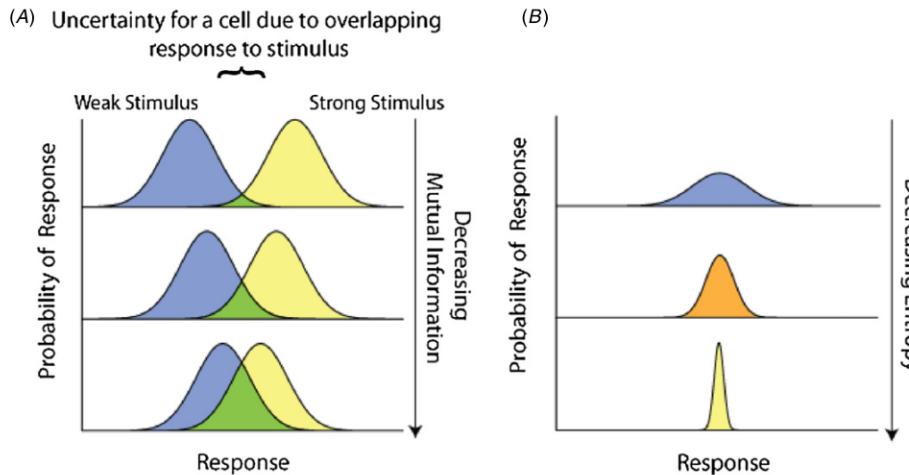


Figure 1. (A) Noise can limit the amount of information a cell can obtain about a stimulus. The magnitude of noise is evidenced in the breadth of the probability distribution of the response to a given stimulus. For sufficiently large noise, a cell which can encounter strong or weak stimuli cannot use its response to discern which stimulus was encountered with absolute precision. Consequently, from the cell's perspective, noise leads to a loss of information about the input. The amount of mutual information between the stimulus and cellular response also suffers such that the greater the overlap between distributions, the less mutual information is communicated. (B) Entropy can be understood as a measure of dispersion. A wider probability distribution corresponds to an increase in the uncertainty of the cellular response and consequently, entropy.

A workflow of measurement of channel capacity

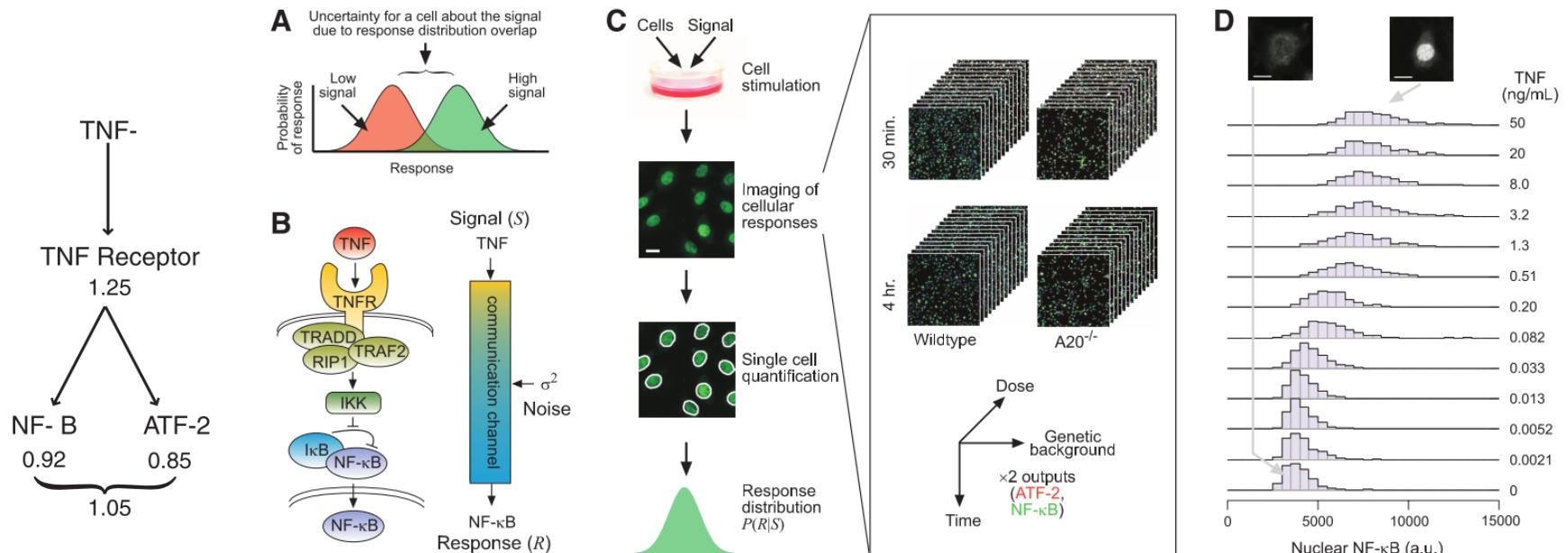


Fig. 1. Information theoretic analysis of cell signaling fidelity. **(A)** Schematic showing information loss due to overlapping noisy response distributions. **(B)** Diagram of the TNF–NF- κ B signaling pathway represented in biochemical form (left) and as a noisy communication channel (right). **(C)** Experimental flowchart for using immunocytochemistry to sample the conditional response distribution at single-cell resolution and resulting four-dimensional compen-

dium of multiple responses in cells of multiple genetic backgrounds to multiple TNF concentrations, at multiple time points. The data were collected in a single experiment, allowing controlled, quantitative comparisons along each dimension. **(D)** Distributions of noisy NF- κ B nuclear translocation responses to 30-min TNF exposure (examples shown at top) used to compute the channel capacity of the TNF–NF- κ B pathway. Scale bars, 20 μ m.

Signaling networks

- Prediction of channel capacity based on bush and tree model

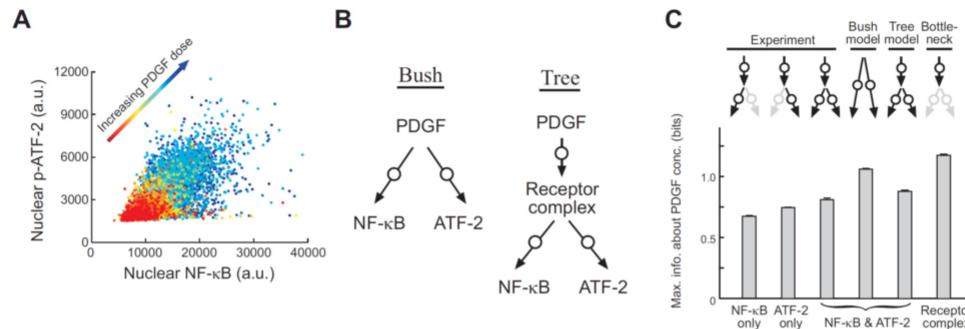


Fig. S11. The PDGF signaling network contains an information bottleneck. (A) Scatter plot showing nuclear NF-κB and ATF-2 responses to 30 min. stimulation of PDGF. Each datapoint represents a single cell, and each concentration of TNF examined is shown using a distinct color. (B) Schematics of information flow through the PDGF signaling network highlighting the experimentally testable hypotheses of whether the network lacks (bush model, left) or contains (tree model, right) an information bottleneck due to the steps of receptor complex activation common to multiple PDGF signaling pathways. (C) Comparison of bush and tree model predictions for the capacity of the PDGF network to experimental values. At 30 min., the NF-κB and ATF-2 pathways together capture more information about PDGF concentration than either pathway alone (bars 1-3), and the tree rather than bush model accurately predicts this increase (bars 3-5). The tree model further predicts a receptor level bottleneck of 1.18 ± 0.01 bits (bar 6).

Discussion

- Is MMP homogeneous among the mitochondrial population?
 - Confocal image
- How to quantitatively measure MMP and retrograde responses in single-level and time-series
 - PMPI, TMRM, RTG3p-BFP, mitotracker, H2B-BFP
- What is the biological meaning of the channel capacity of mitochondrial retrograde response
 - Does achieving the channel capacity benefit the cell?
 - How does the biochemical network contribute to the channel properties?
 - Are there predictive properties in retrograde response?

References

1. R.Cheong, A.Rhee, C.Joanne Wang, I.Nemenman, and A.Levchenko, "Information Transduction Capacity of Noisy Biochemical Signaling Networks," 2011.

This is the main paper.

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A. a Gerencser *et al.*, "Quantitative measurement of mitochondrial membrane potential in cultured cells: calcium-induced de- and hyperpolarization of neuronal mitochondria," *J. Physiol.*, vol. 590, pp. 2845–71, 2012.

This methodology is what I plan to use in my project, while it uses confocal instead of cytometry.

H.Rottenberg and S.Wu, "Quantitative assay by flow cytometry of the mitochondrial membrane potential in intact cells," *Biochim. Biophys. Acta*, vol. 1404, pp. 393–404, 1998.

Another way to measure MMP.

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