# Exploring Transcriptional Regulation through Genetic Dissection

**Nir Friedman**

Hebrew University

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- “Next time when you do this, run the sample through BioAnalyzer as well”
- “To check this, you should rerun without salt”
- “Who moved my pipette?!?!”

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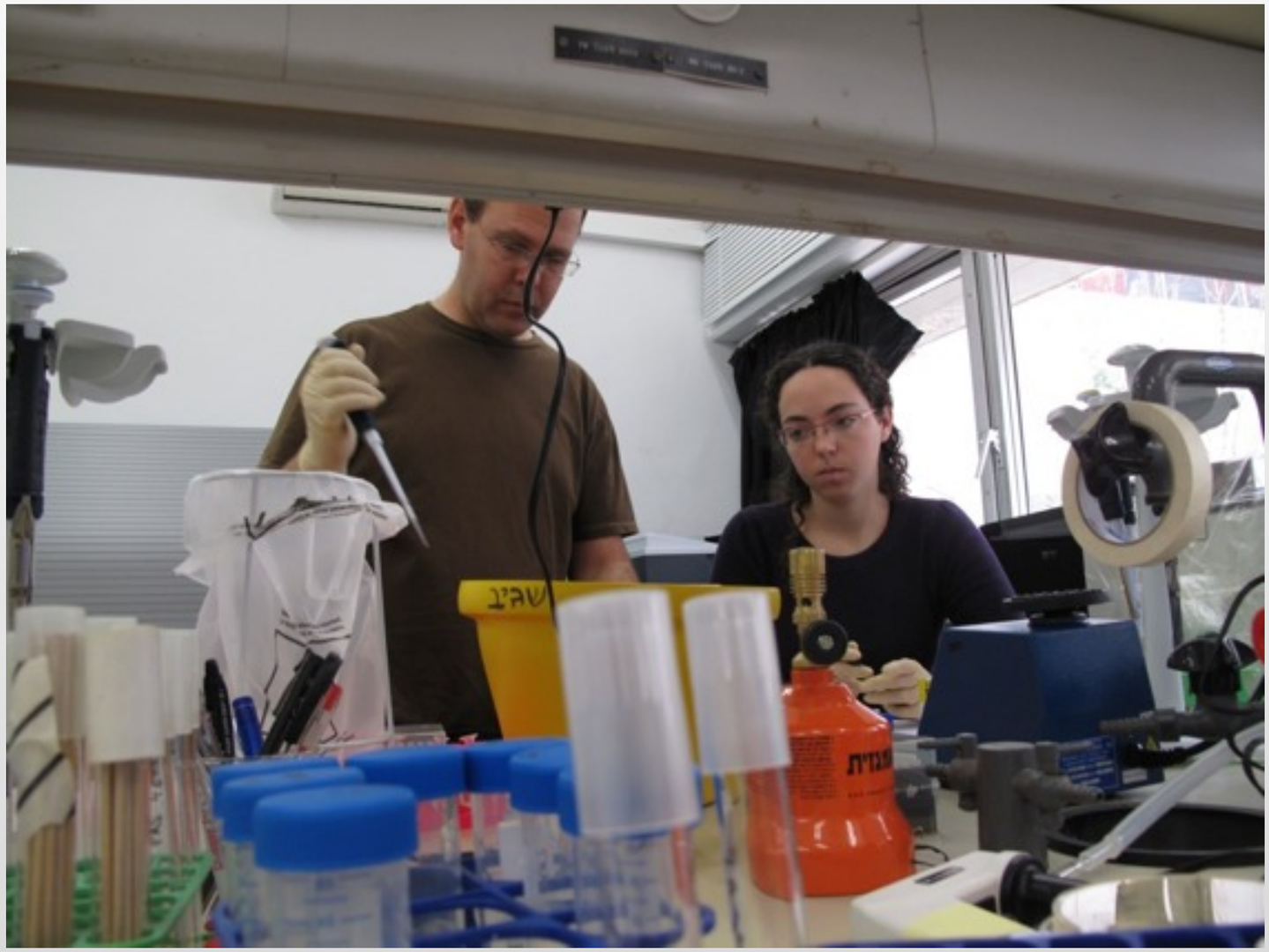

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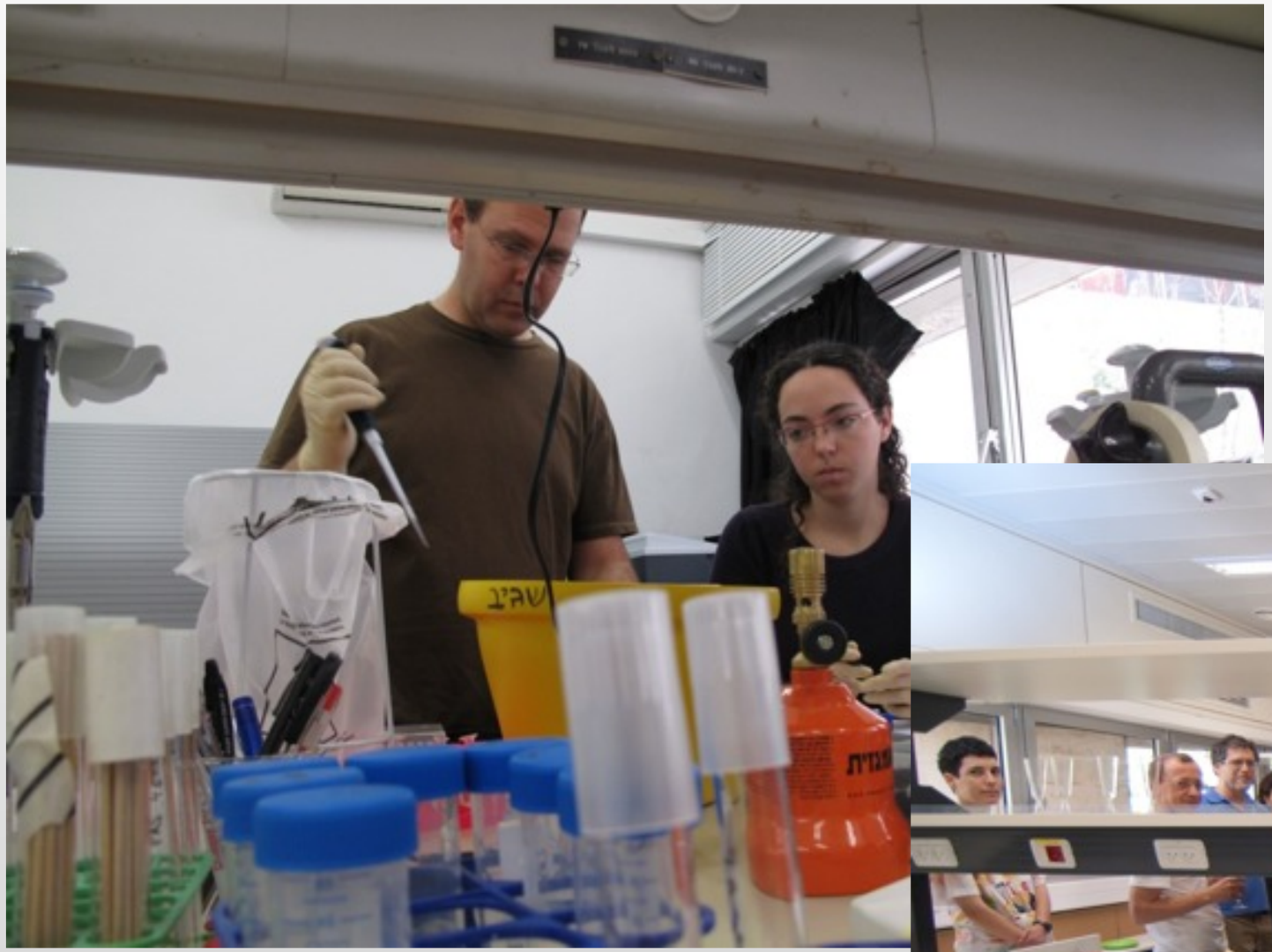
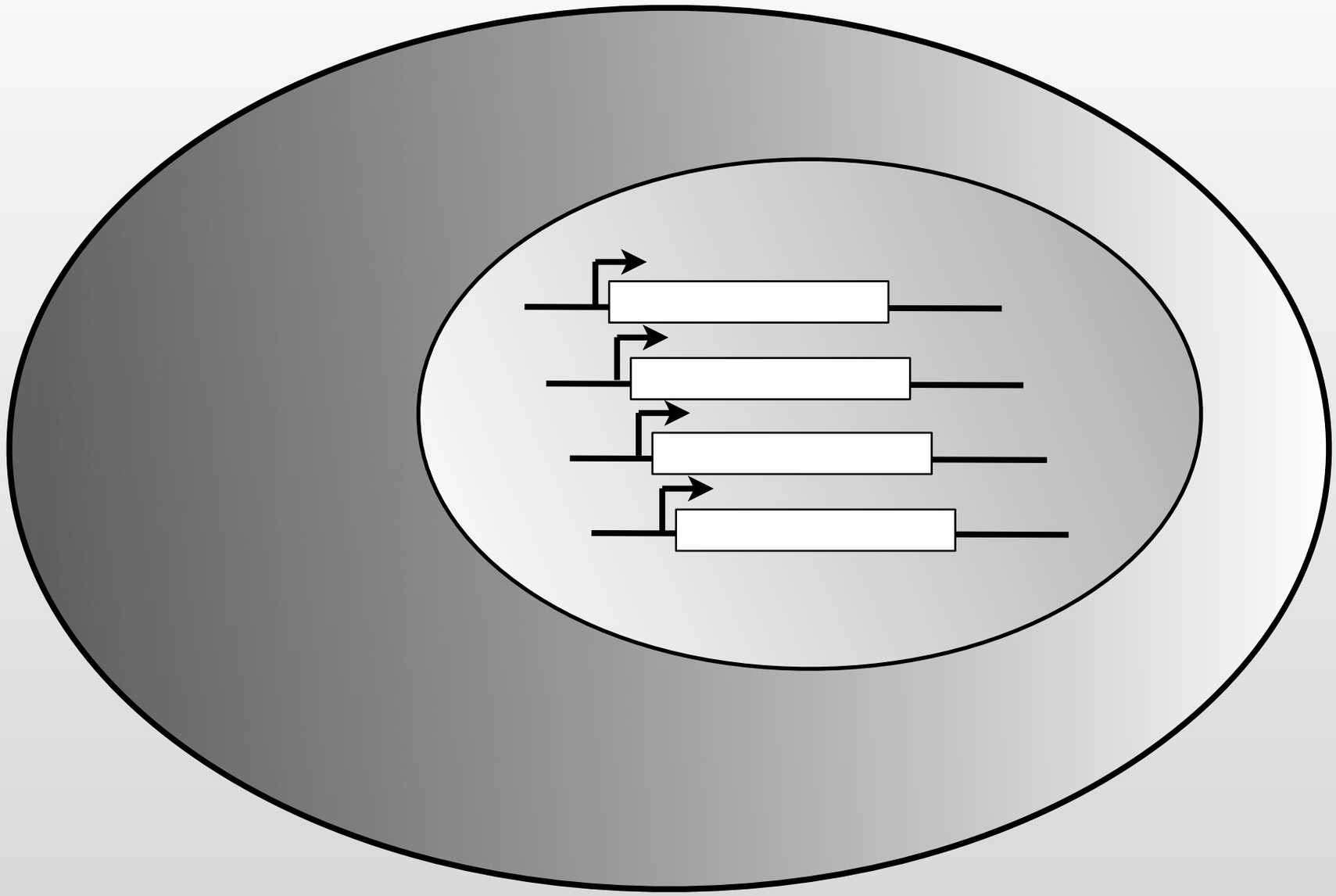


# Big Question



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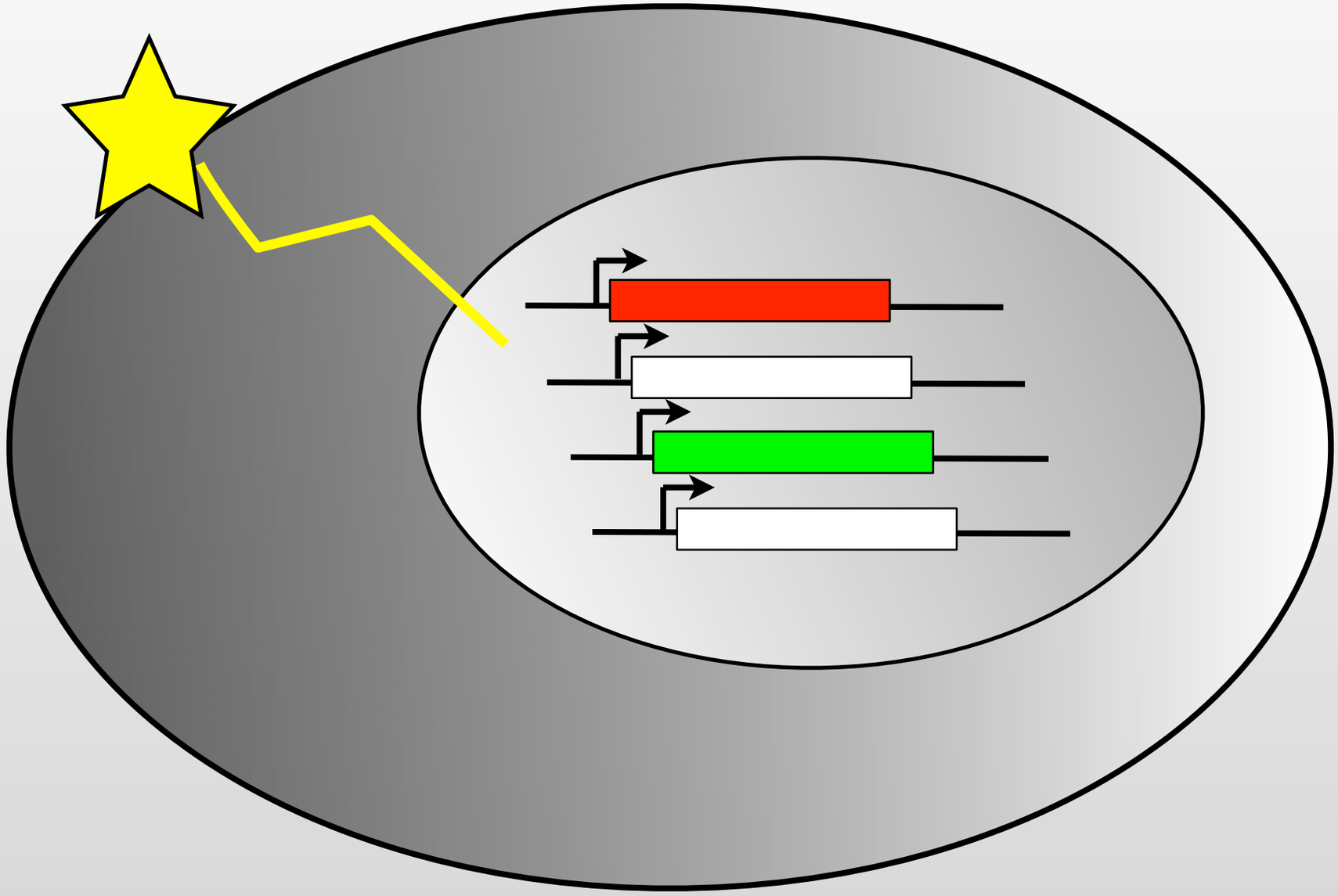
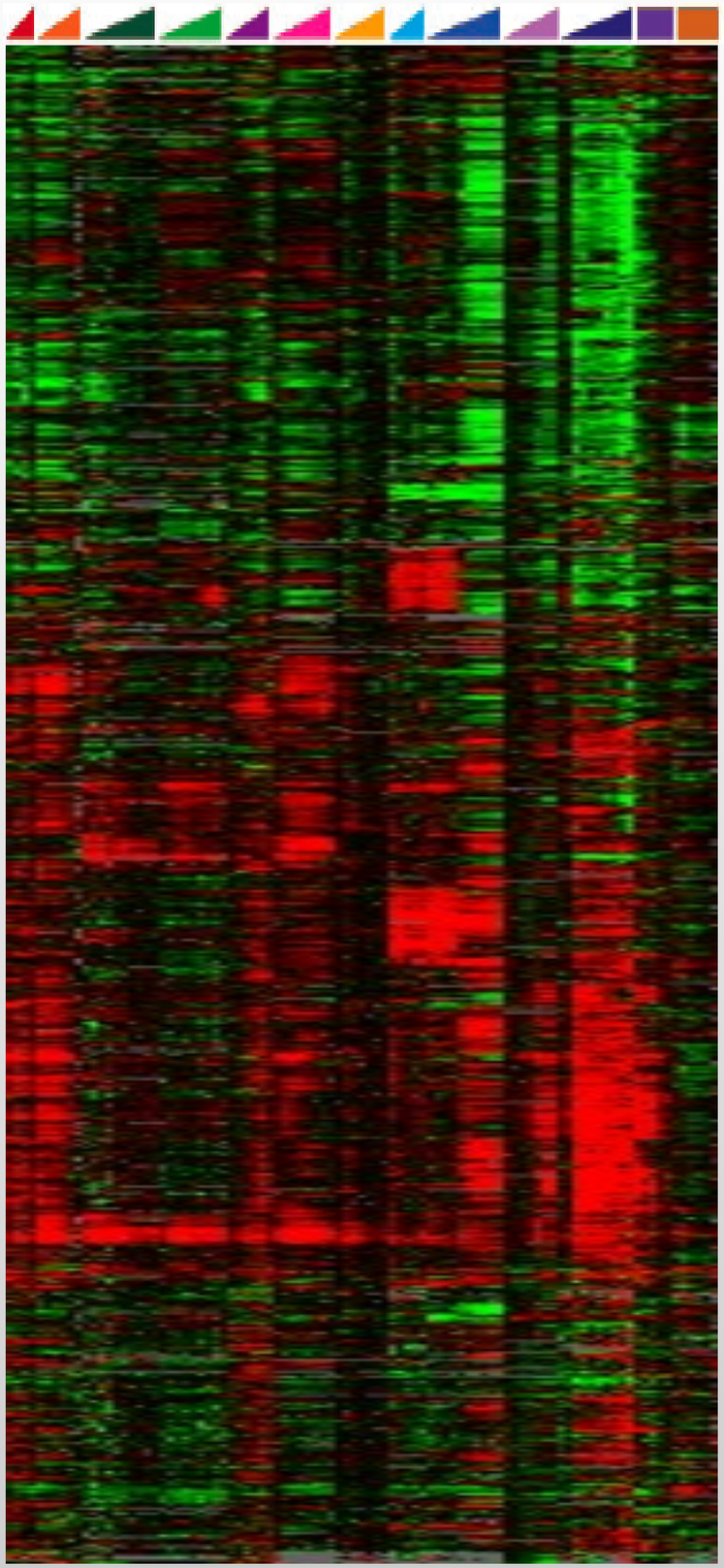
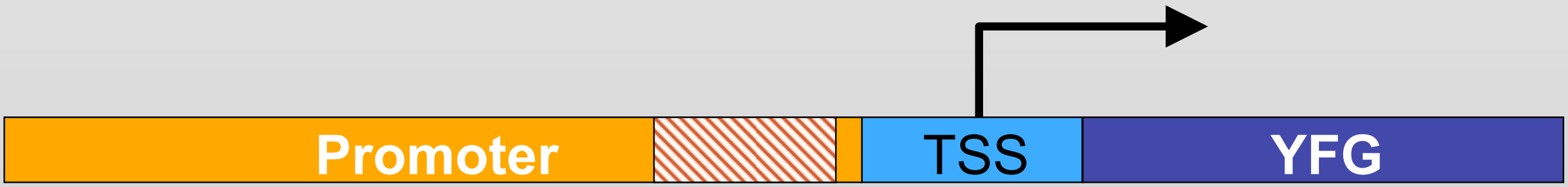




- What determines specific expression of each gene?

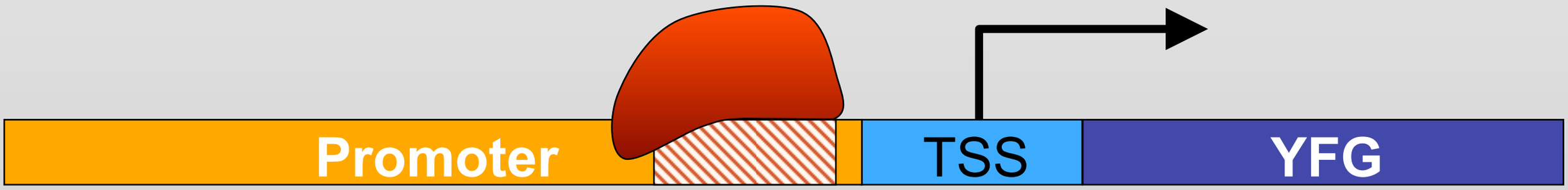
**Gasch *et al*, *Mol. Bio Cell.* 2000**

# Transcriptional Regulation 101



# Transcriptional Regulation 101

- Transcription factors bind to regulatory regions



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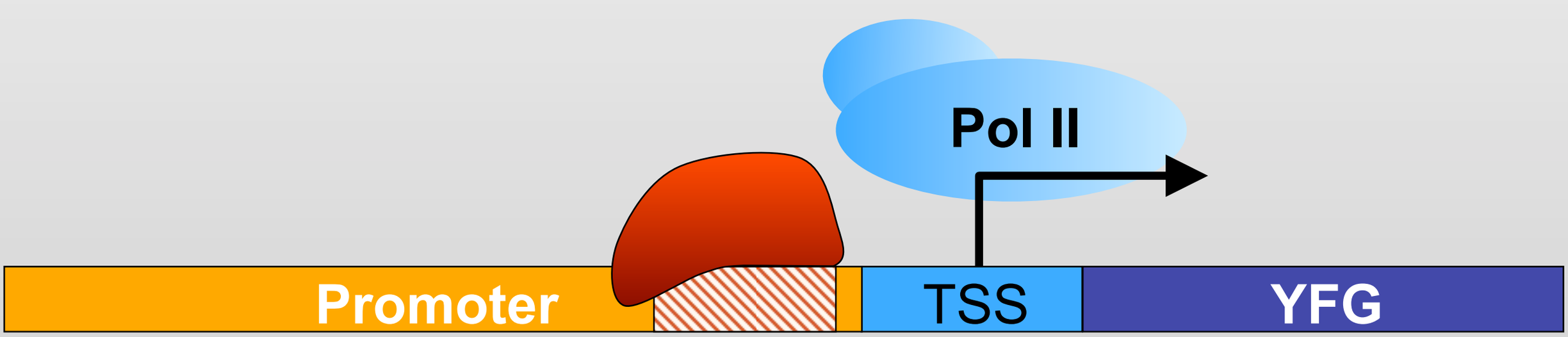

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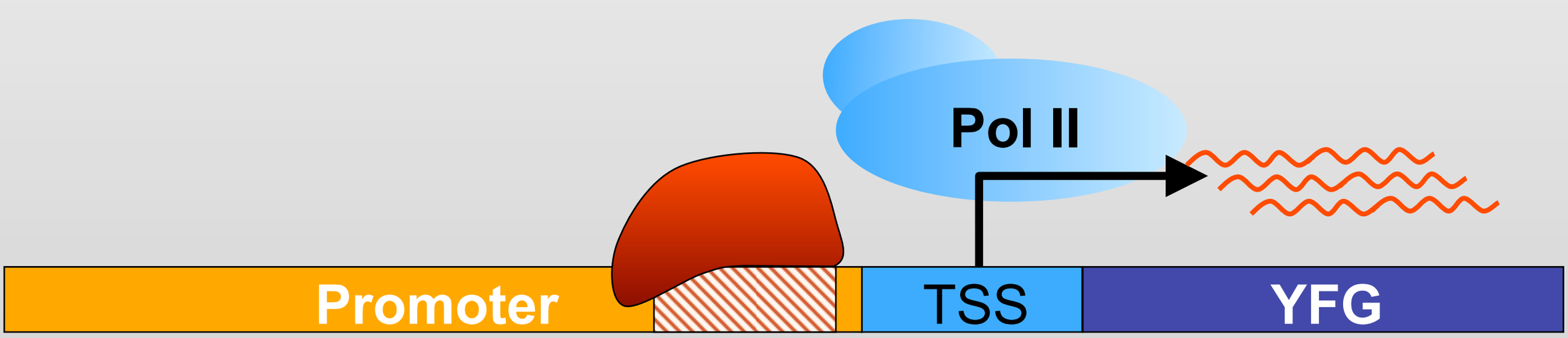
- Transcription factors bind to regulatory regions
- Recruitment of Polymerase II
- Transcription



# The HOG1 pathway

Turgor Pressure

Mbs2

Sho1

Sln1

MAPKK Pbs2

MAPK Hog1

Stress

Msn2/4

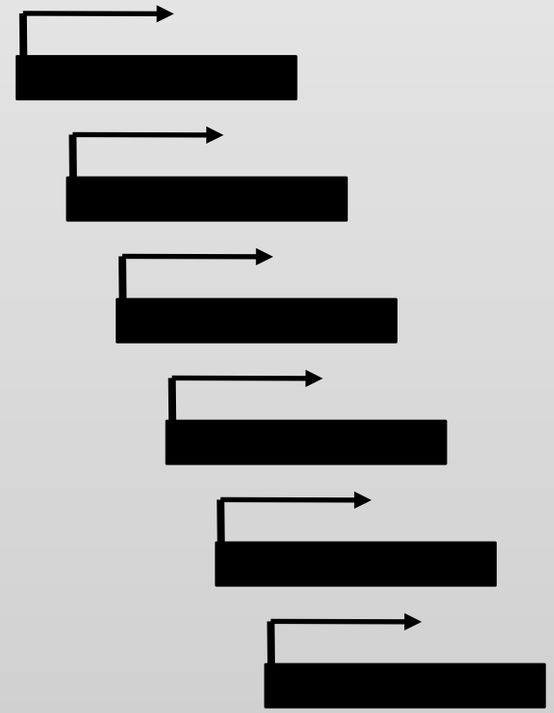
Msn2/4

TF activation

PP

Hog1

Sko1
Hot1
Msn1
Smp1
Cin5
Skn7

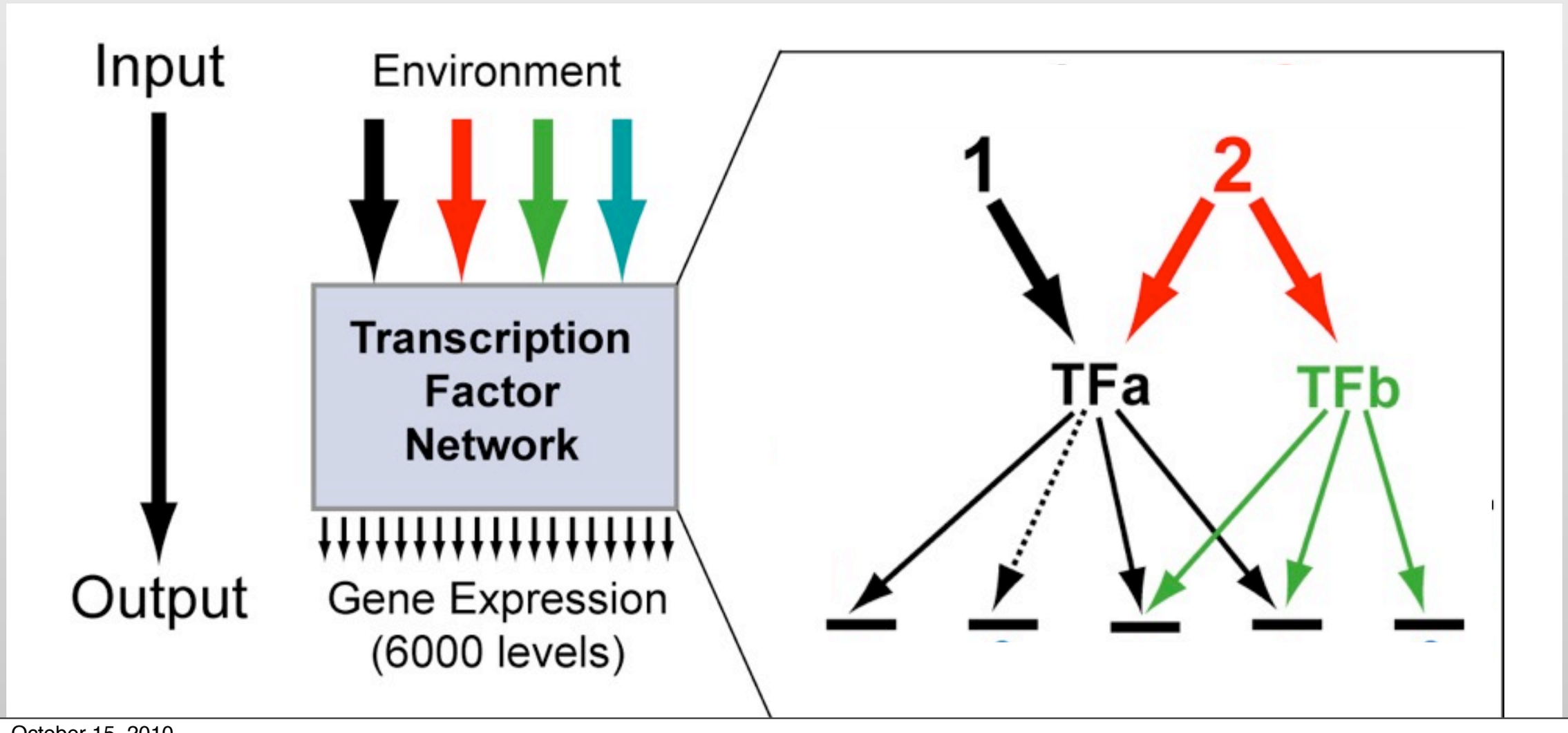


Cytoplasm | Nucleus

# Key Questions

## Mechanisms

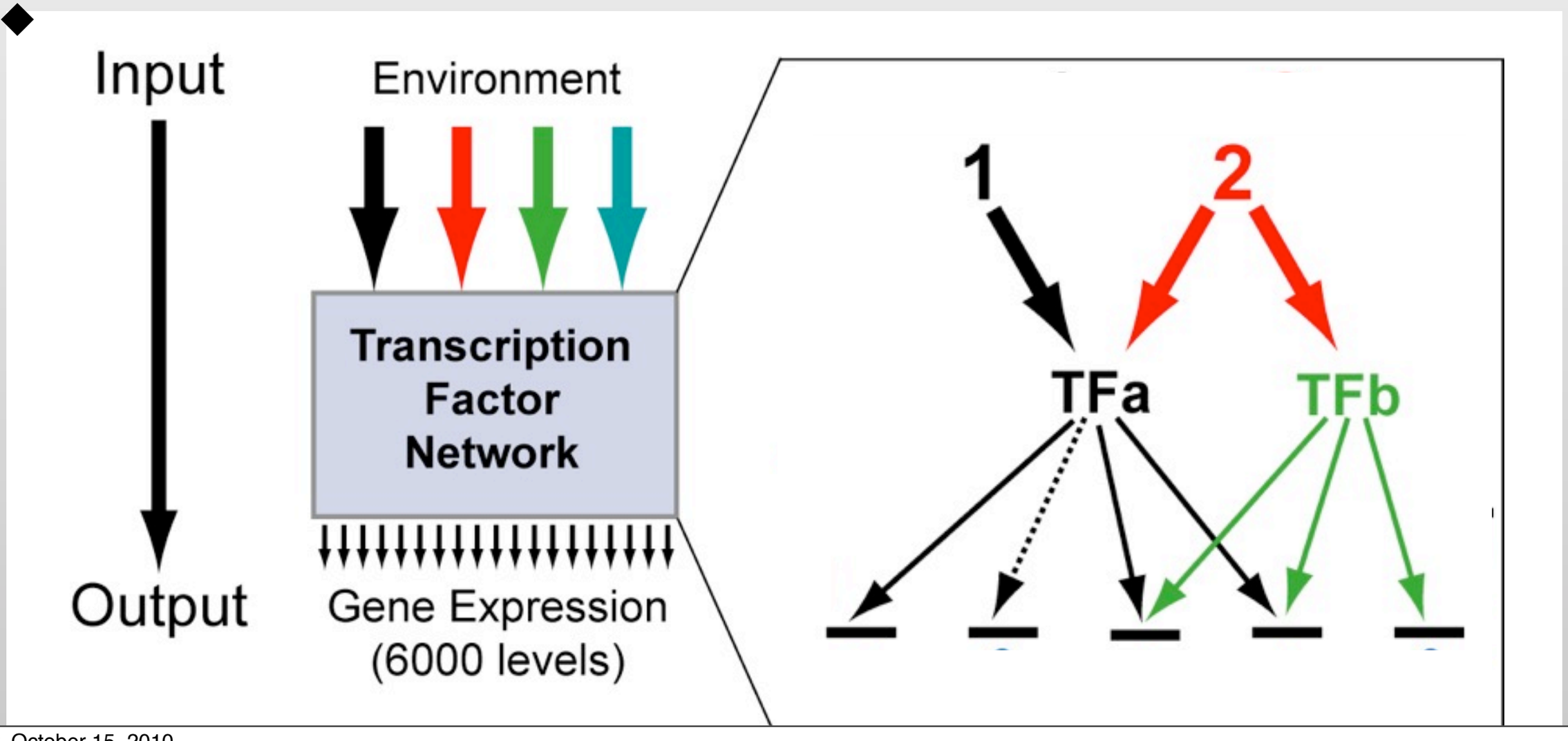
- Signal transduction
- Transcriptional regulation
- Chromatin



# Key Questions

## Combinatorial Interactions

- Enormous number of responses (modules)
- Limited number of signaling pathways and TFs



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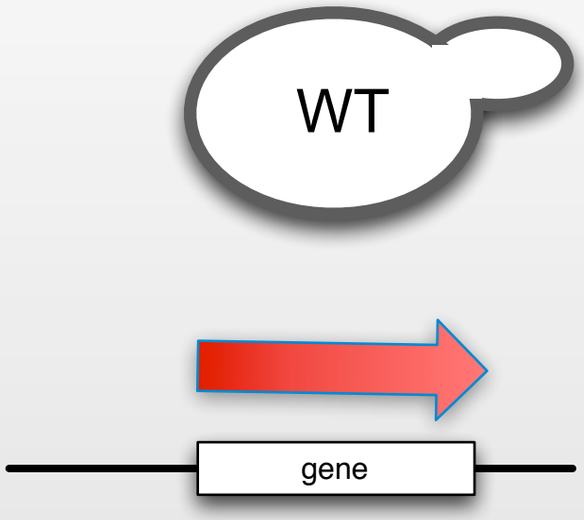

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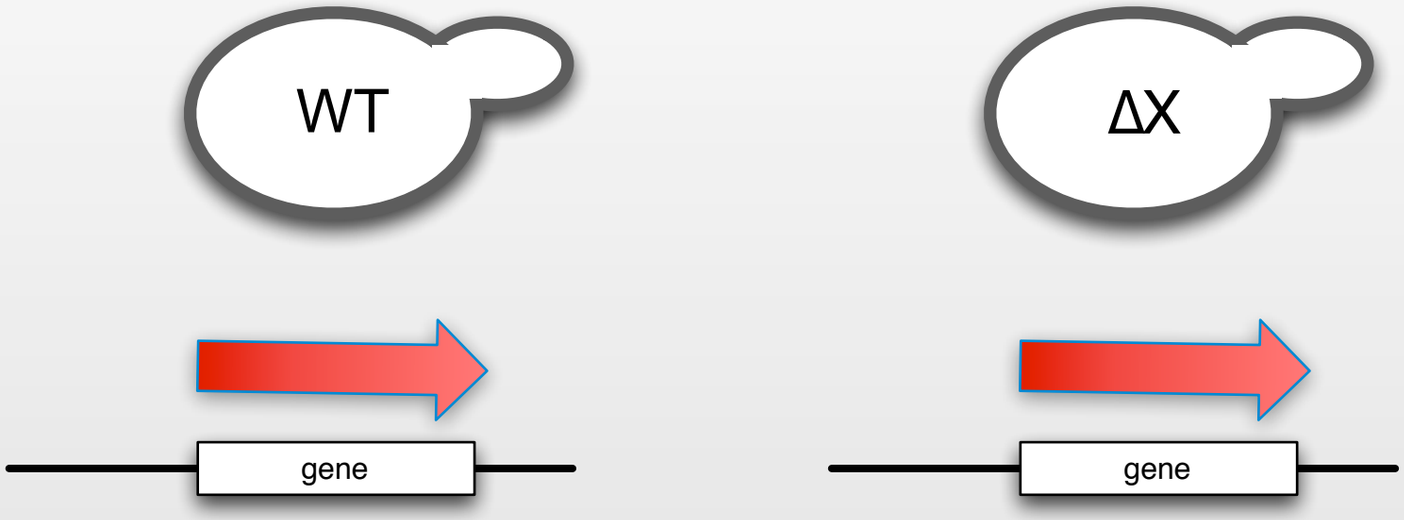

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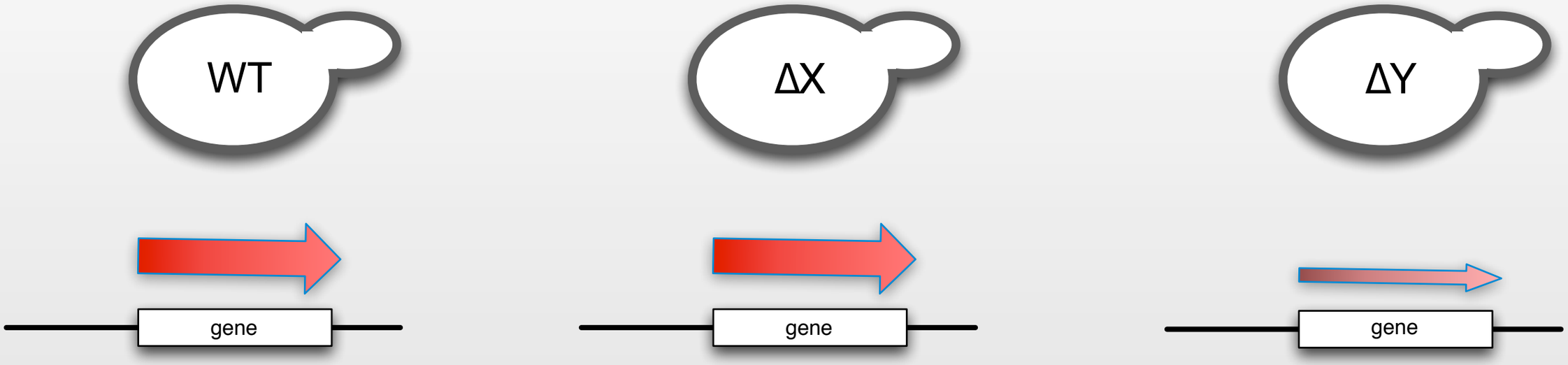

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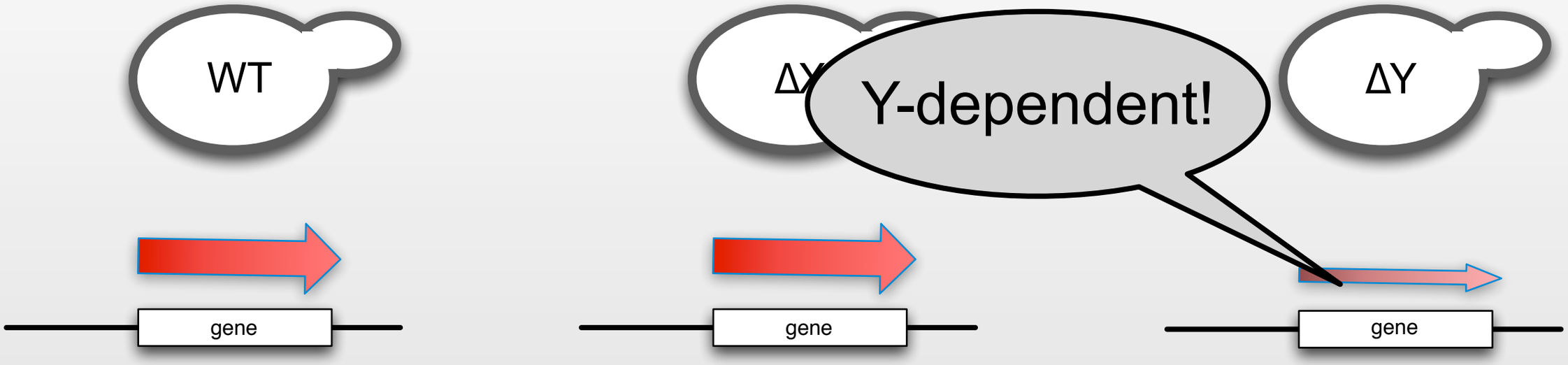

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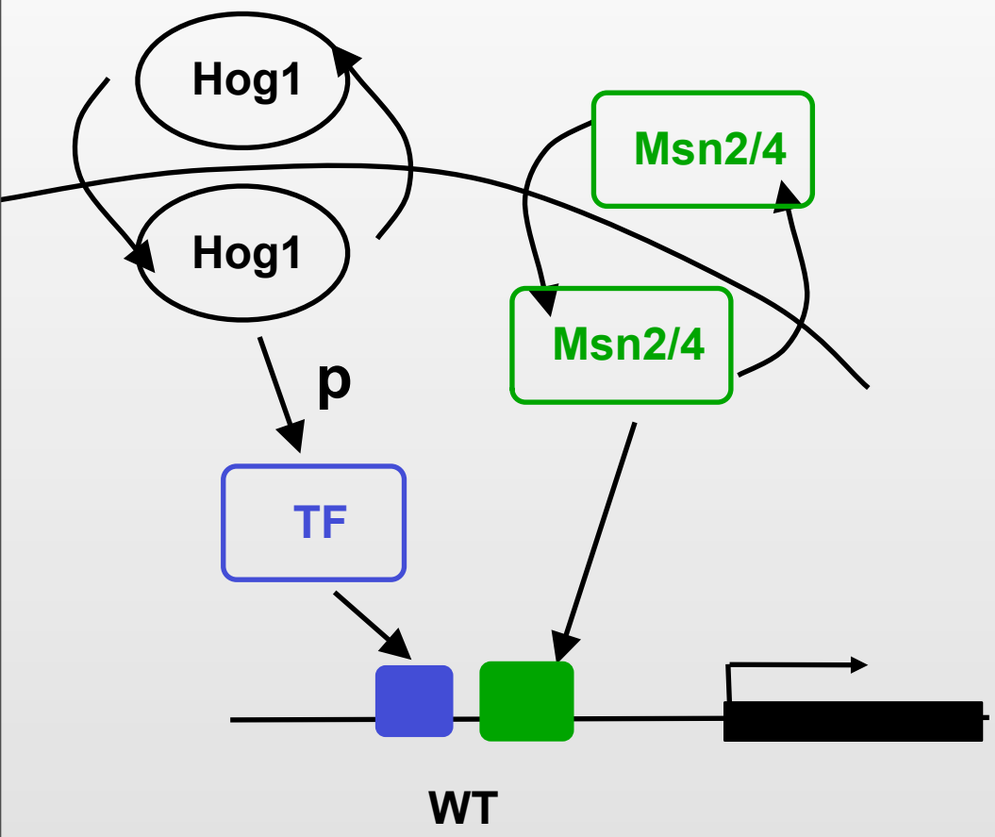


WT

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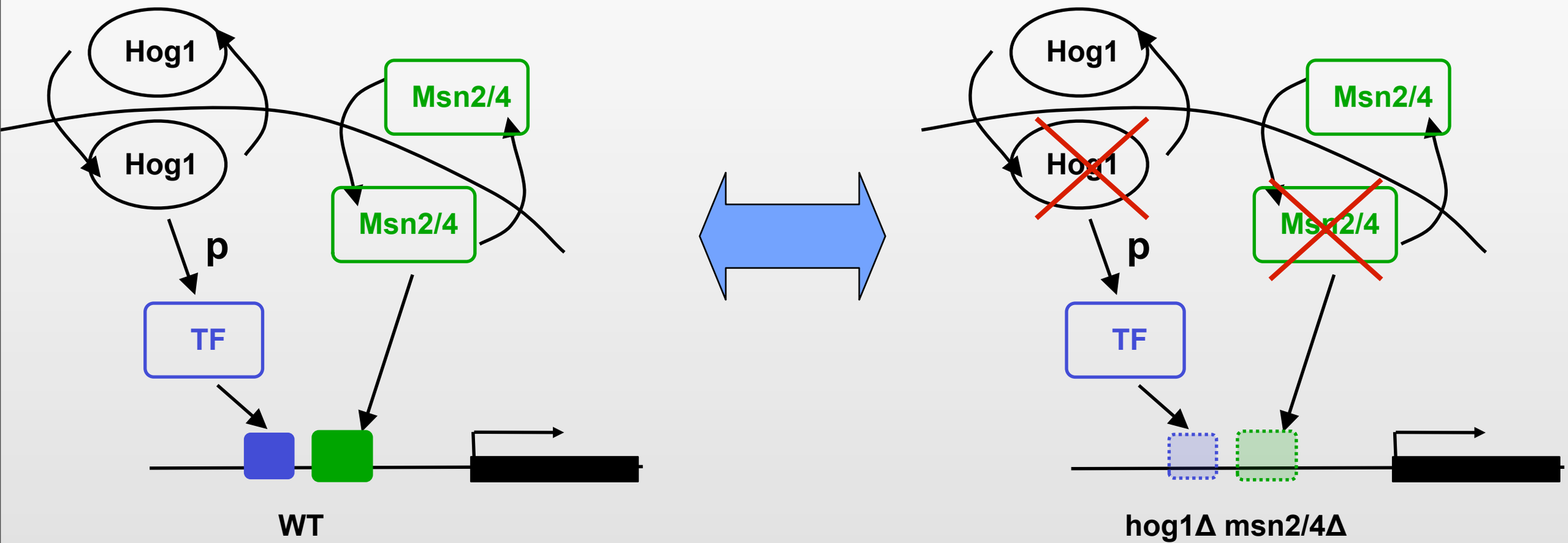


**WT** **hog1Δ msn2/4Δ**

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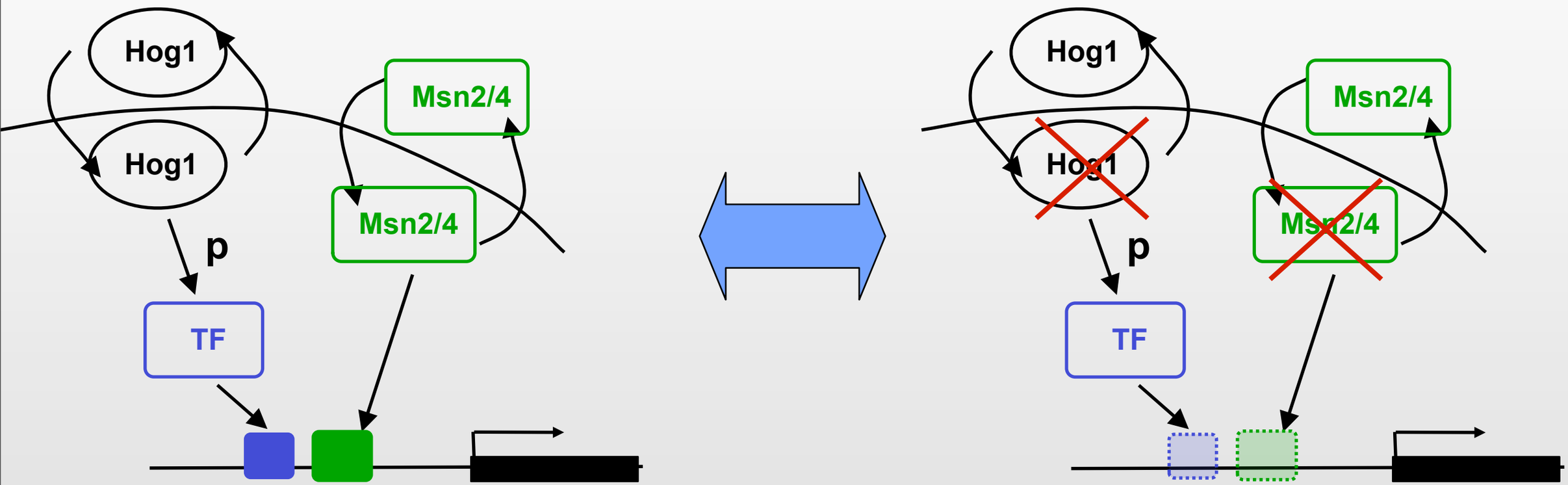


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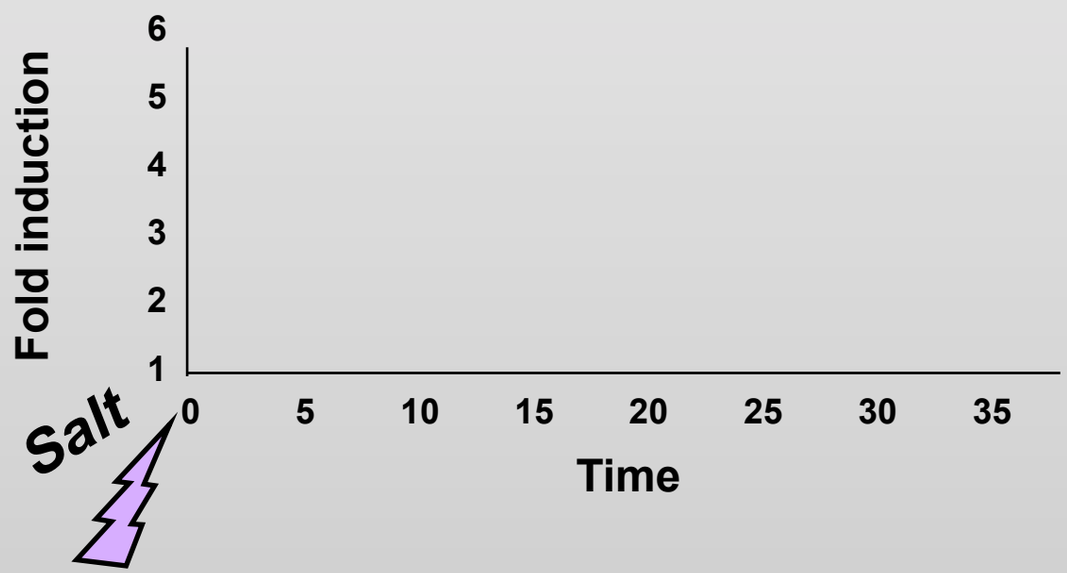

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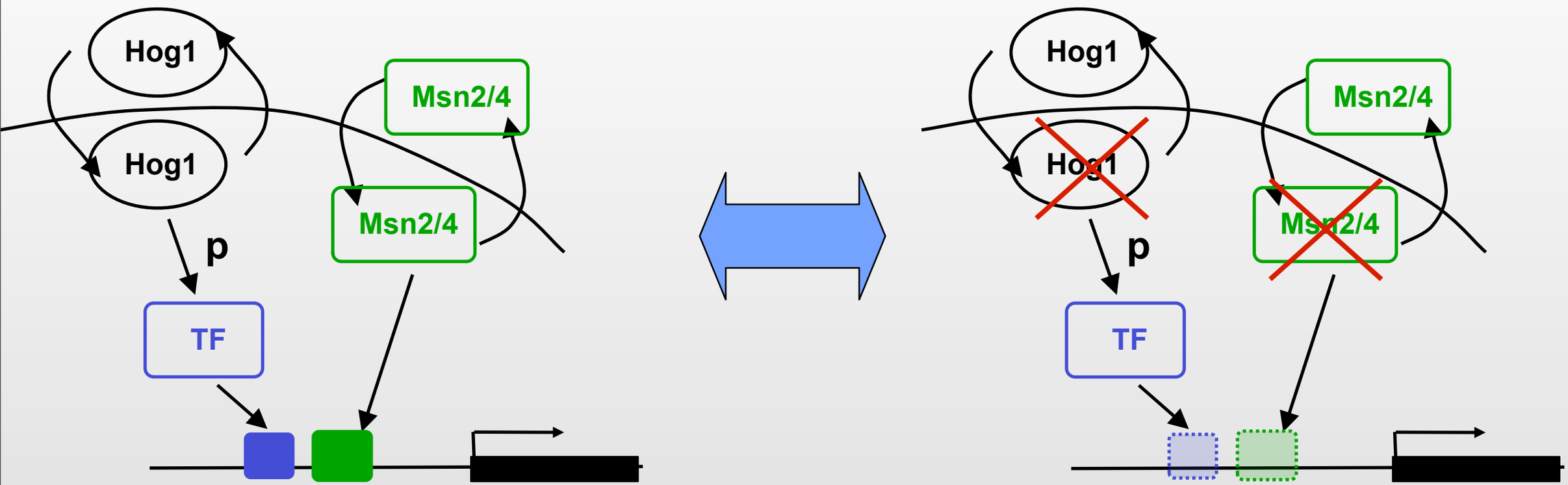


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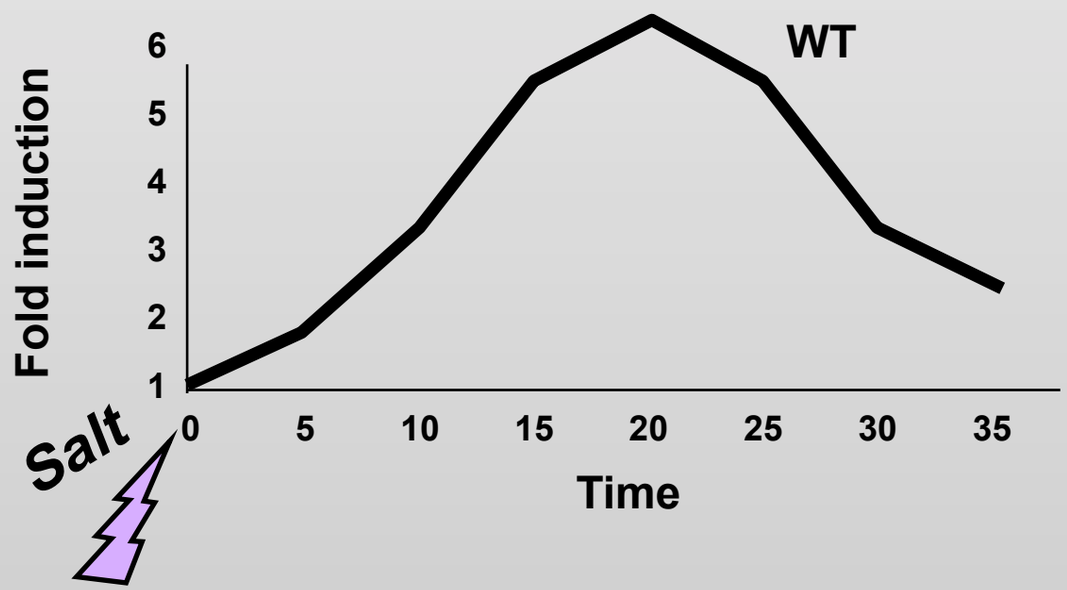

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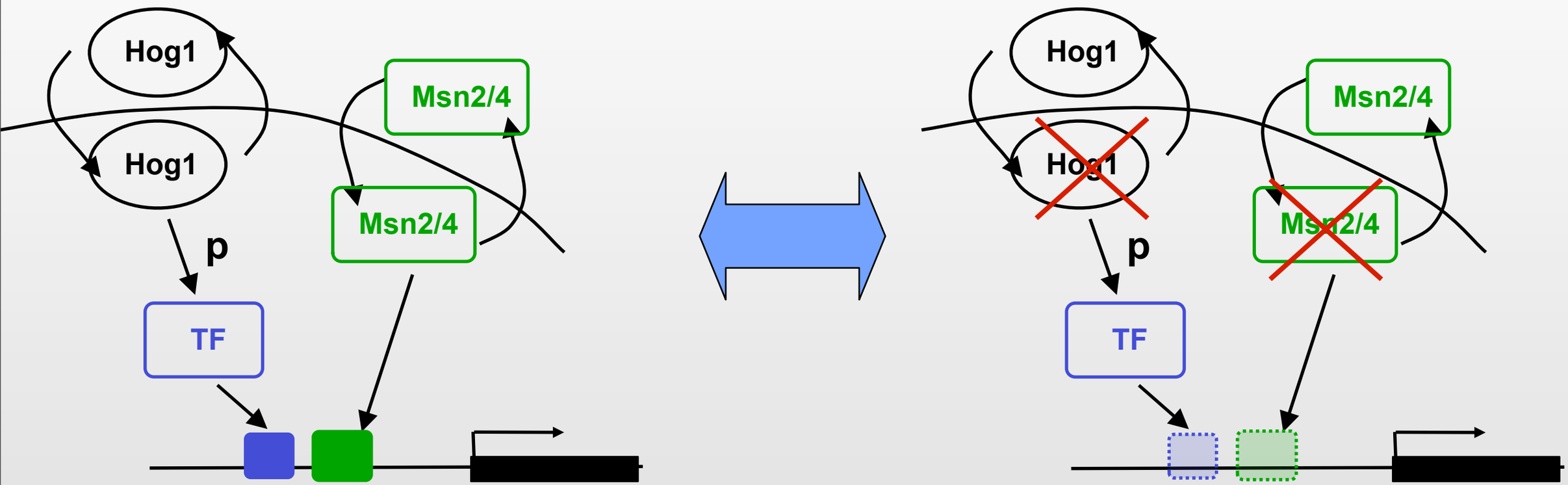


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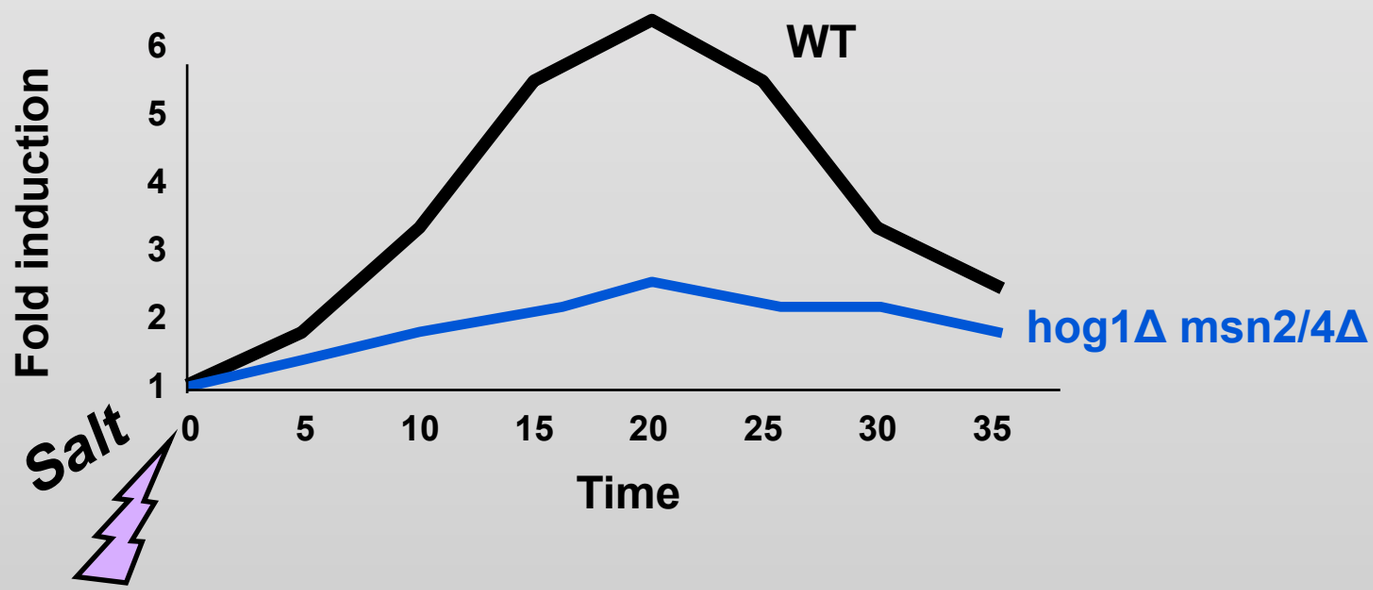

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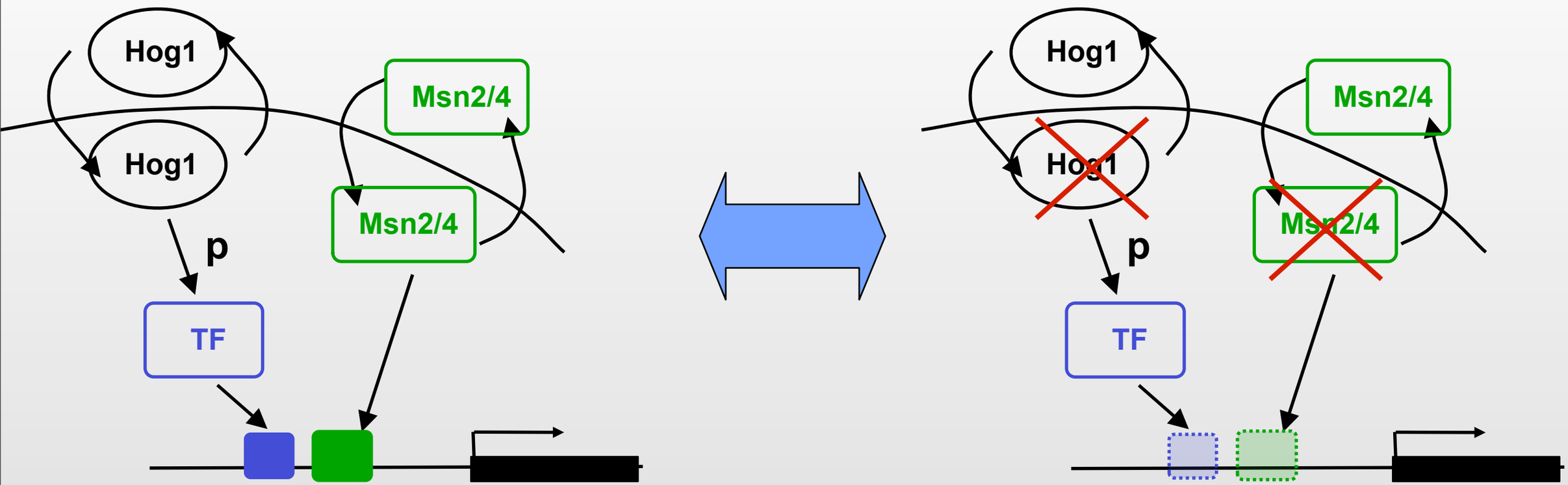


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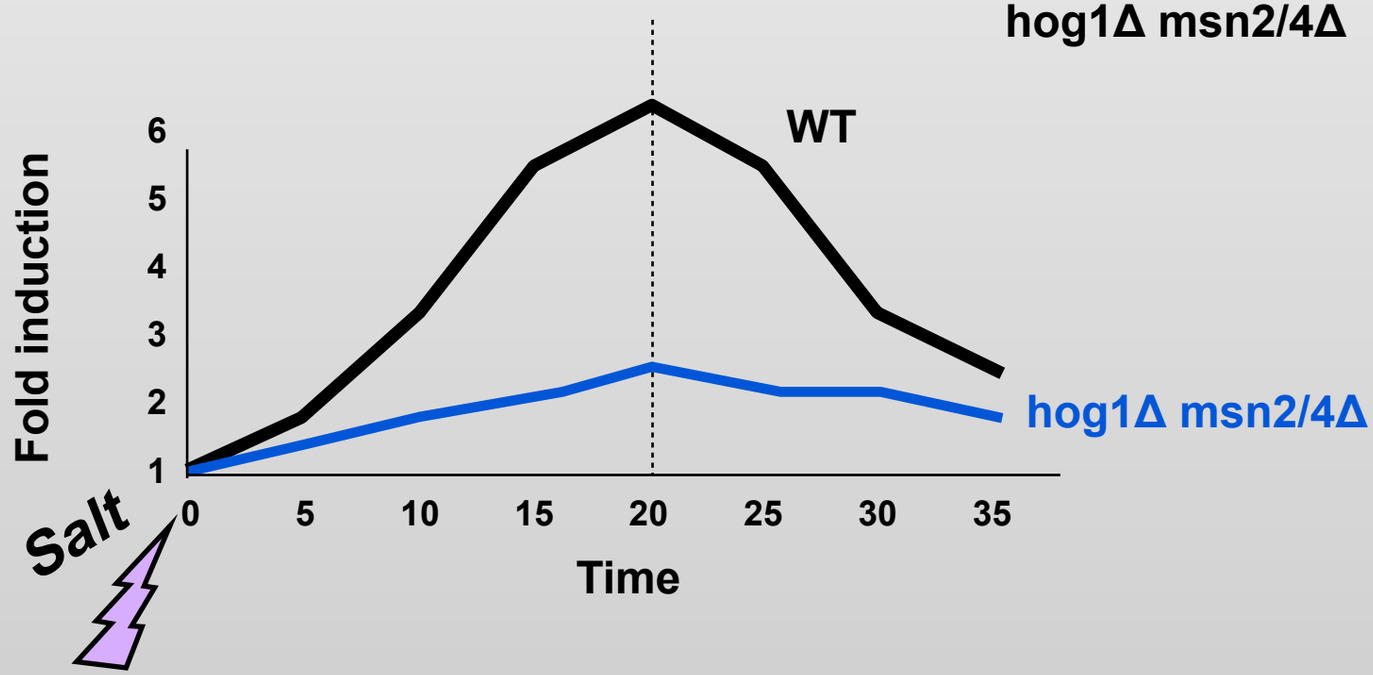

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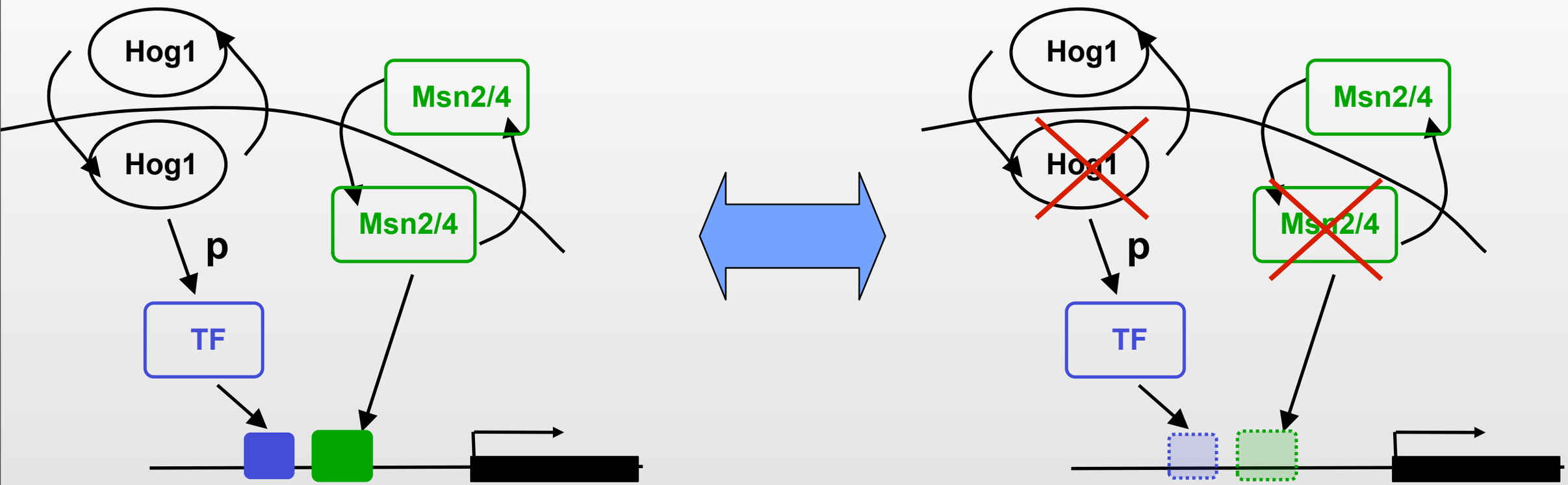


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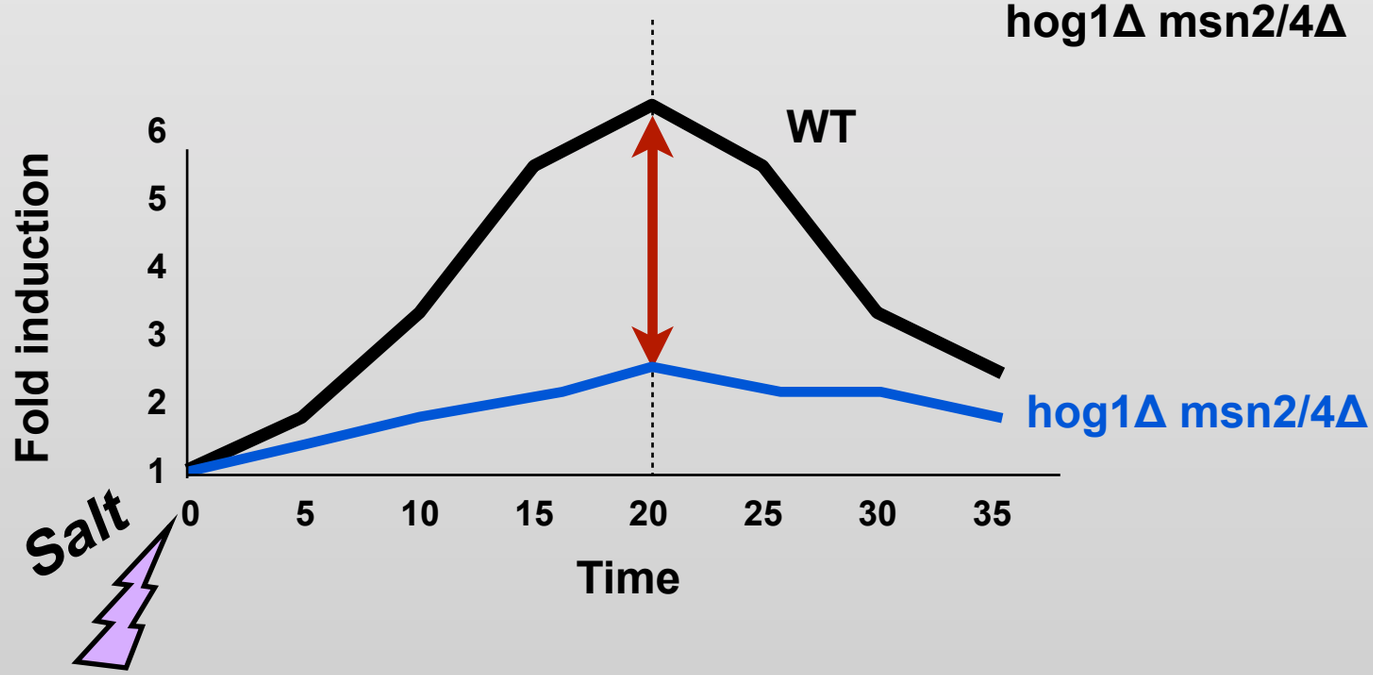

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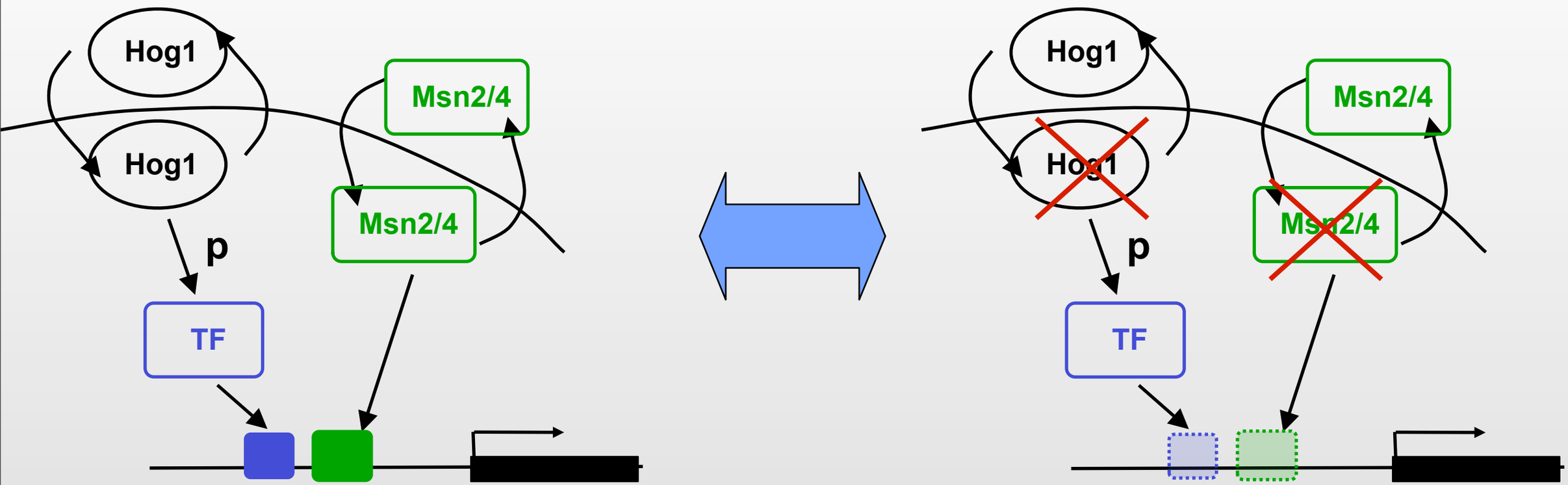
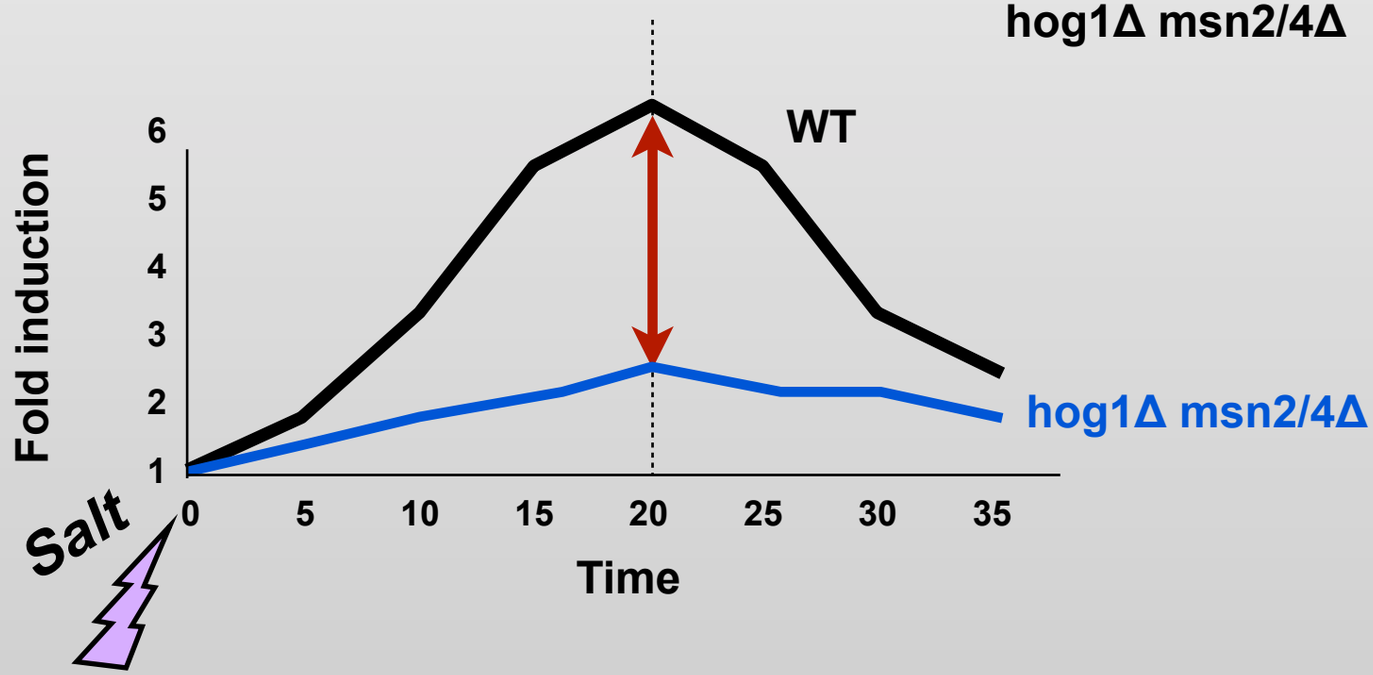
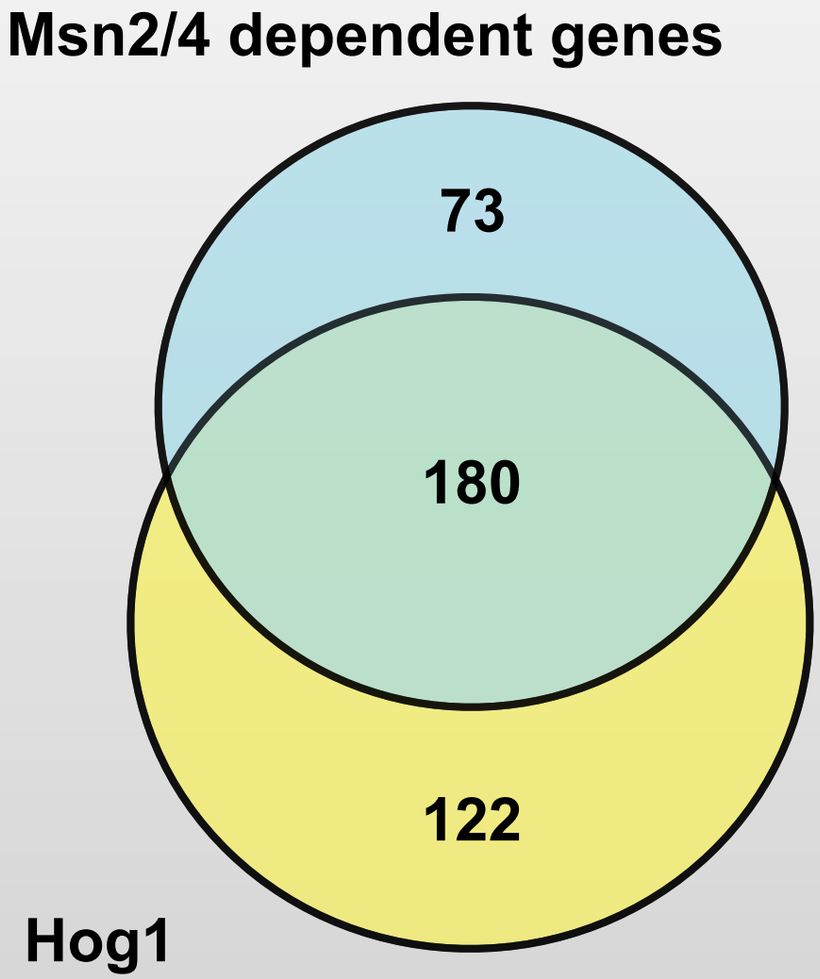



- ~240 Hog1 & Msn2/4-pathway activated genes

# Single KOs Provide Limited Information



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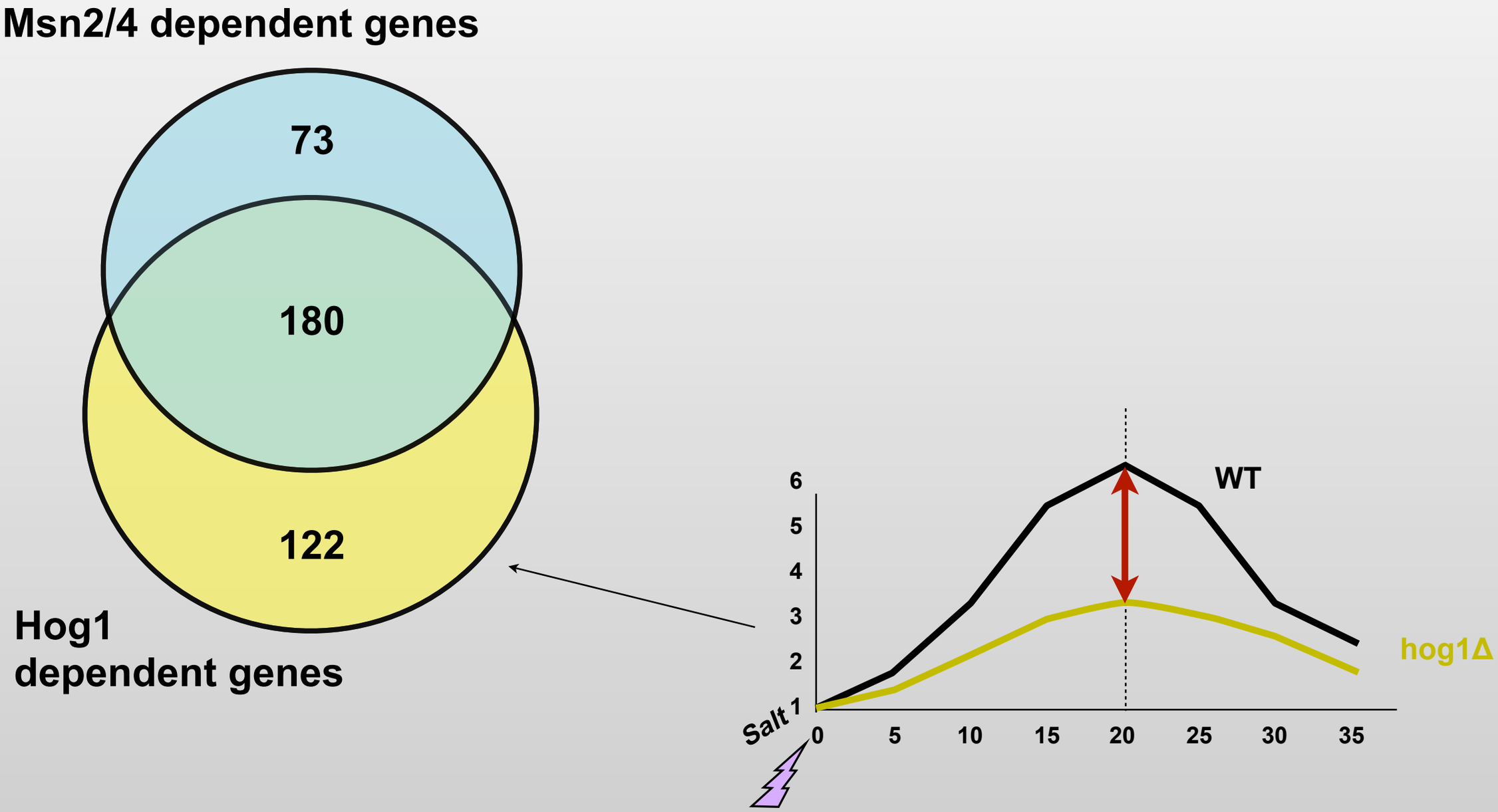

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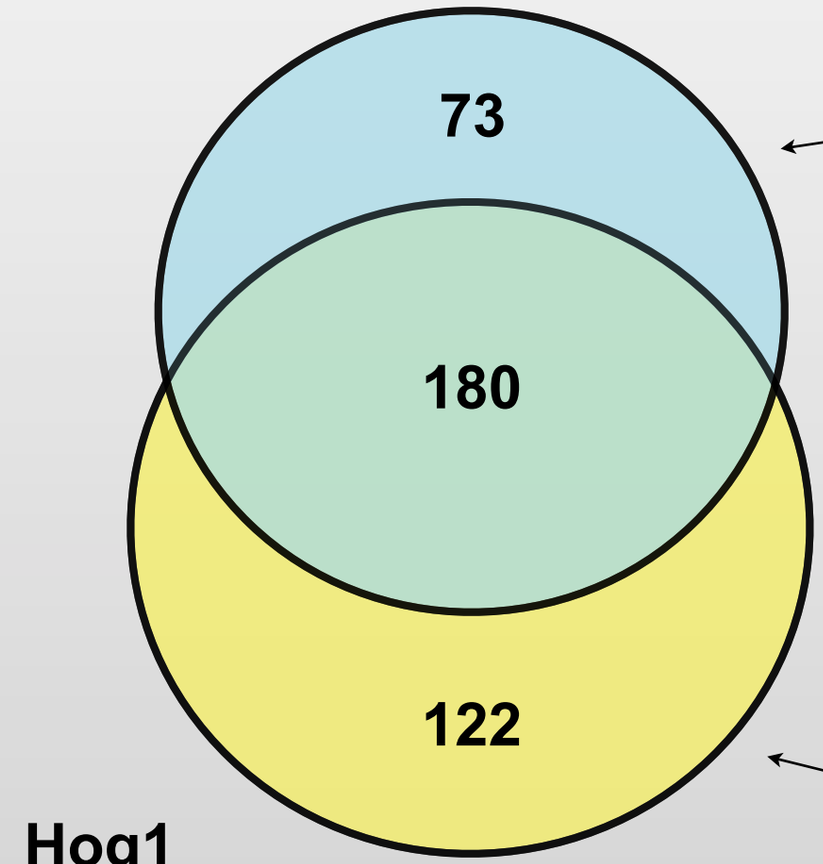


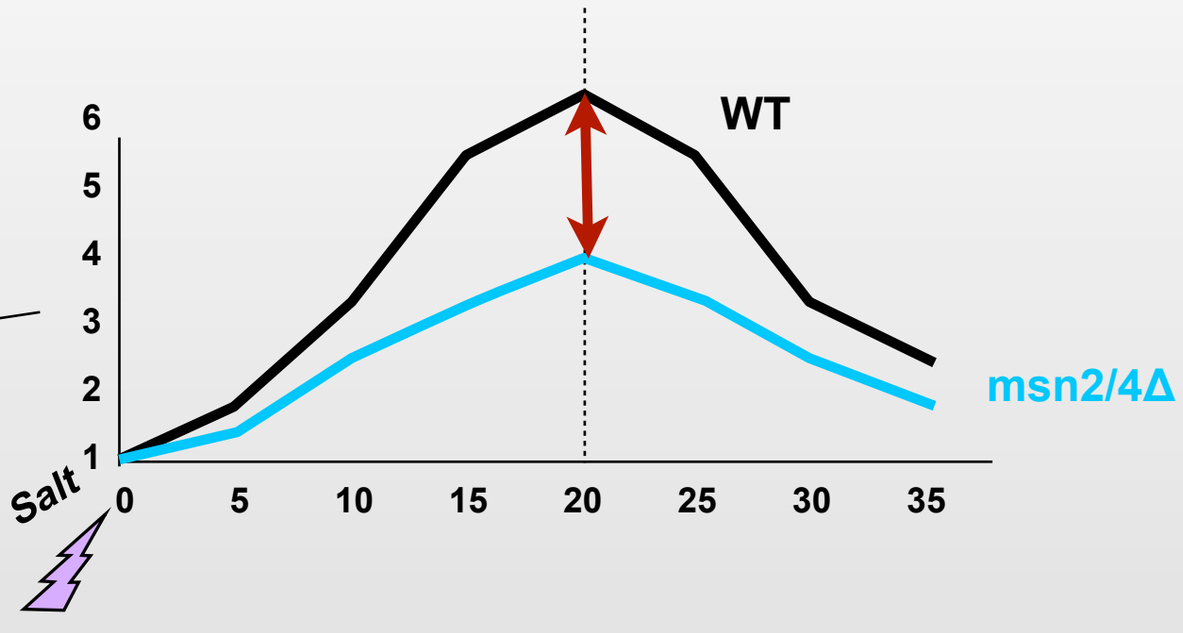


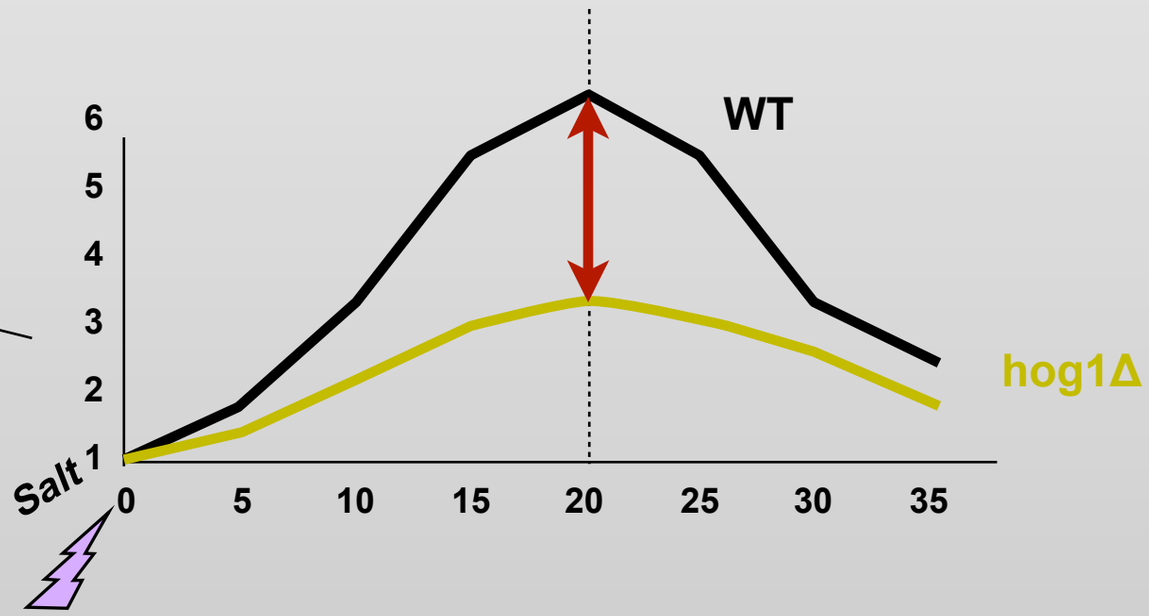

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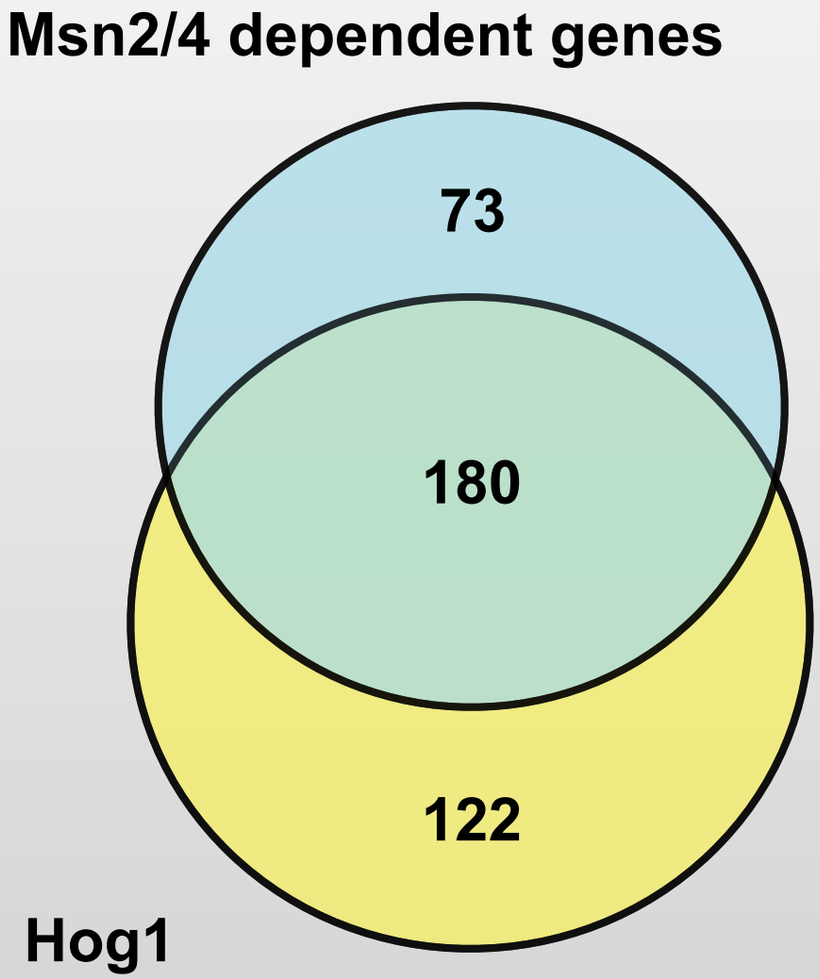

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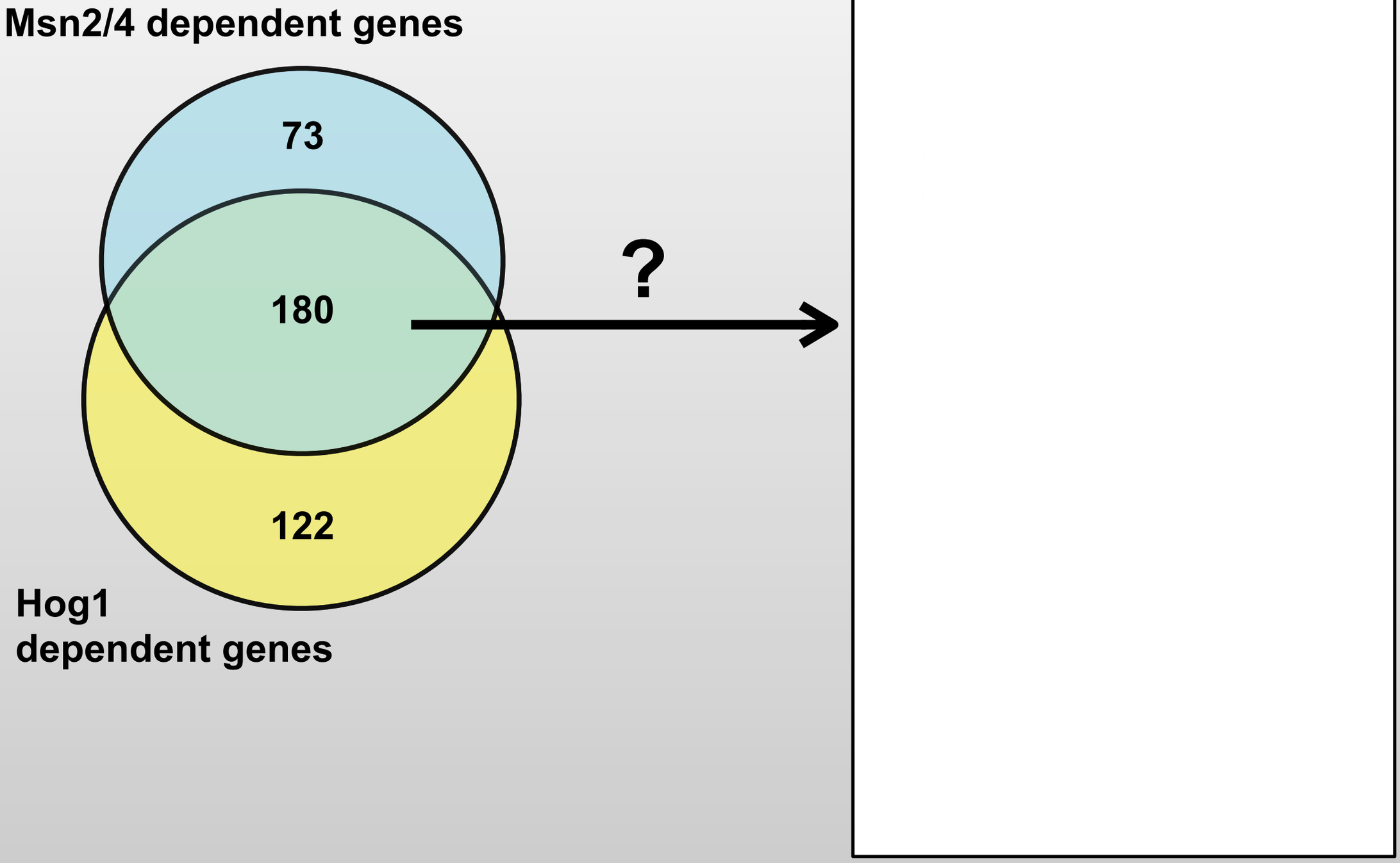

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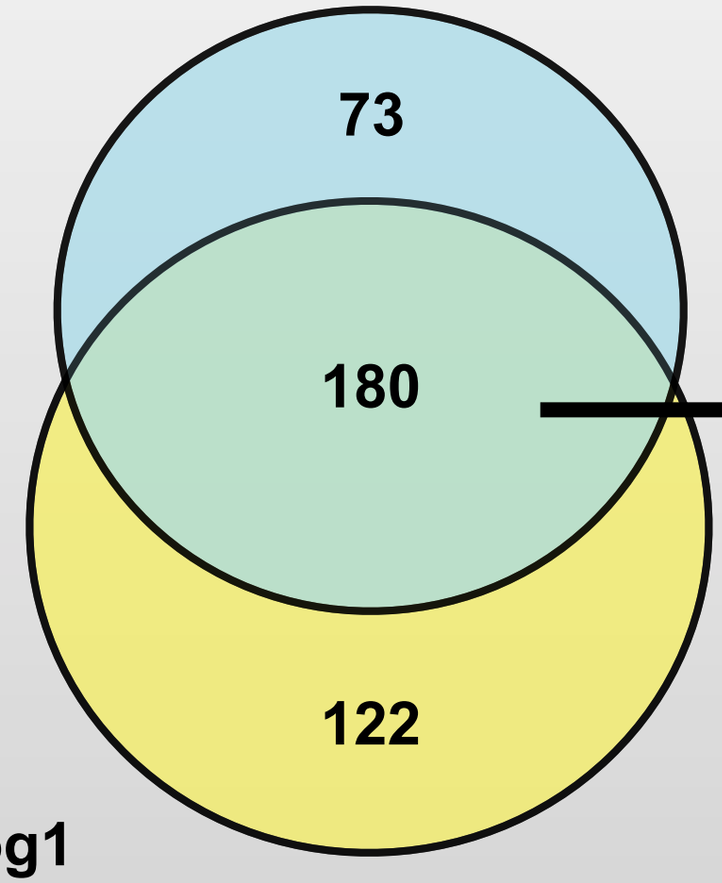


**Hog1 dependent genes**

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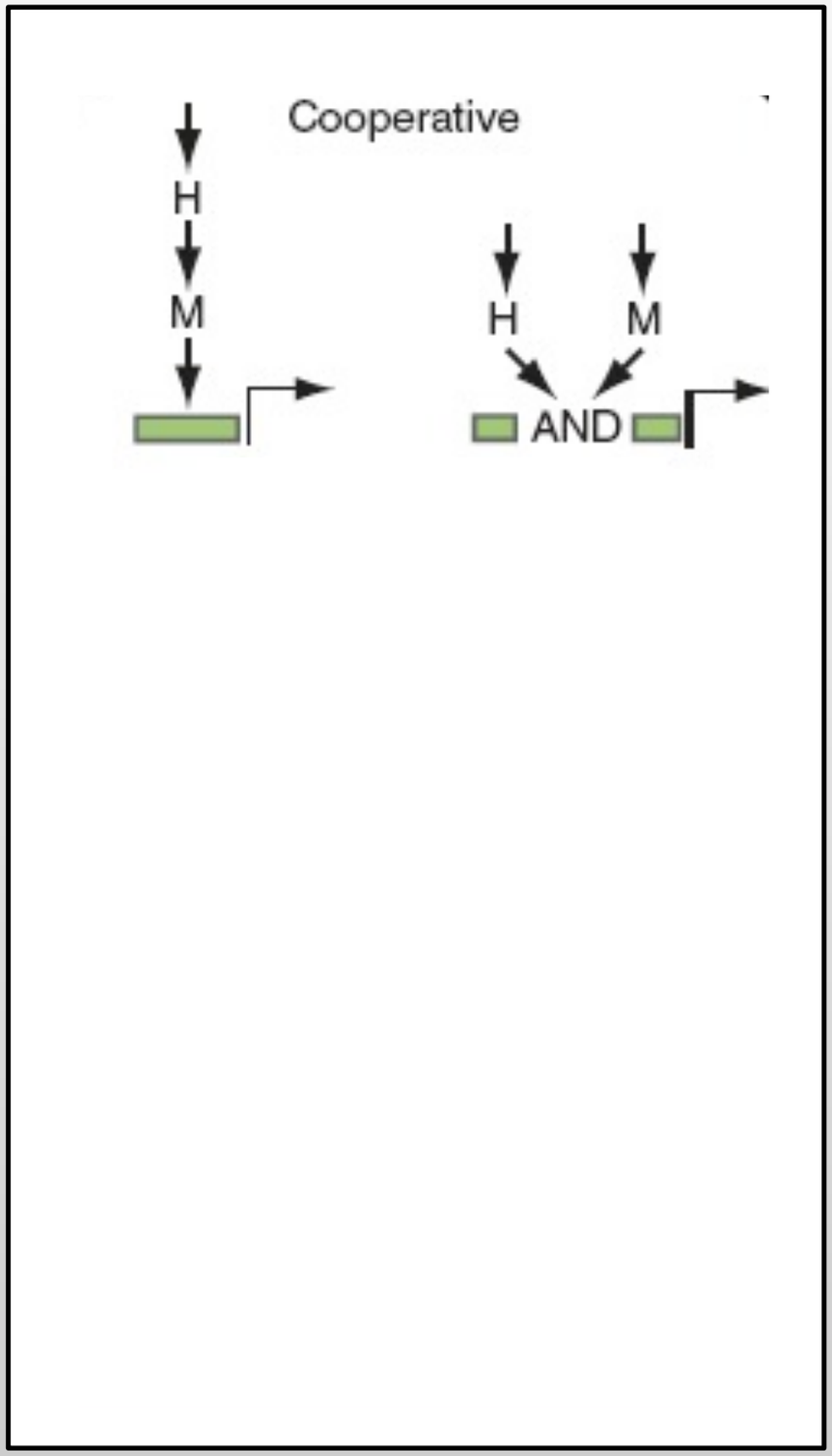

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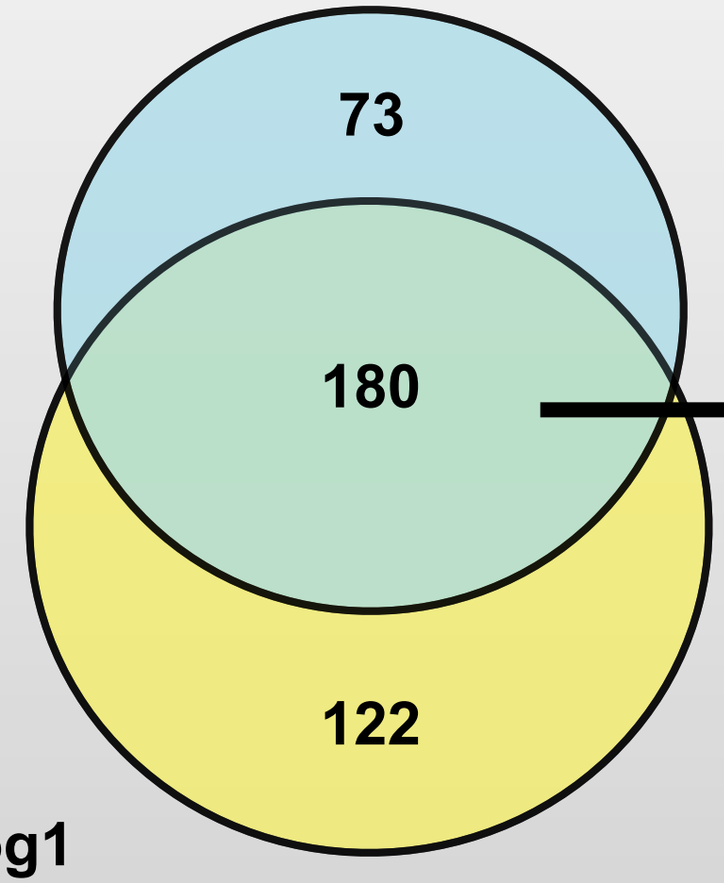


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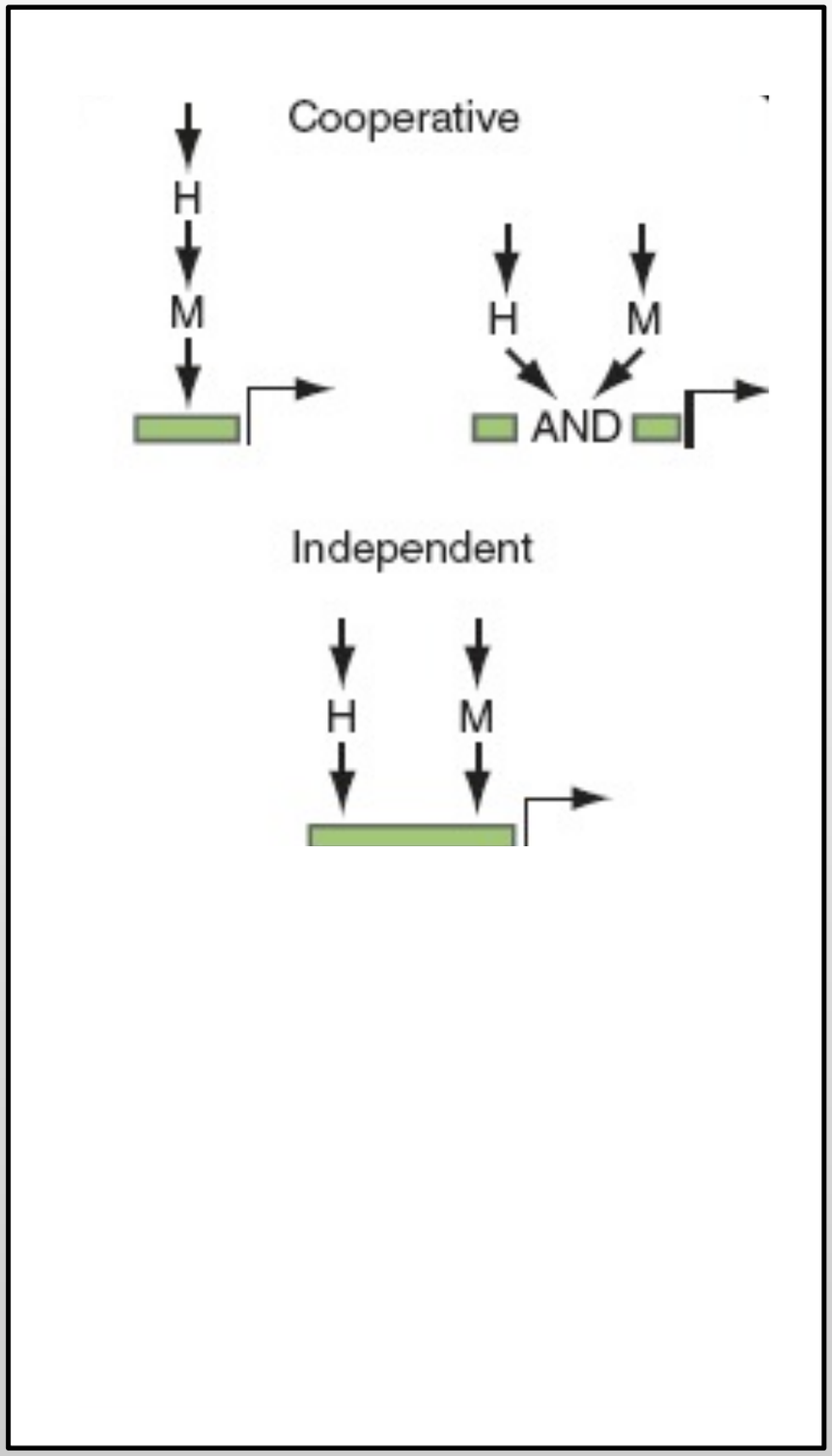

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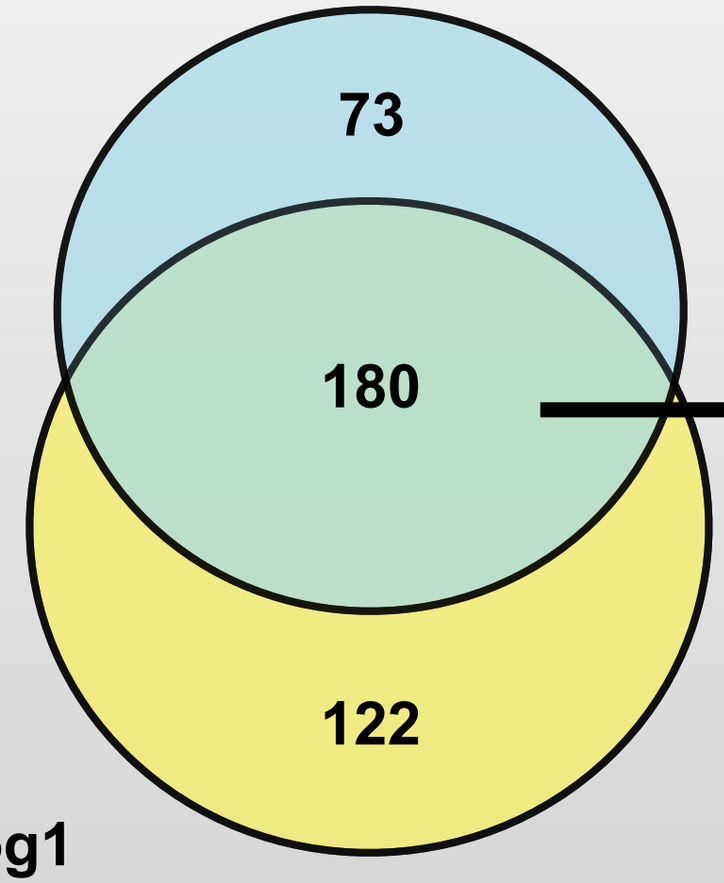


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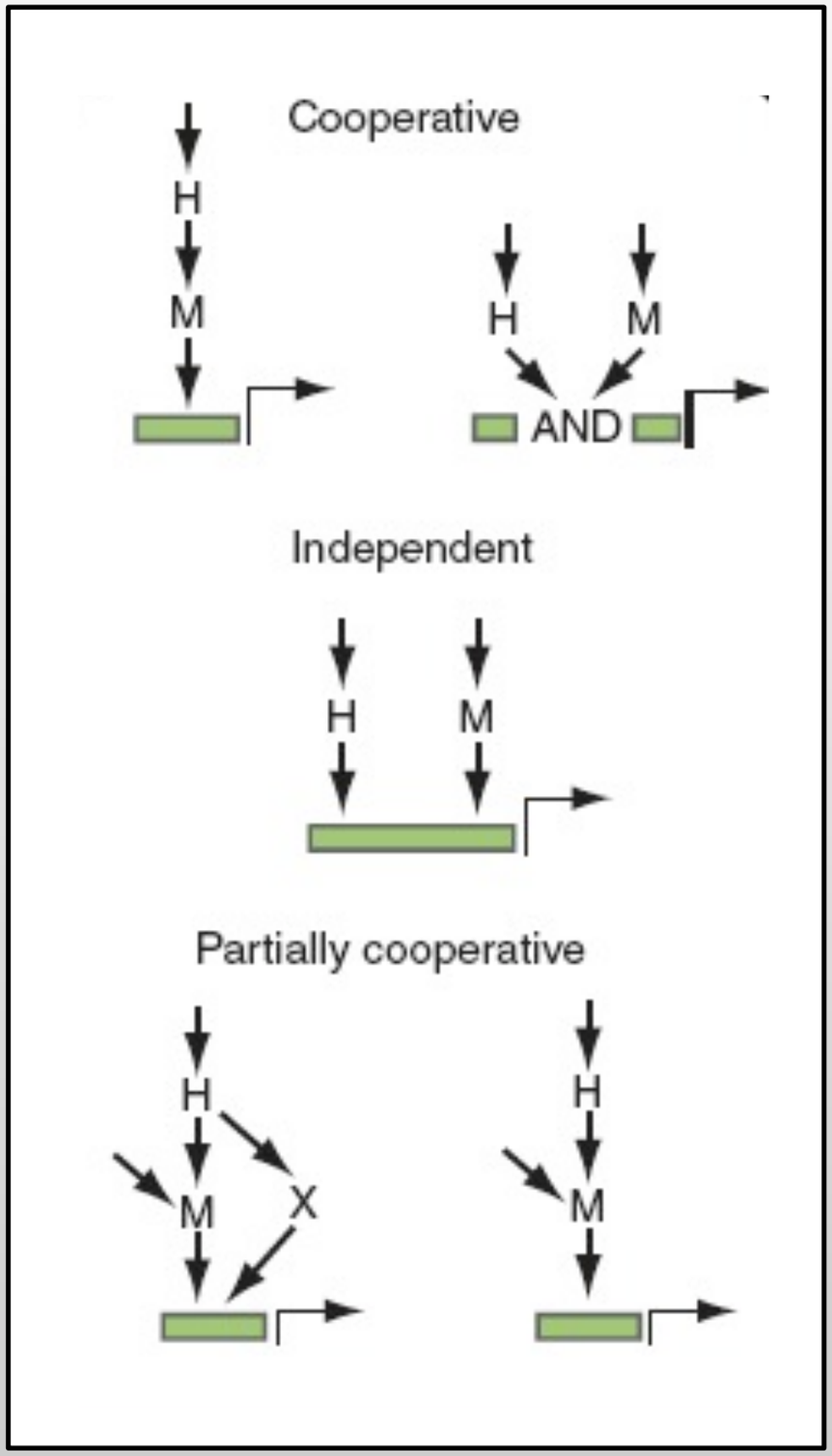
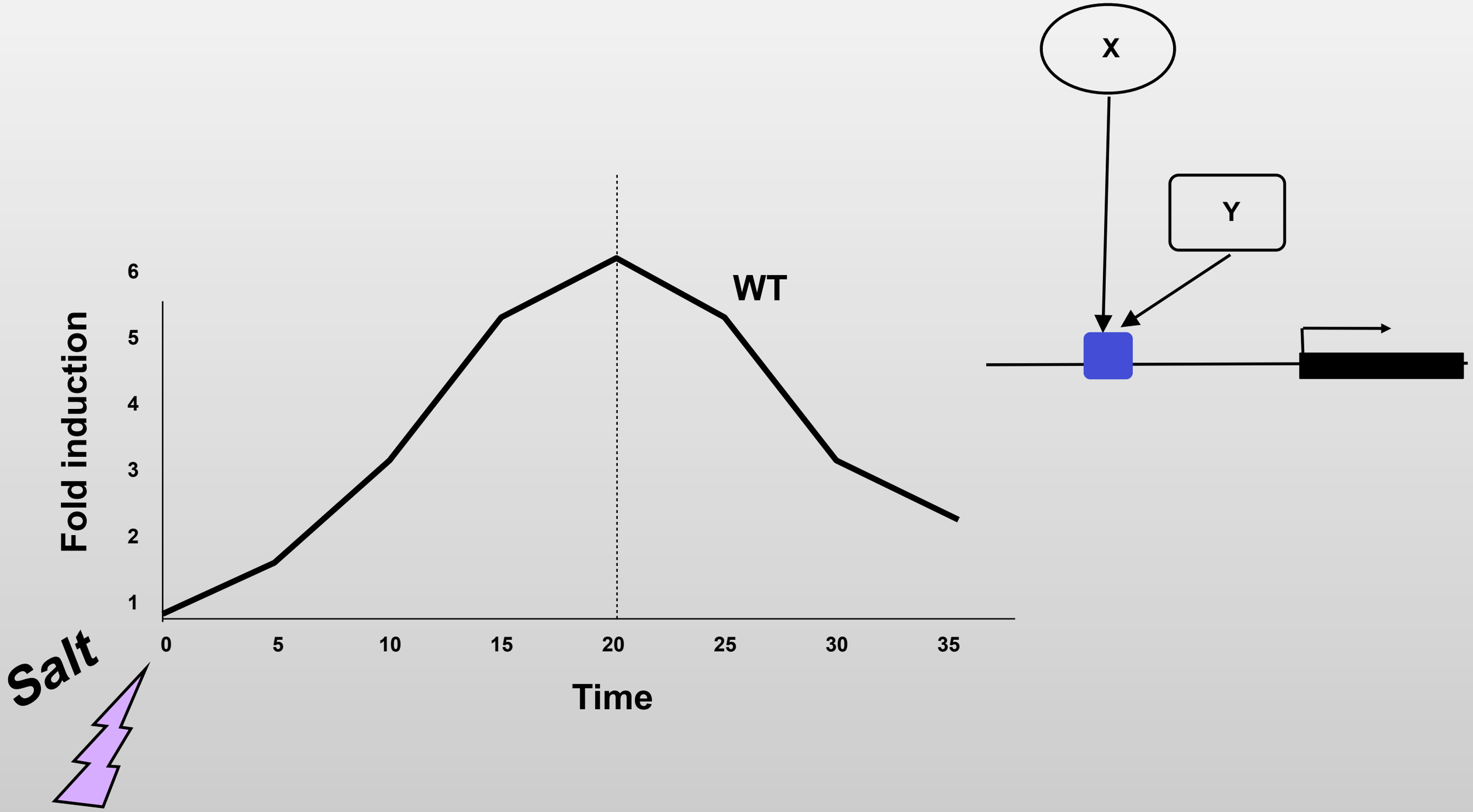


# Genetic Interactions

Understanding pathways by double perturbations



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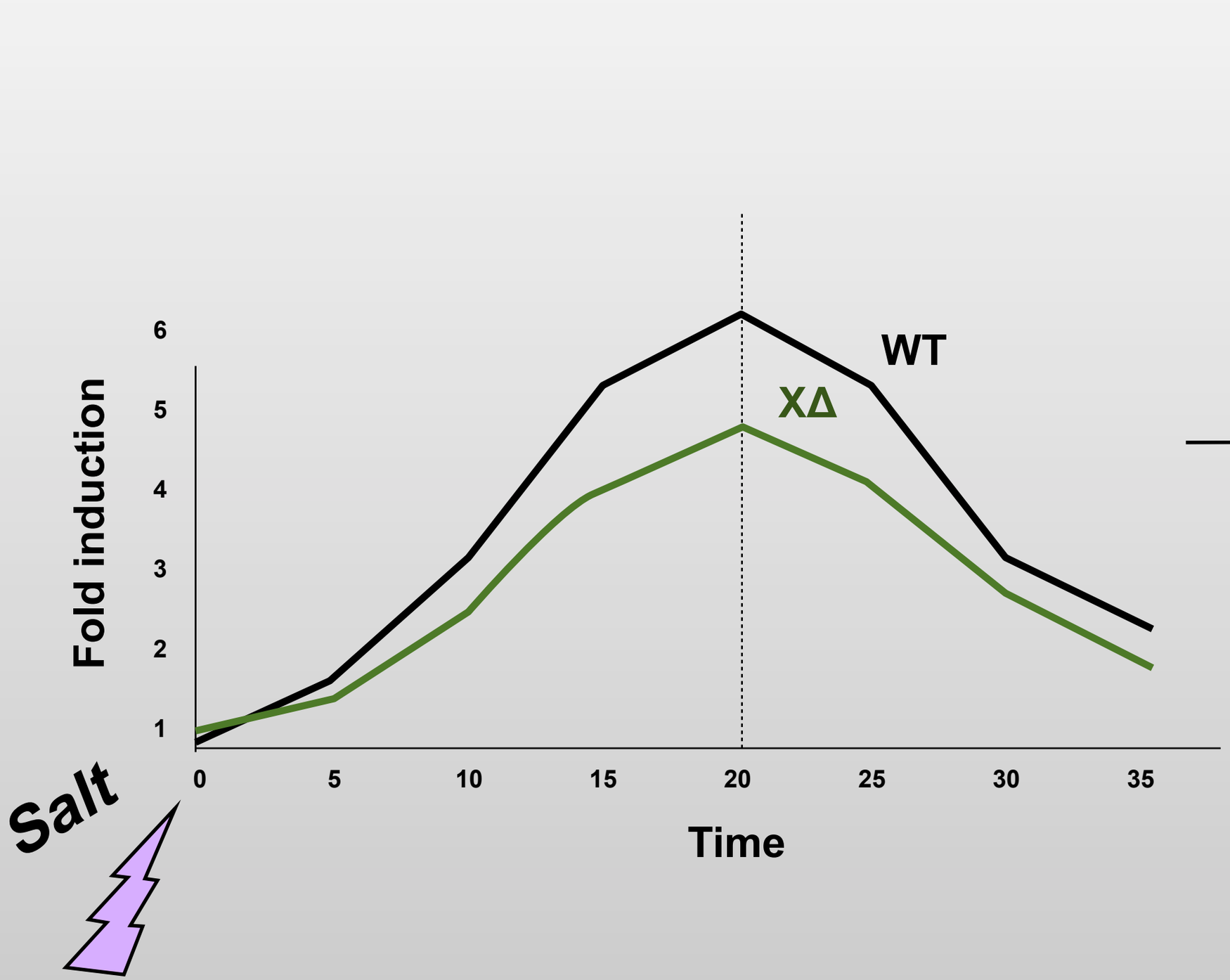


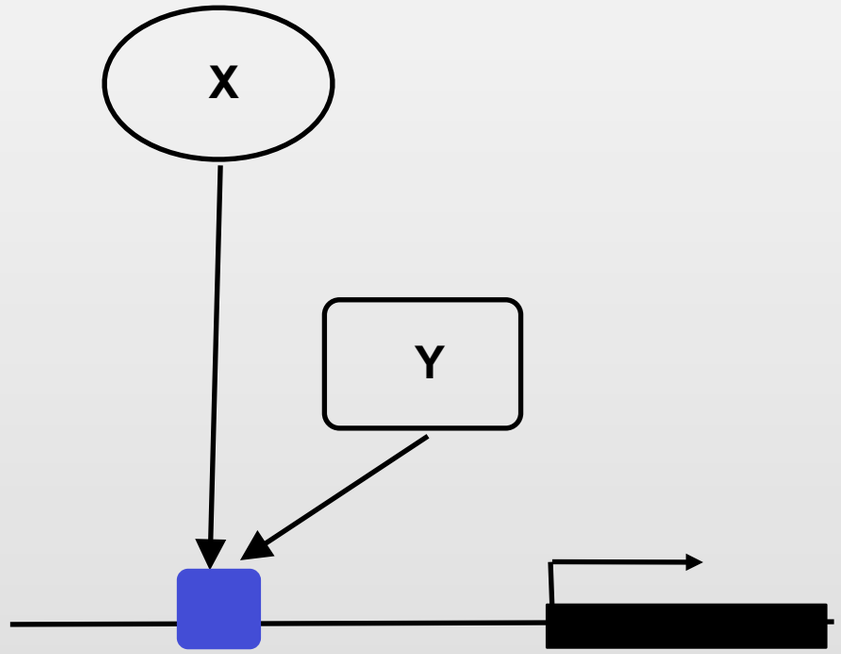

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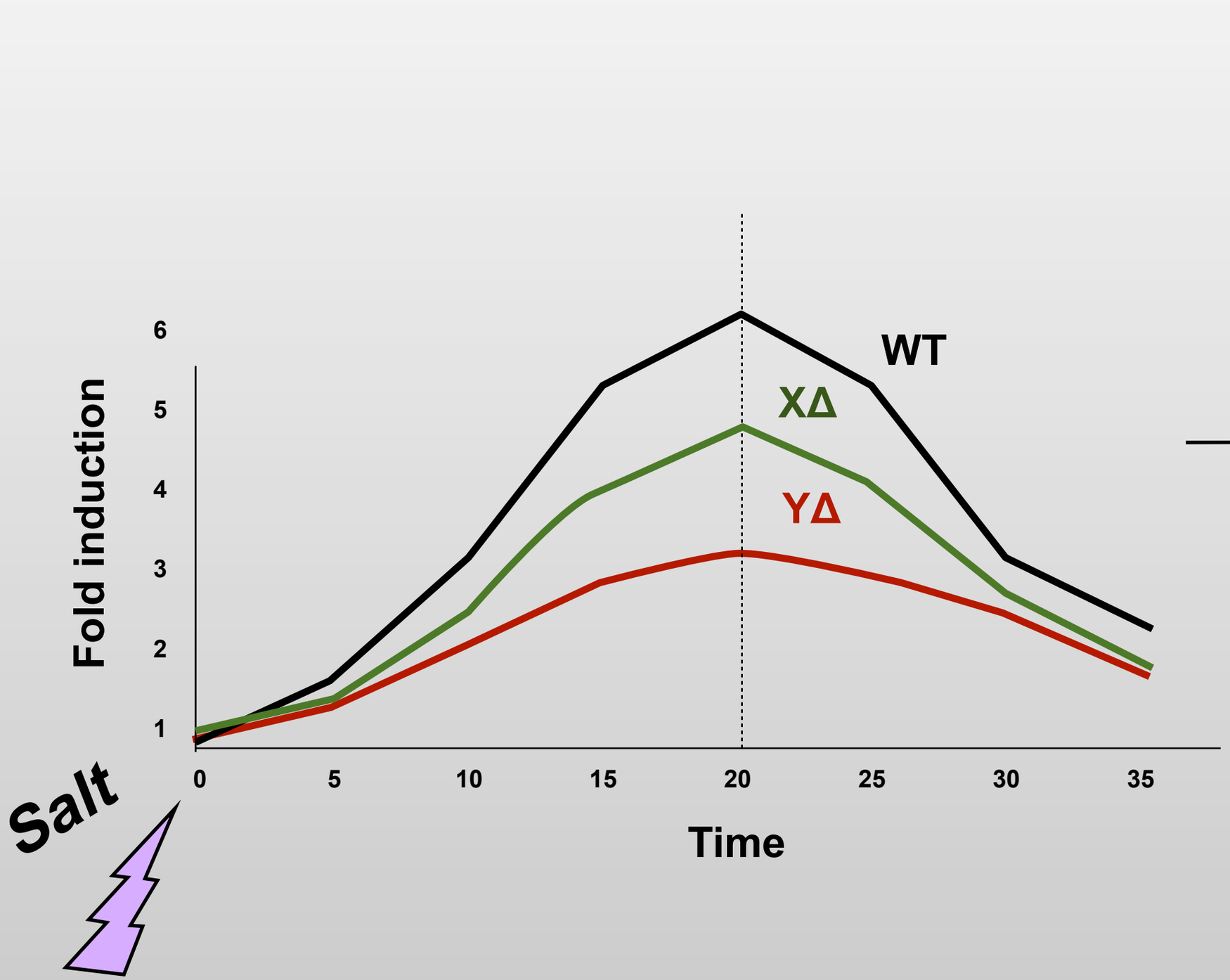


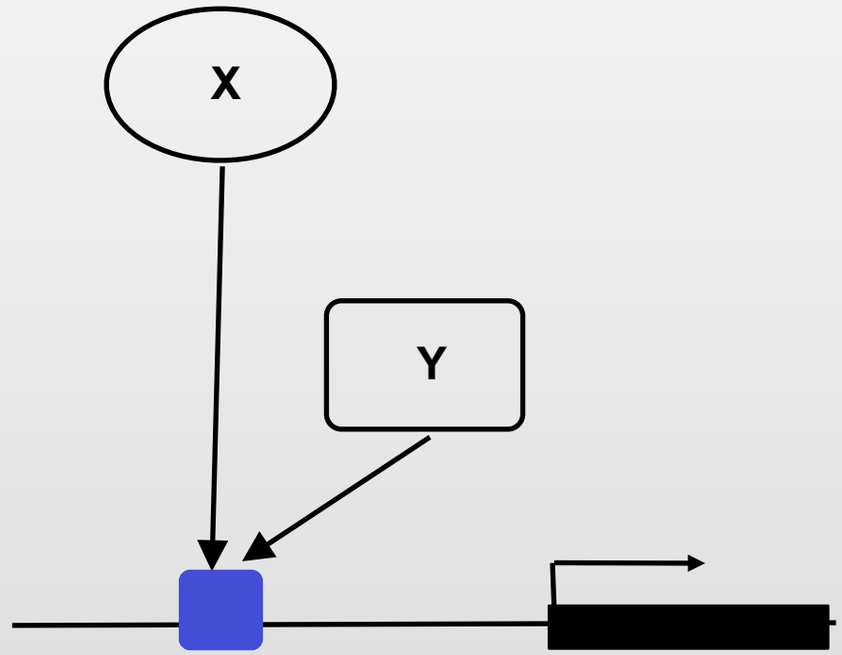

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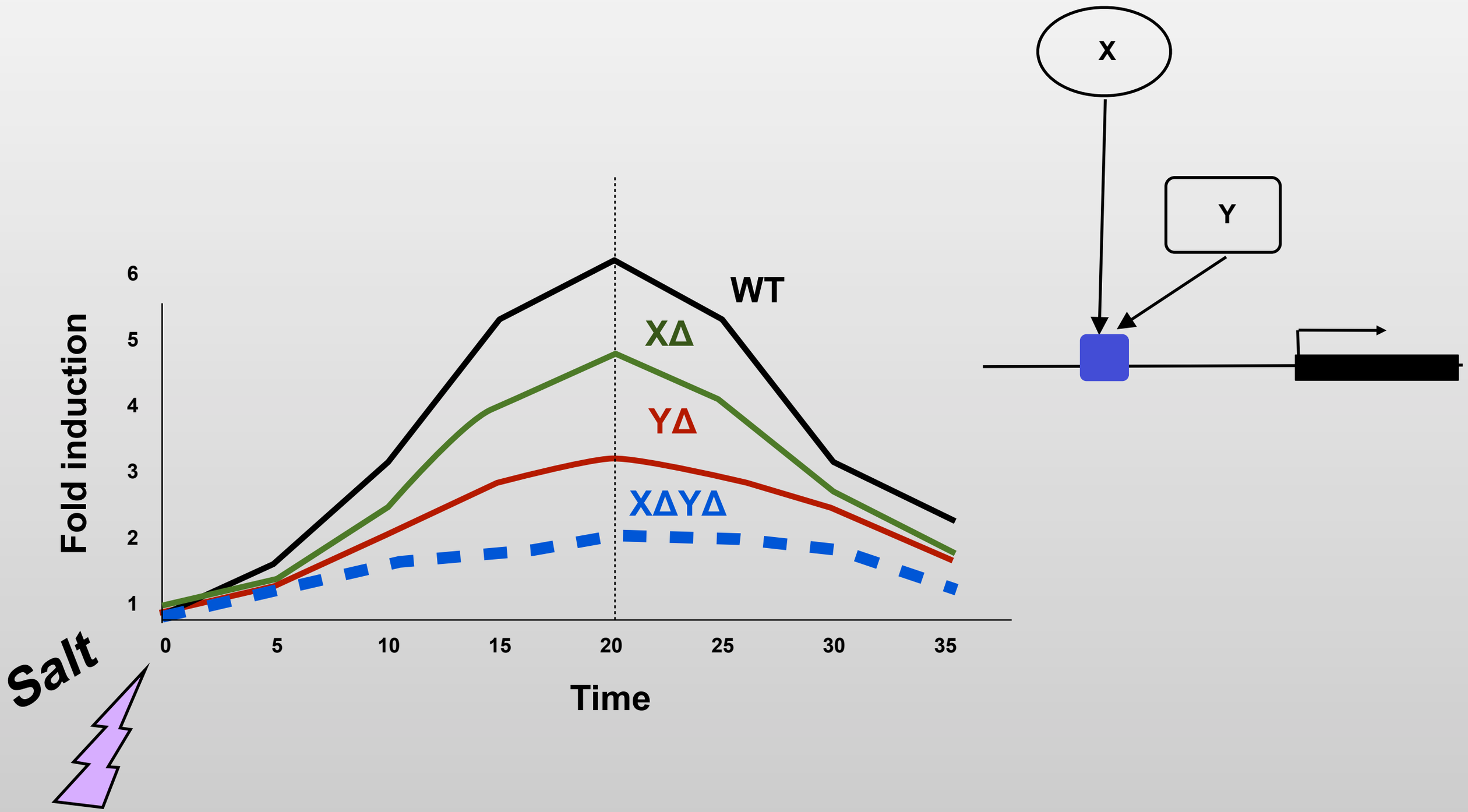

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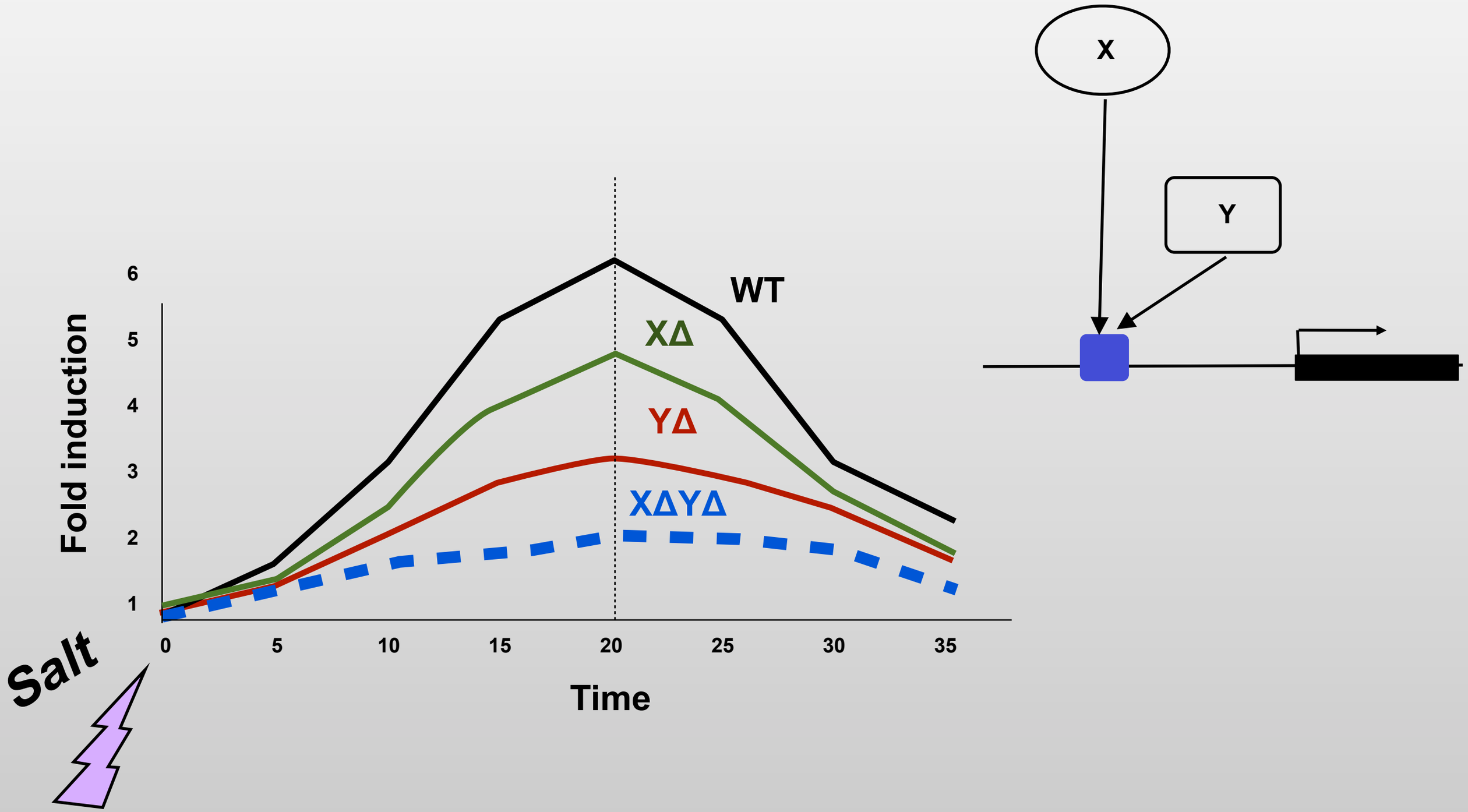

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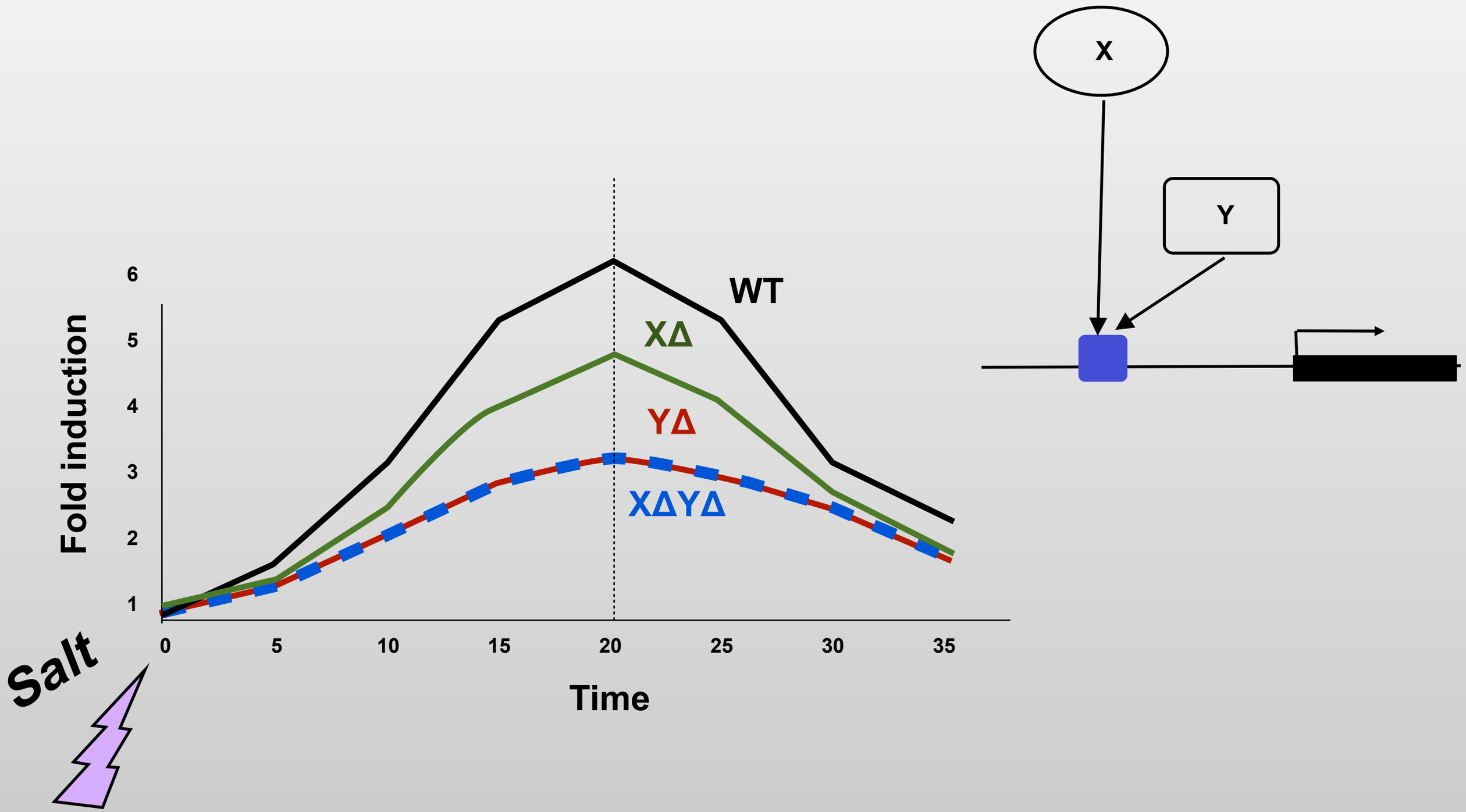

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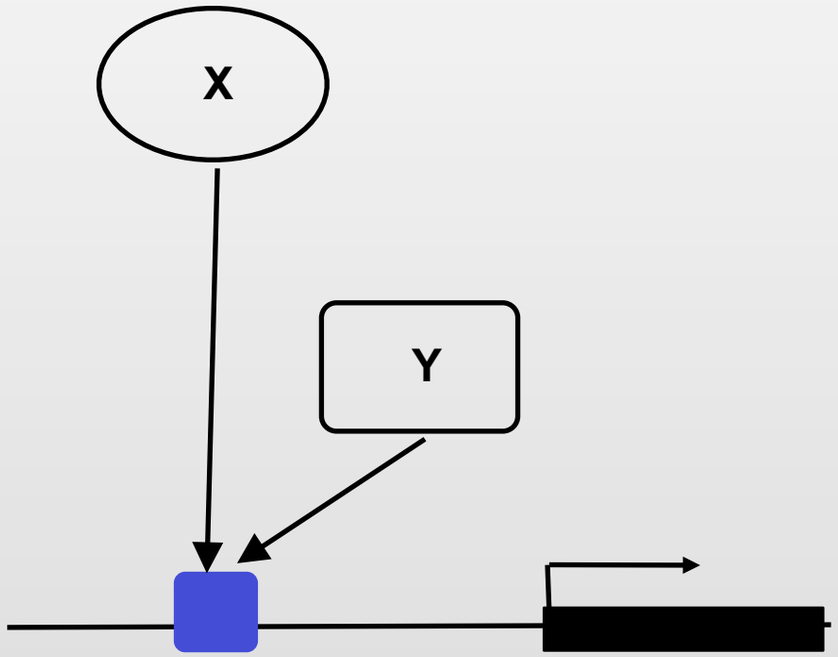
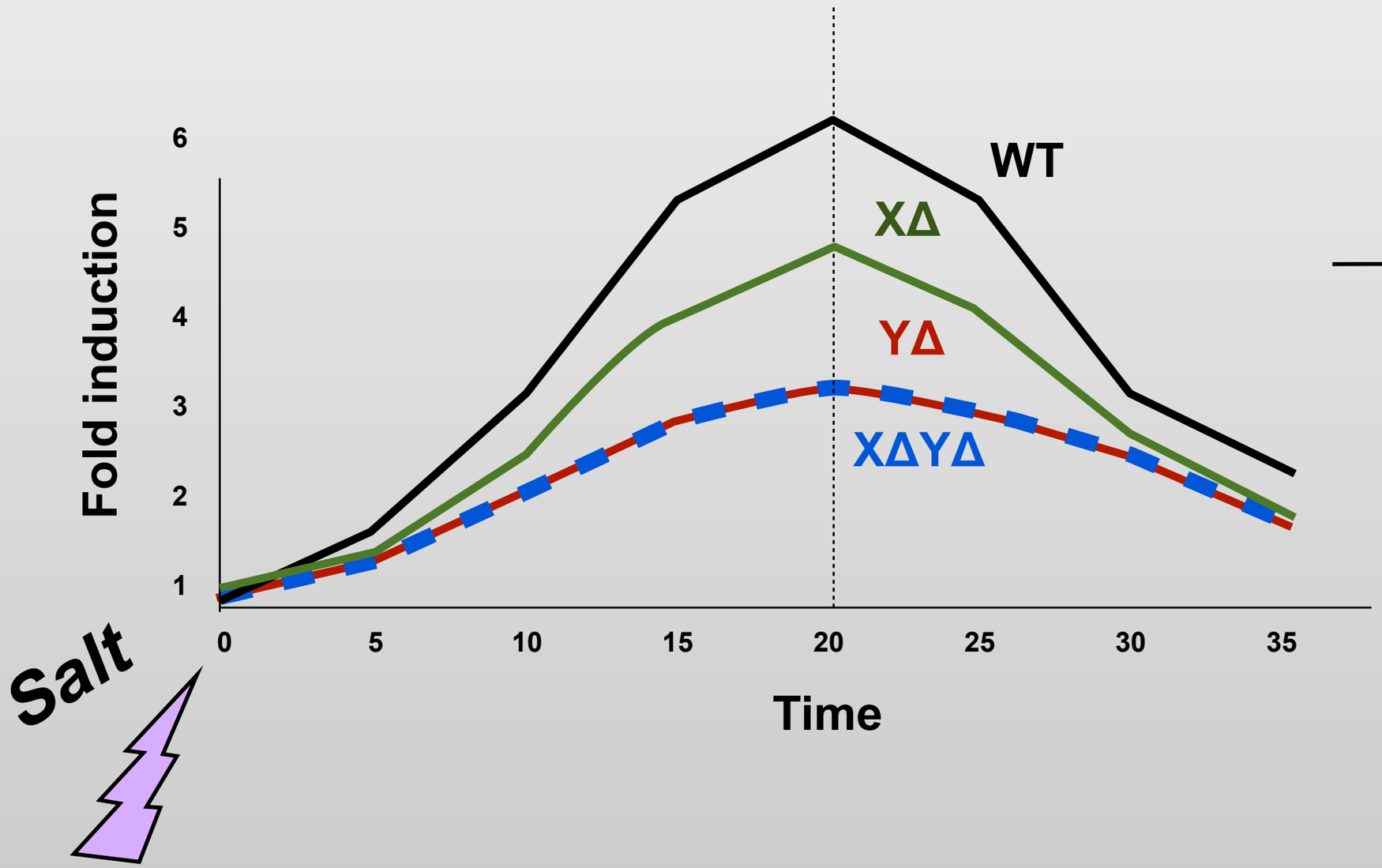
Understanding pathways by double perturbations

## Epistasis

- ΔXΔY = ΔY



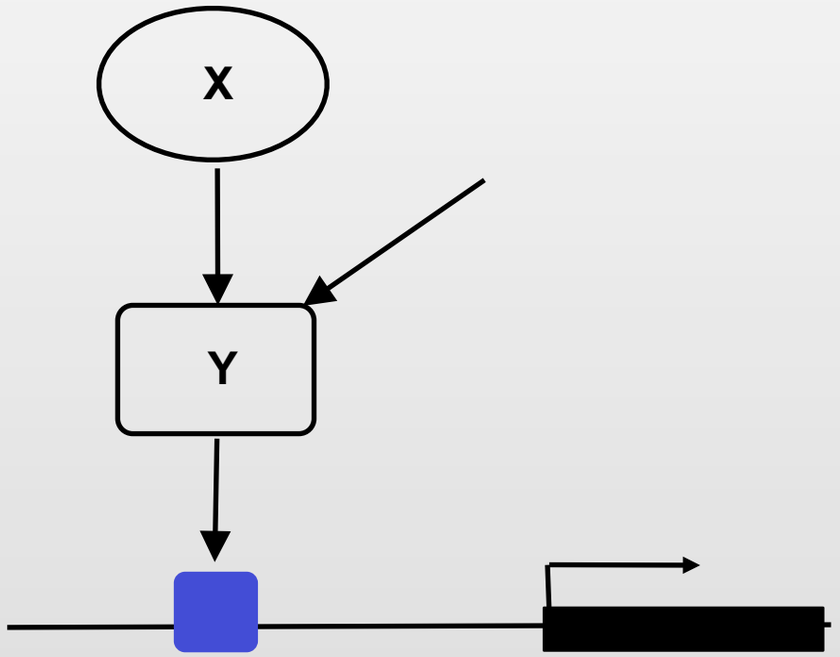



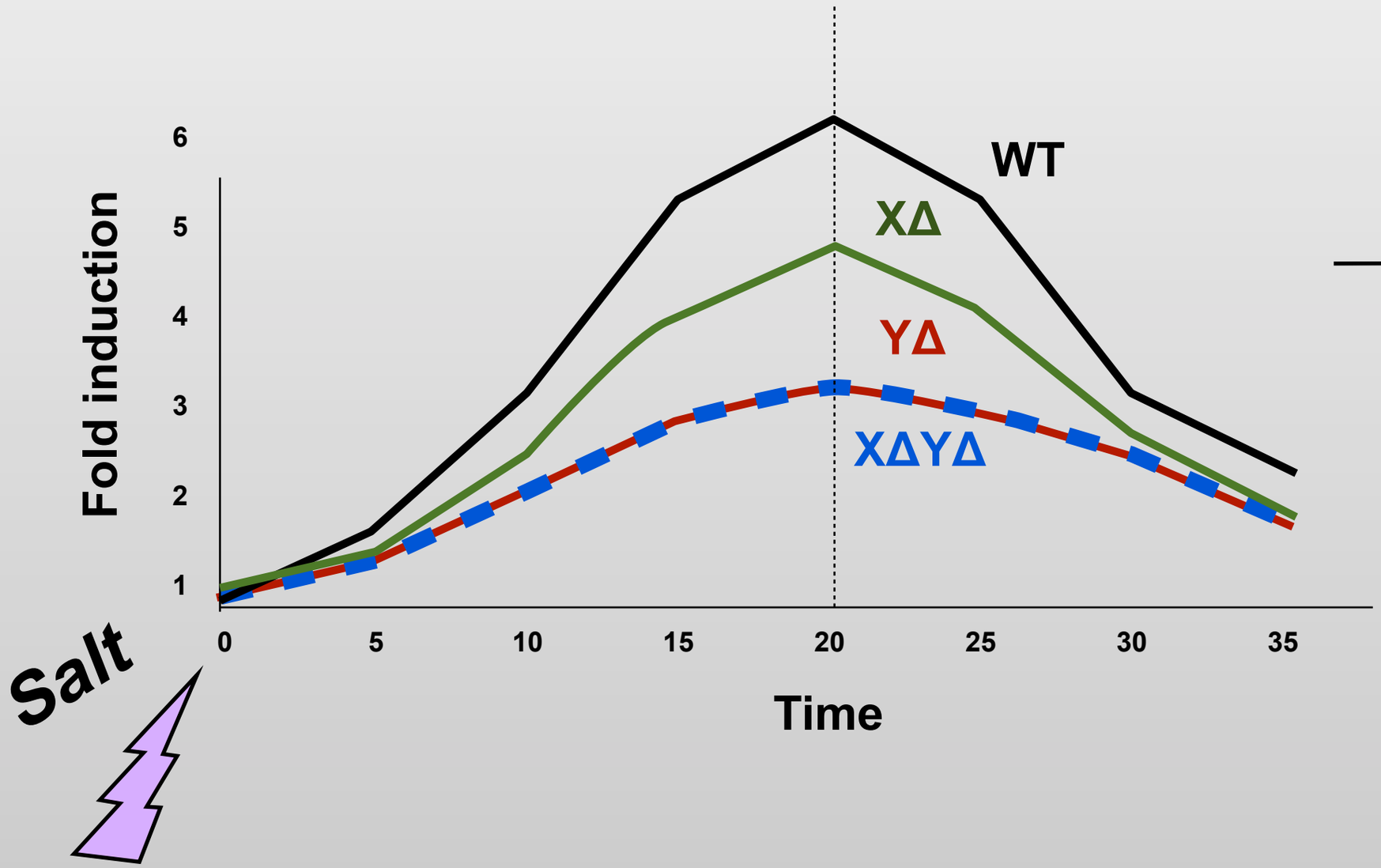
# Genetic Interactions

Understanding pathways by double perturbations

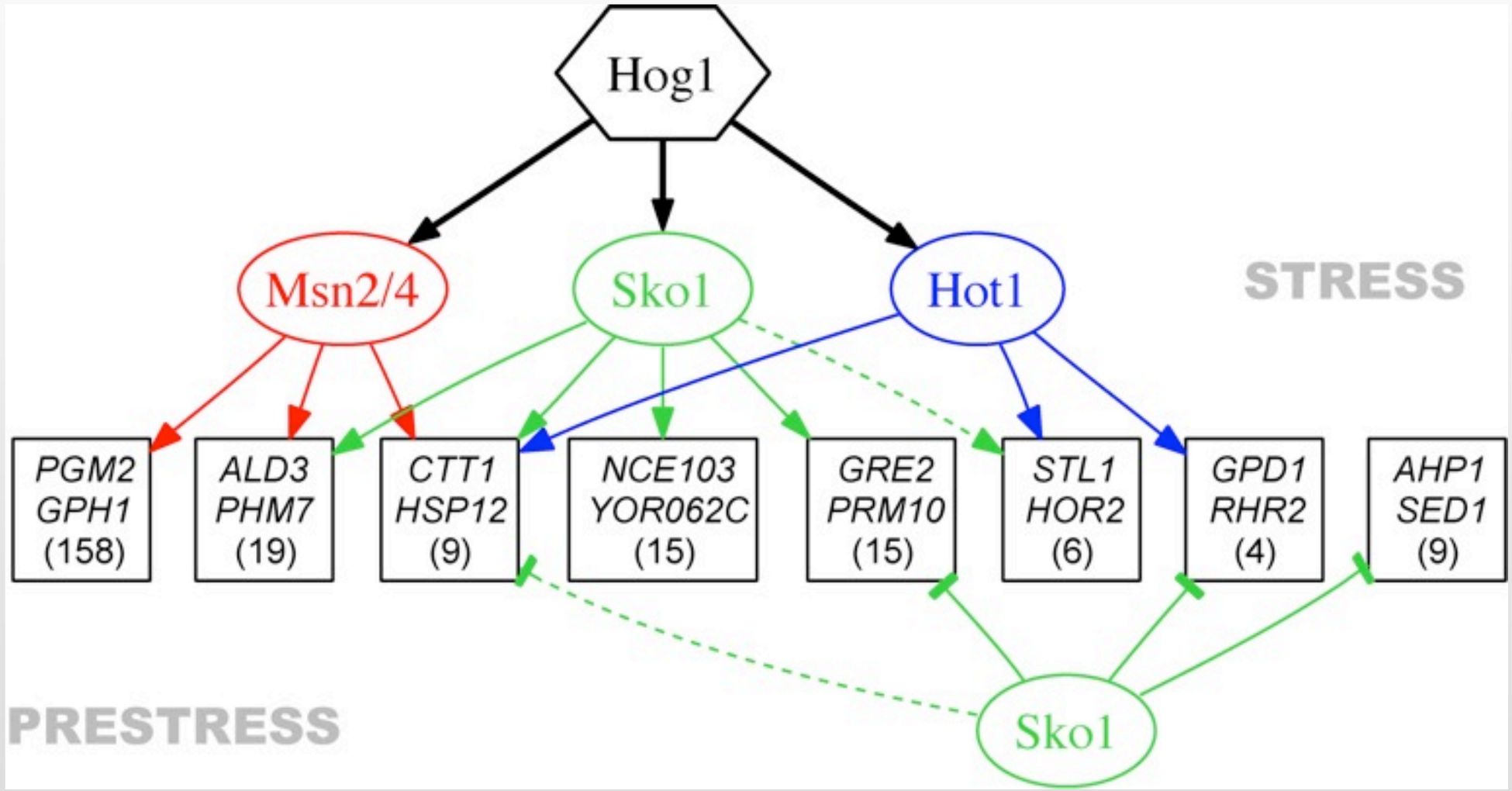
## Epistasis

- ΔXΔY = ΔY



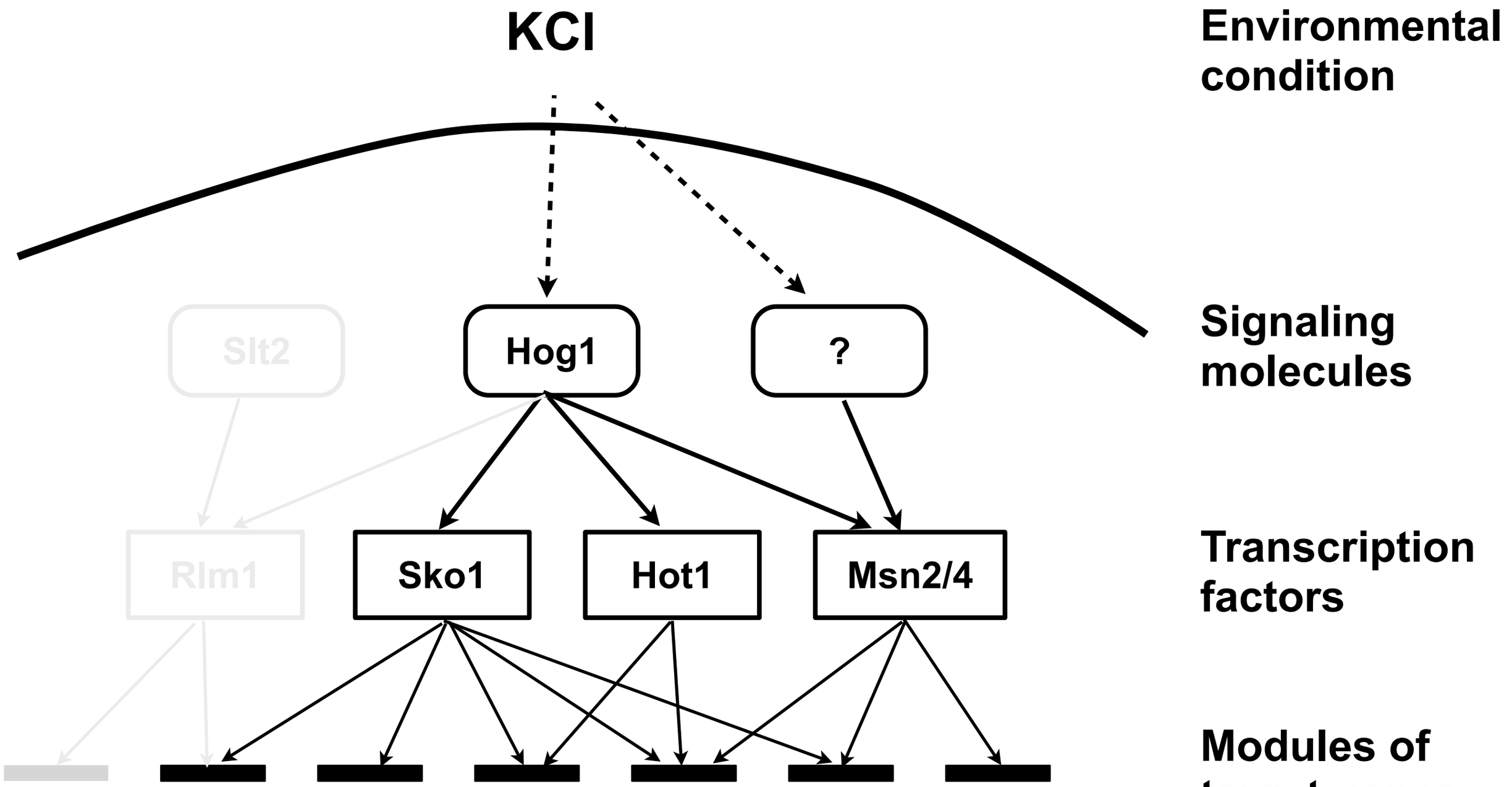
# Combinatorial Network



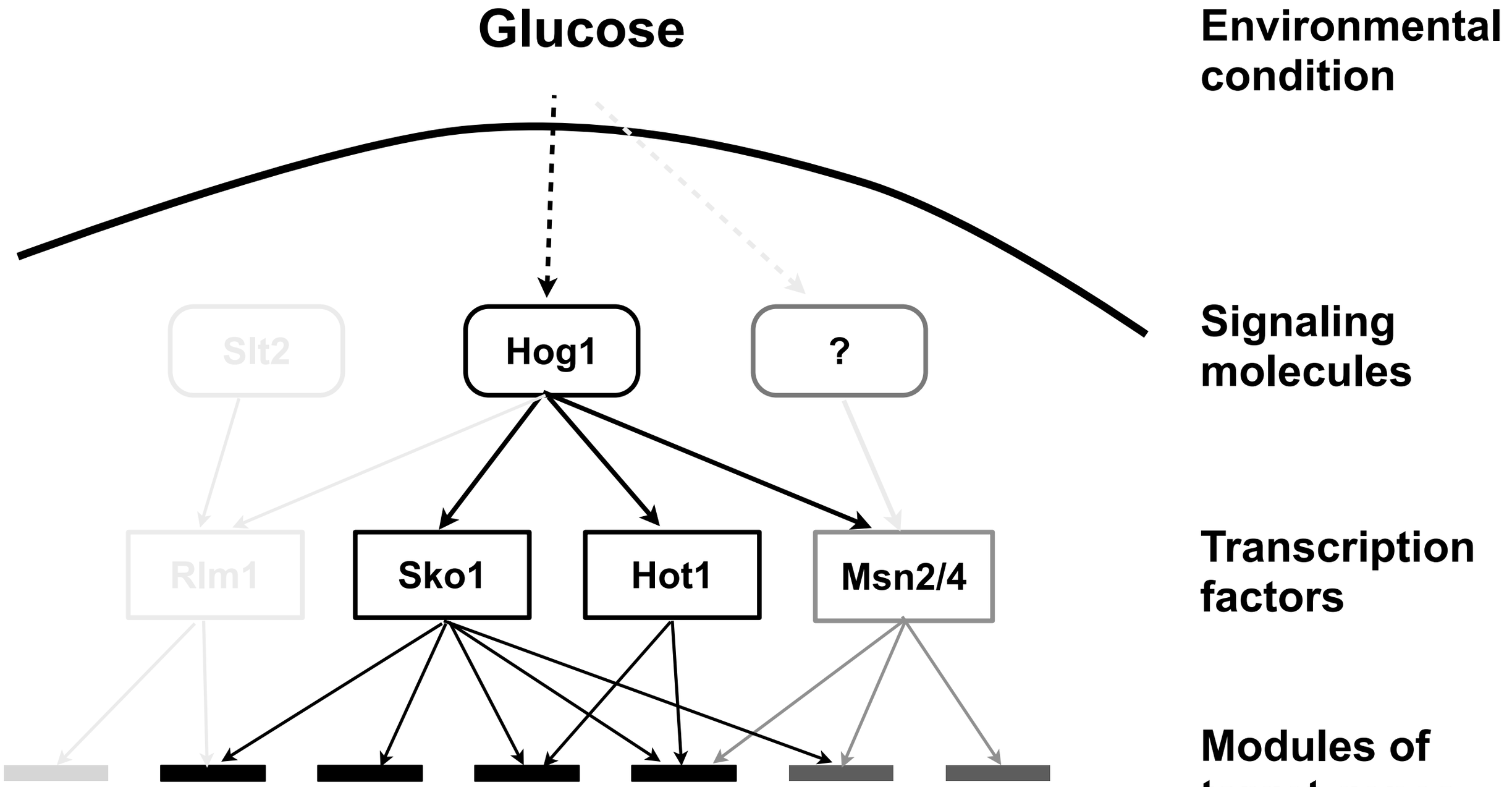

- Hog1 response is mediated through a dense overlapping regulatory circuit

**Why?**

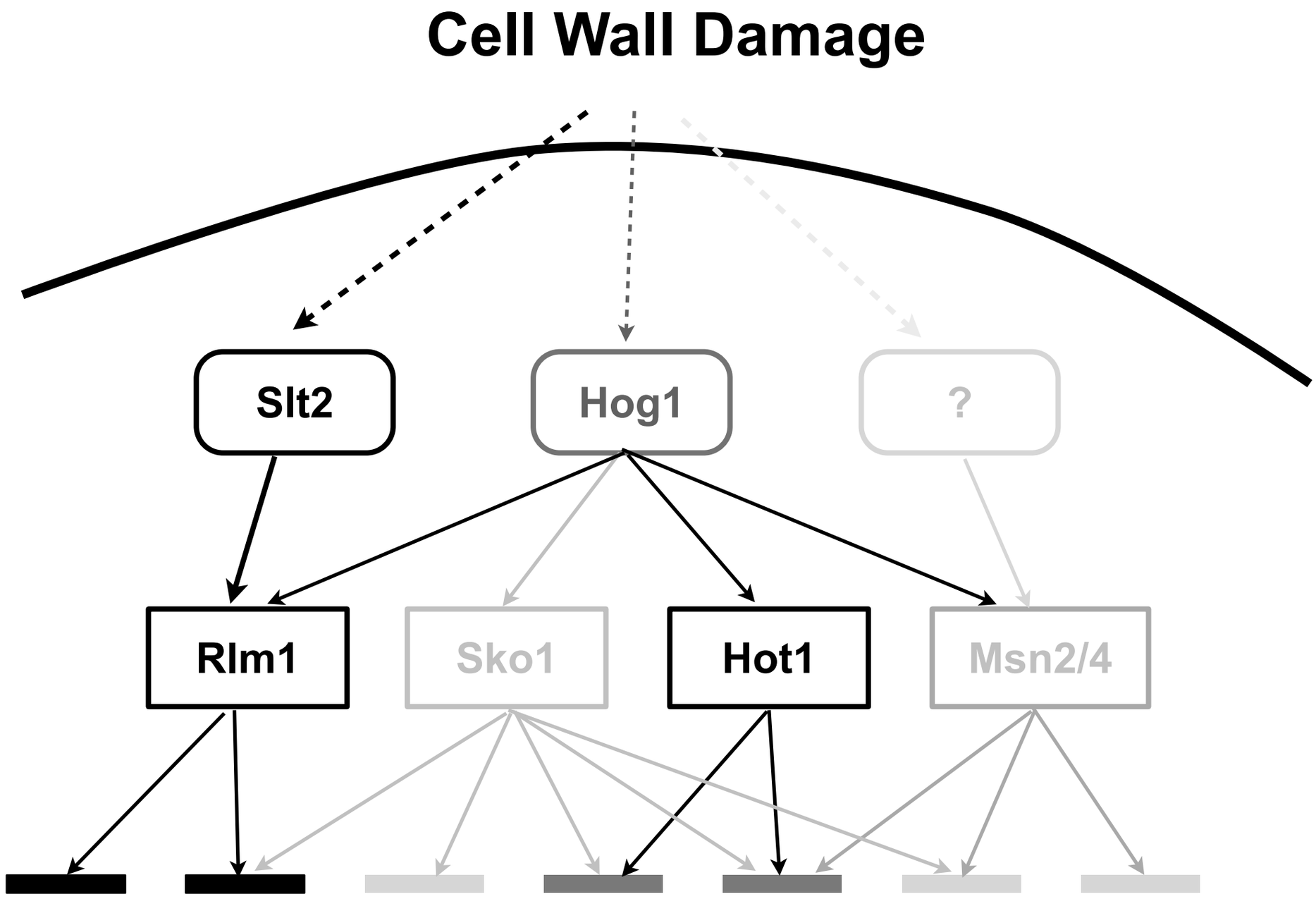
# Information Flow



# Information Flow



# Information Flow



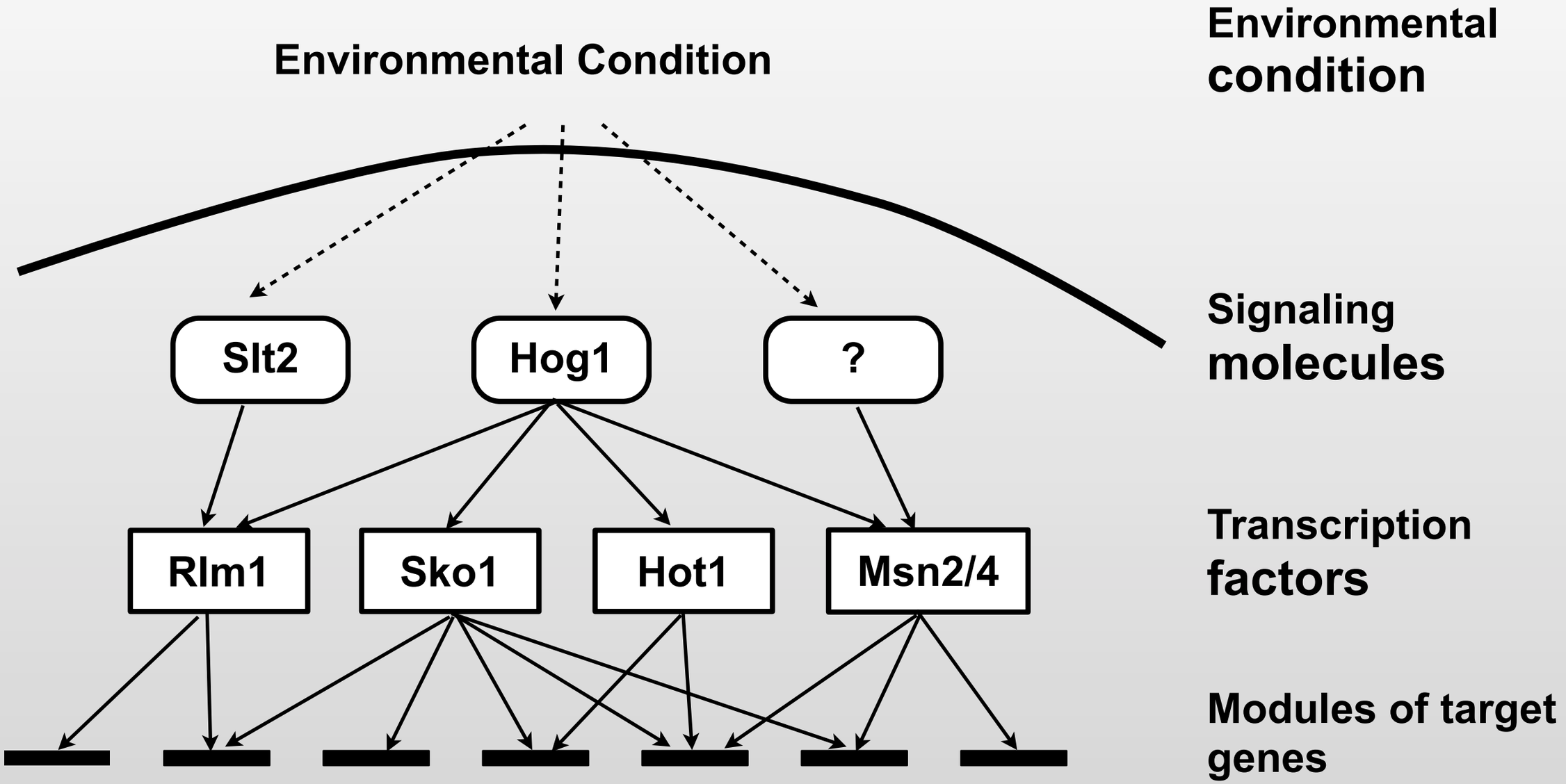

Environmental condition

Signaling molecules

Transcription factors

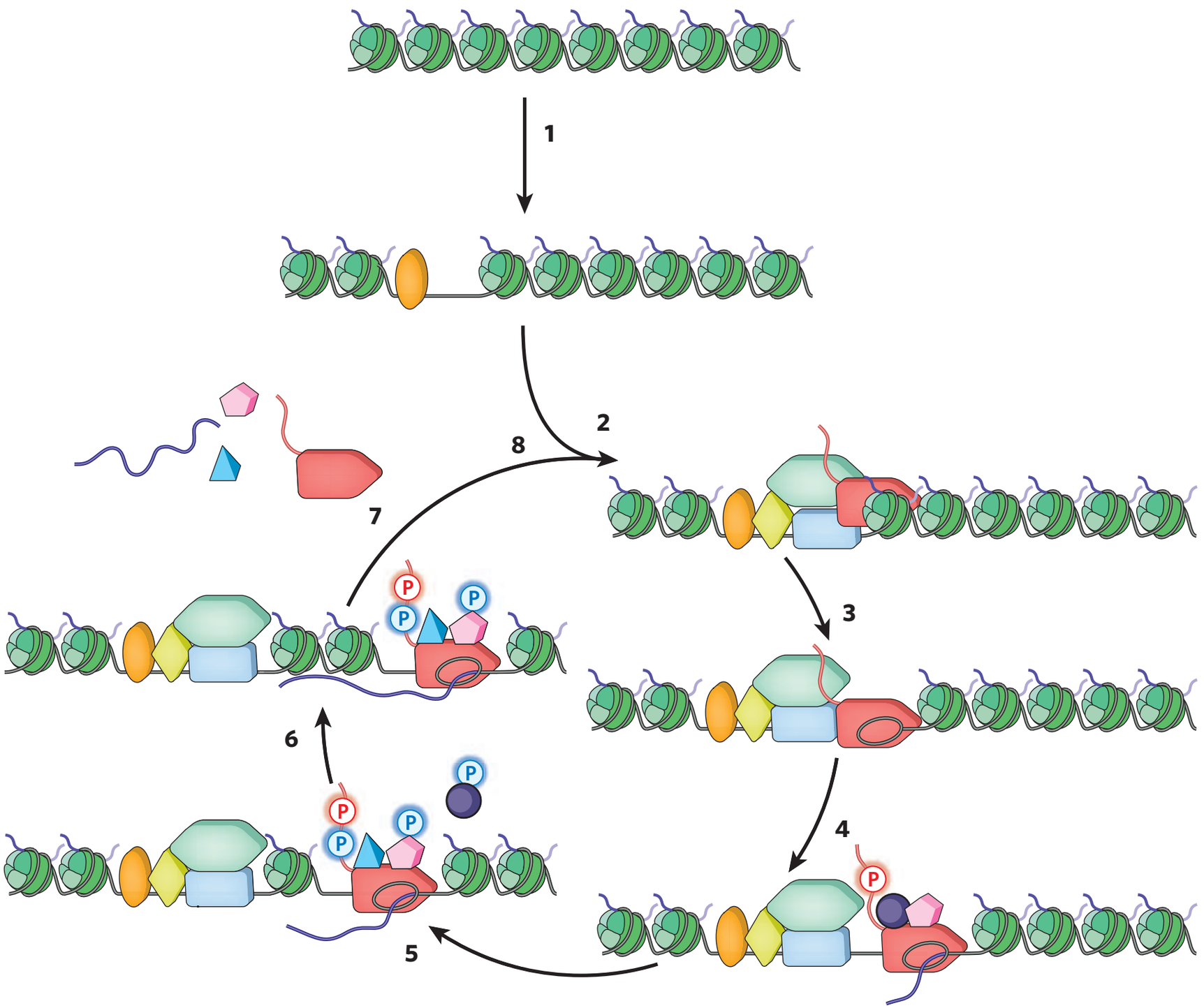
Modules of target genes

# Modulation via Network Reconfiguration

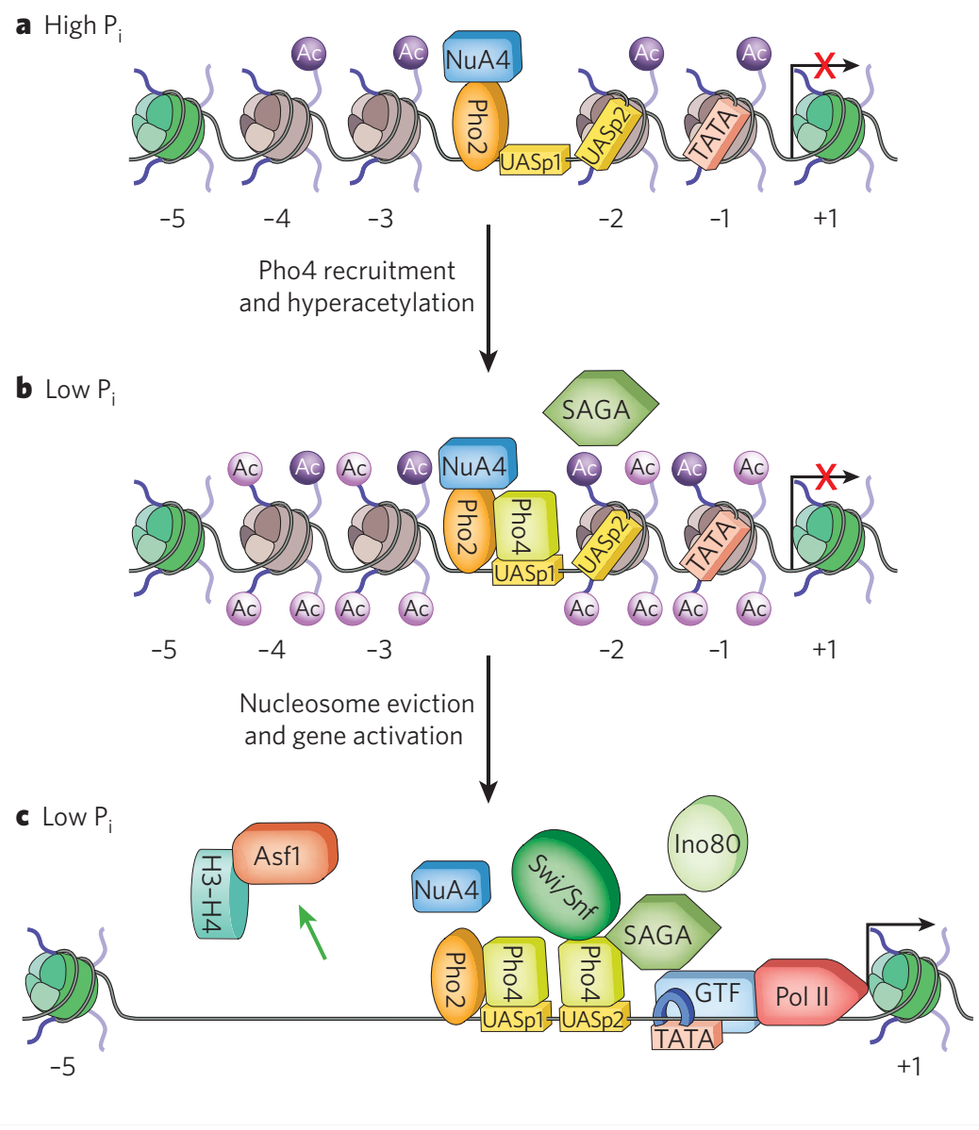


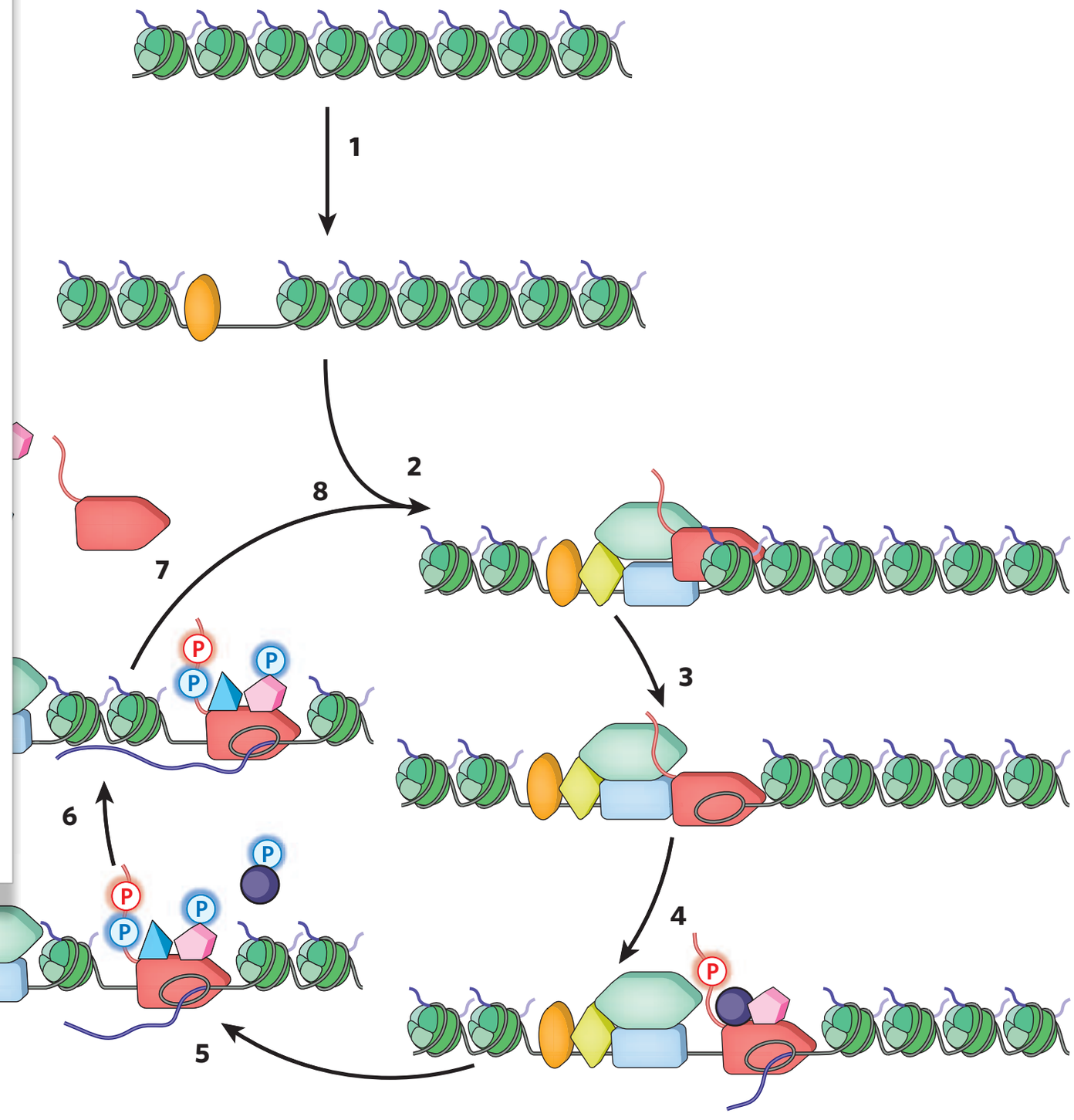

- Cross-talk between Hog1 pathway and general stress response, and Slt2 pathway

# Regulation: Beyond TFs



# Regulation: Beyond TFs



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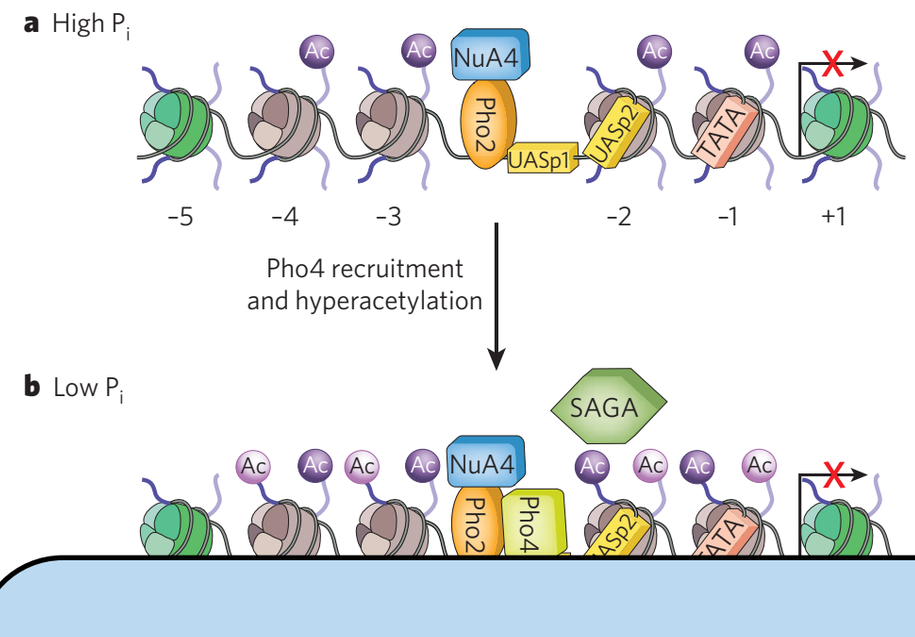
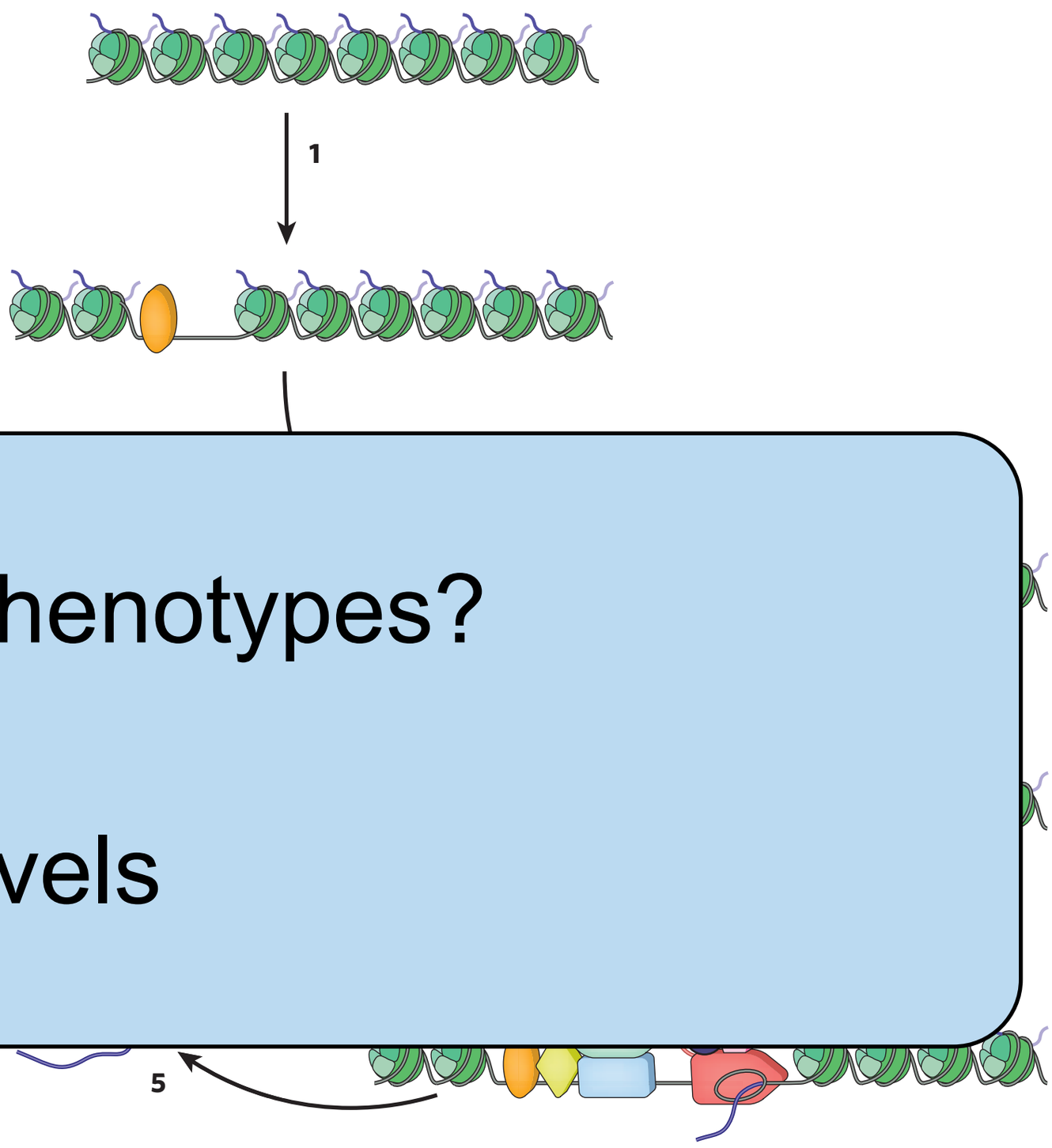


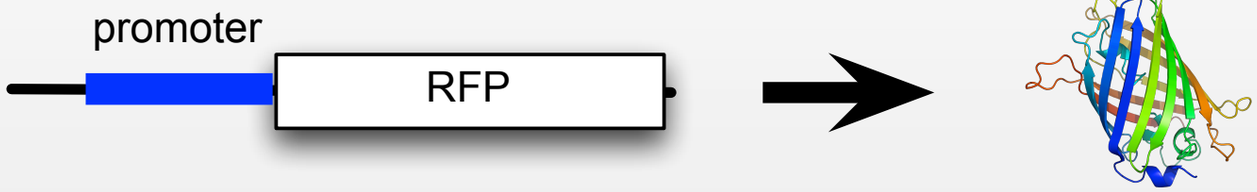




What are suitable phenotypes?

- mRNA levels
- reporter protein levels

# Variability as Phenotype

- Promoter activity reporter



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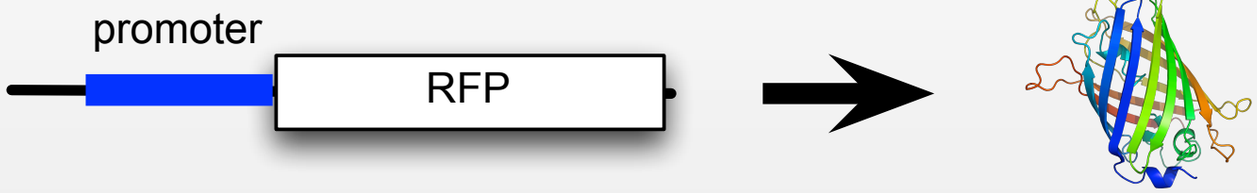
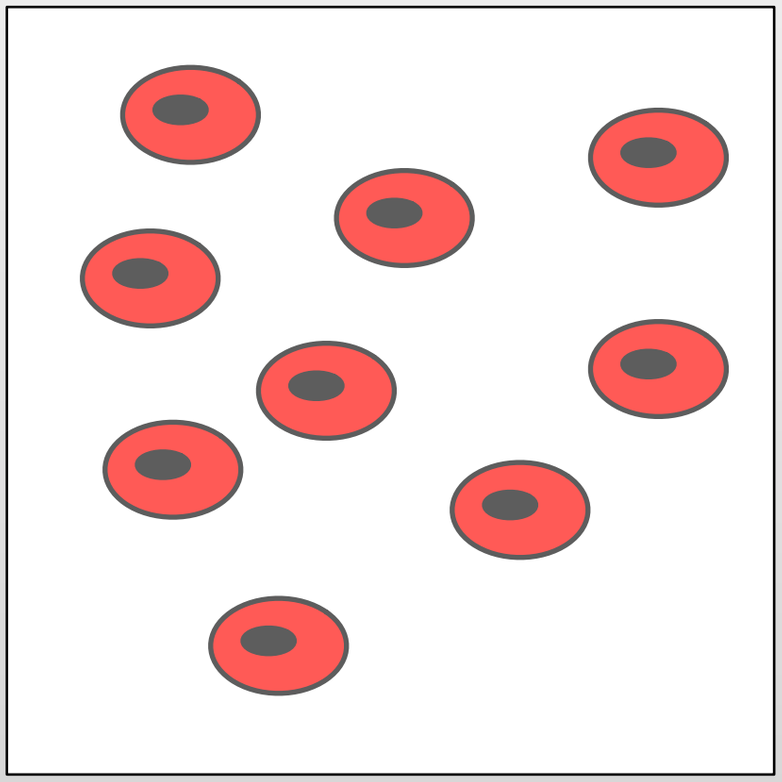
- Promoter activity reporter



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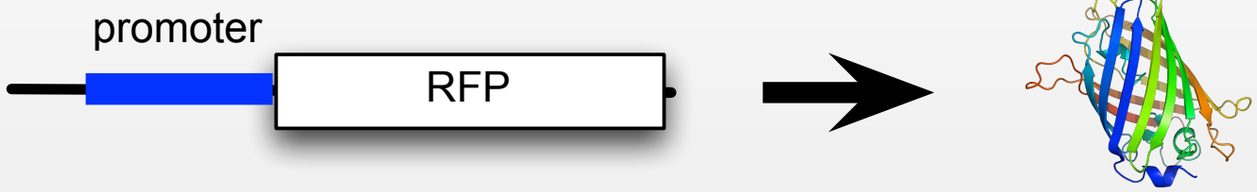
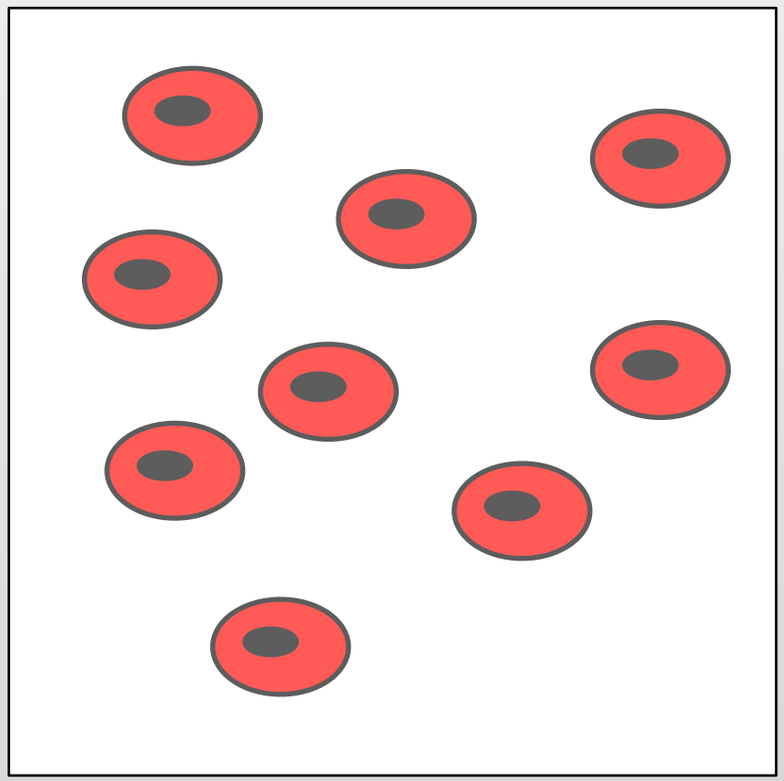
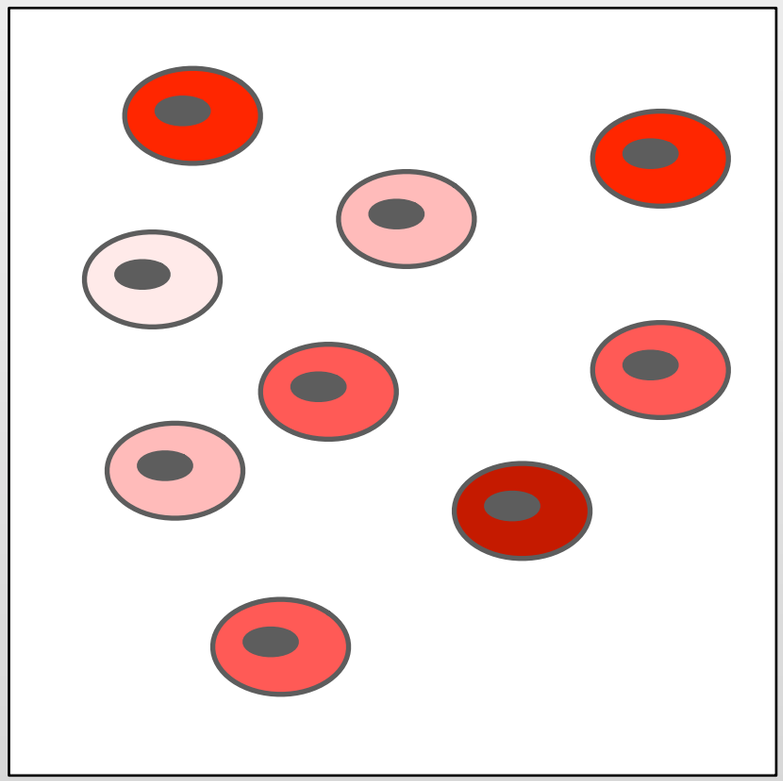
- Promoter activity reporter





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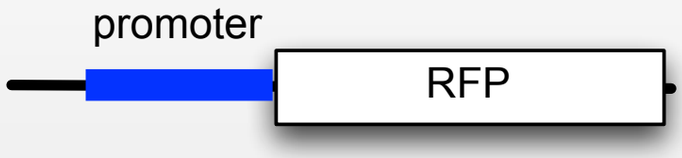
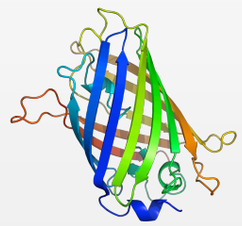
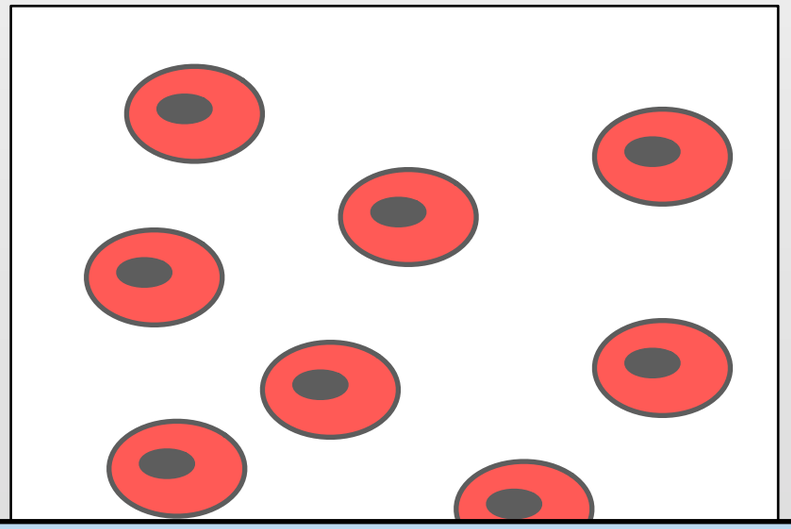
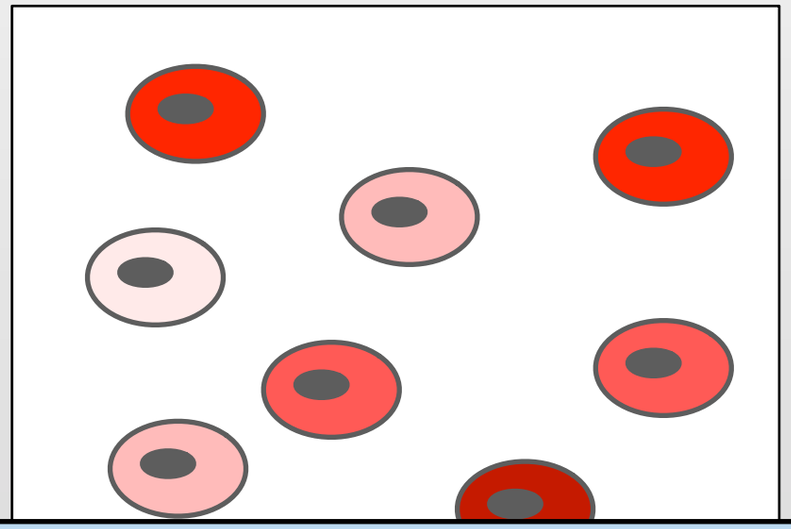
- Promoter activity reporter



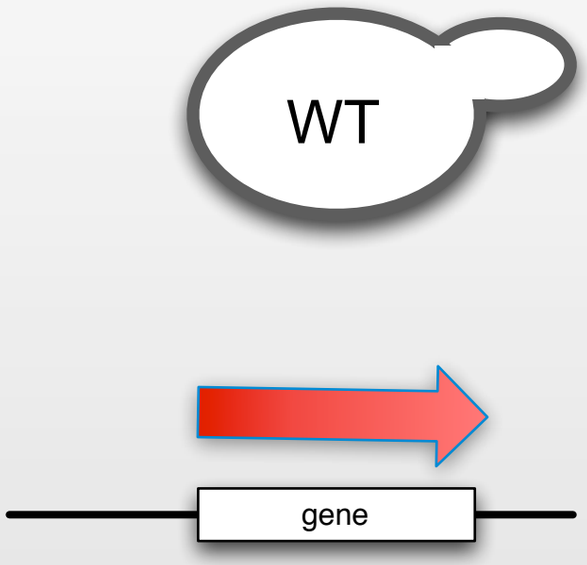




Cell-to-cell variability as phenotype

- Difference from mean expression?
- Relations to mechanism?

# Thought Experiment



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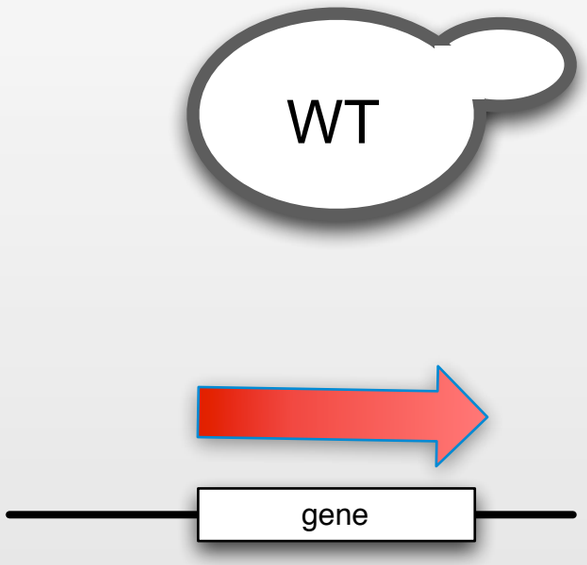
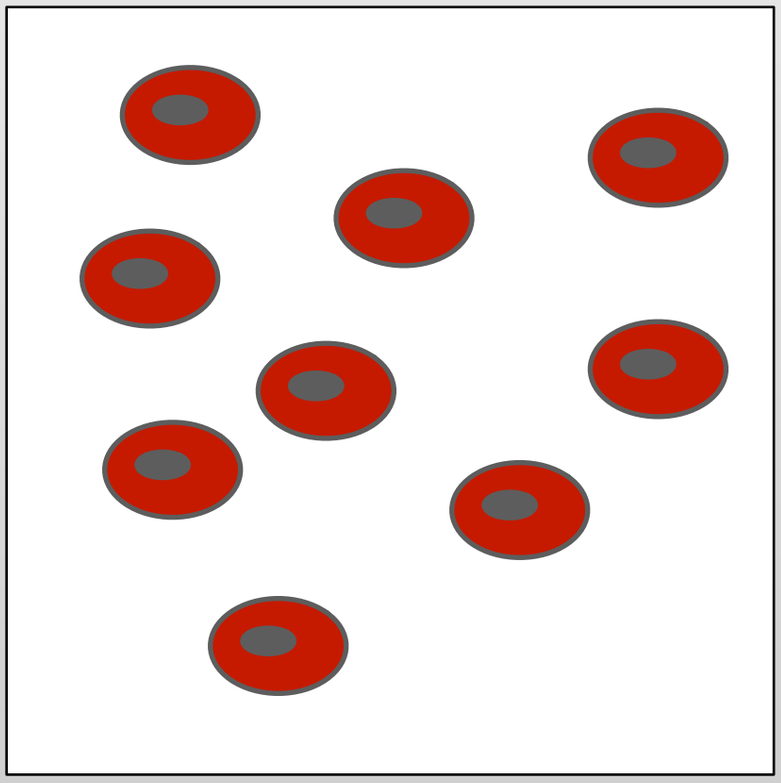
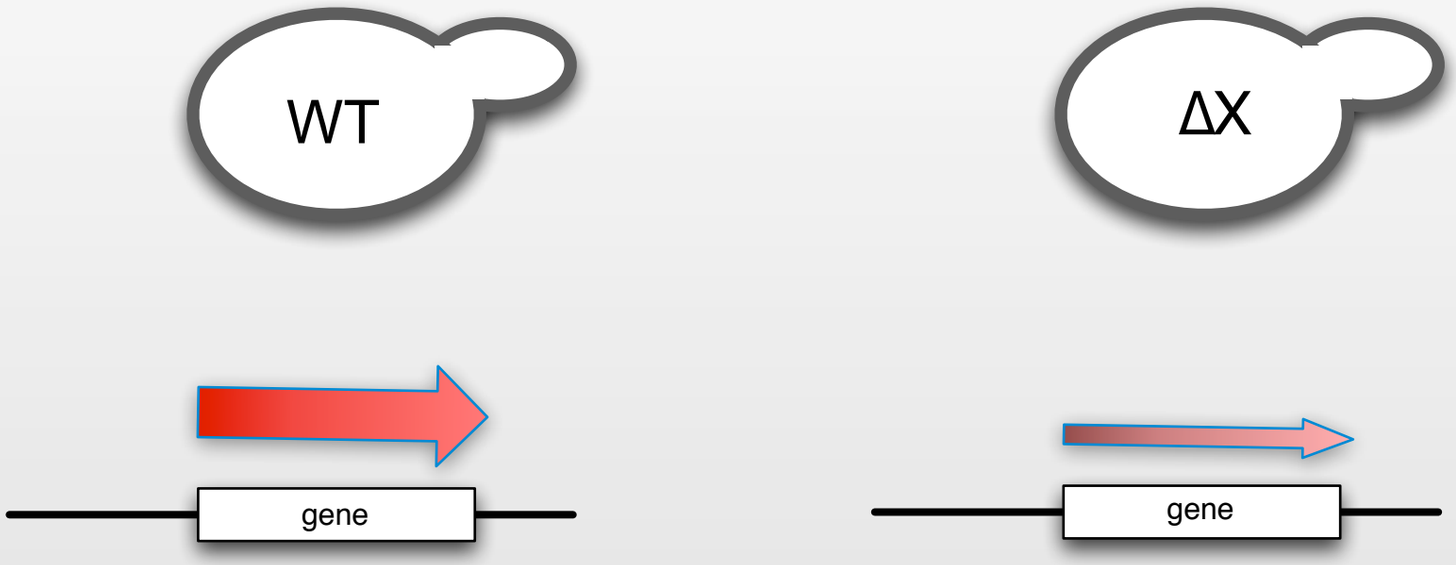
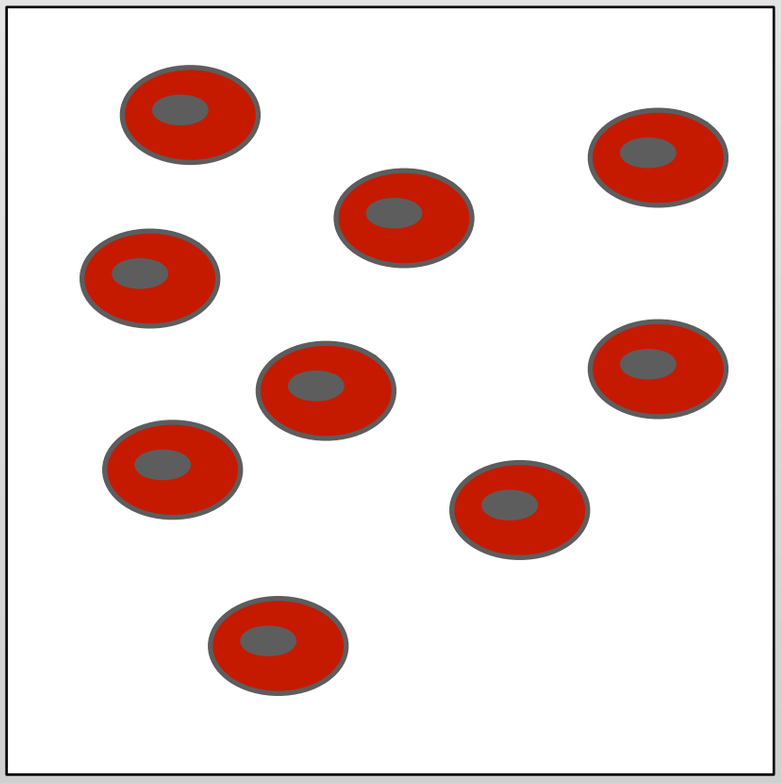


# Thought Experiment



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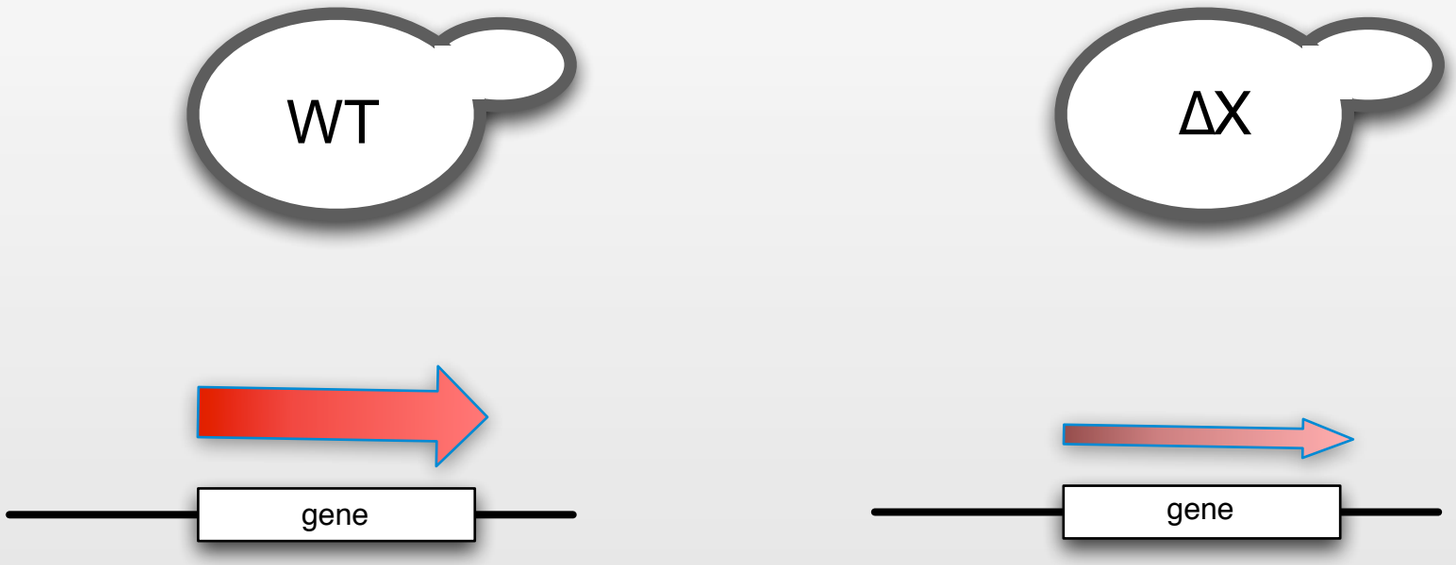
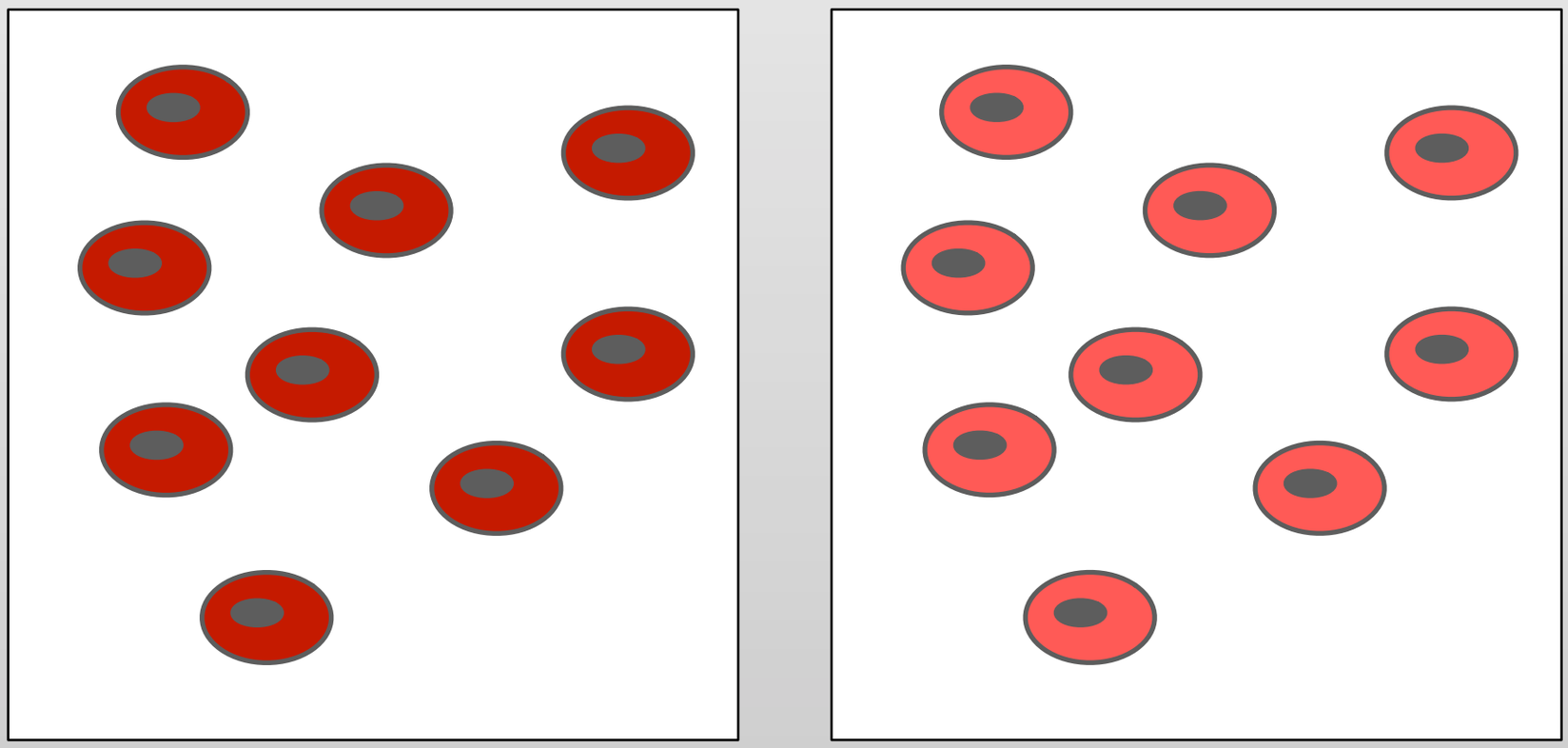
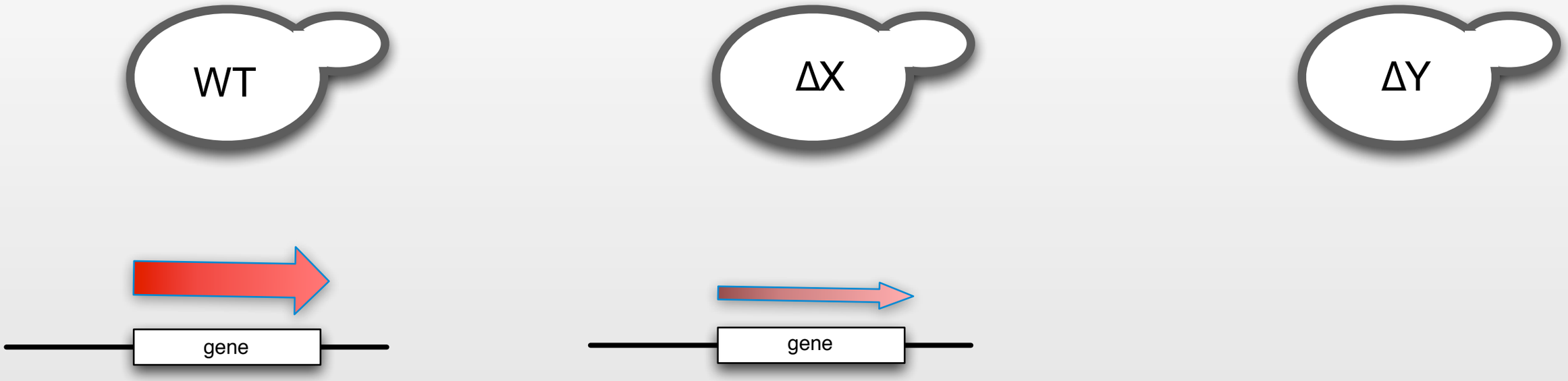
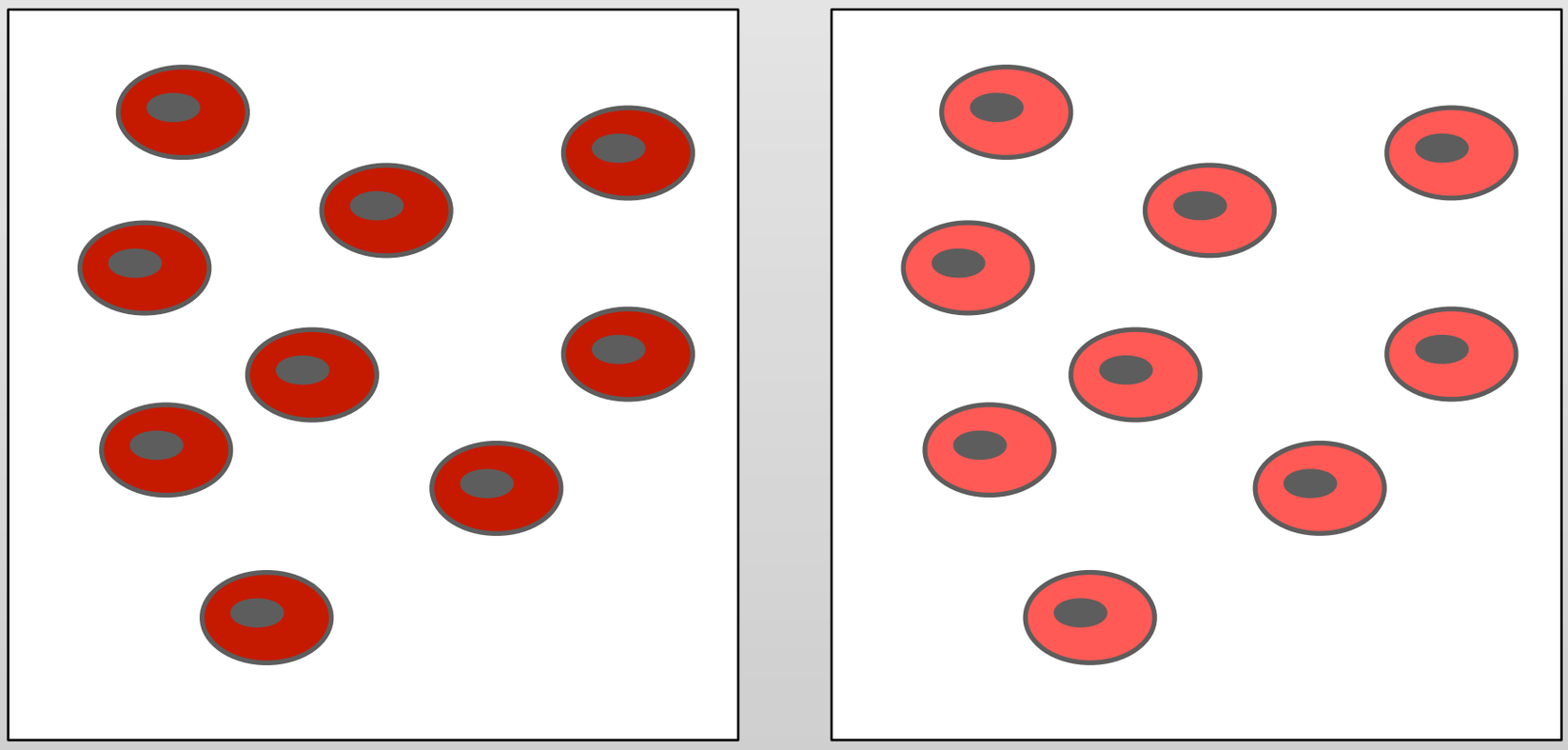


# Thought Experiment



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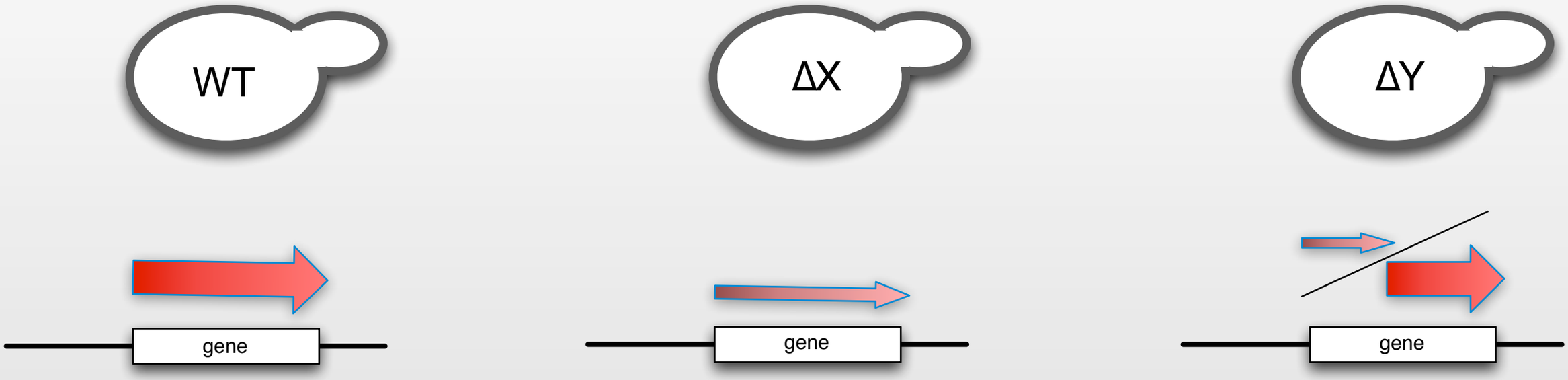
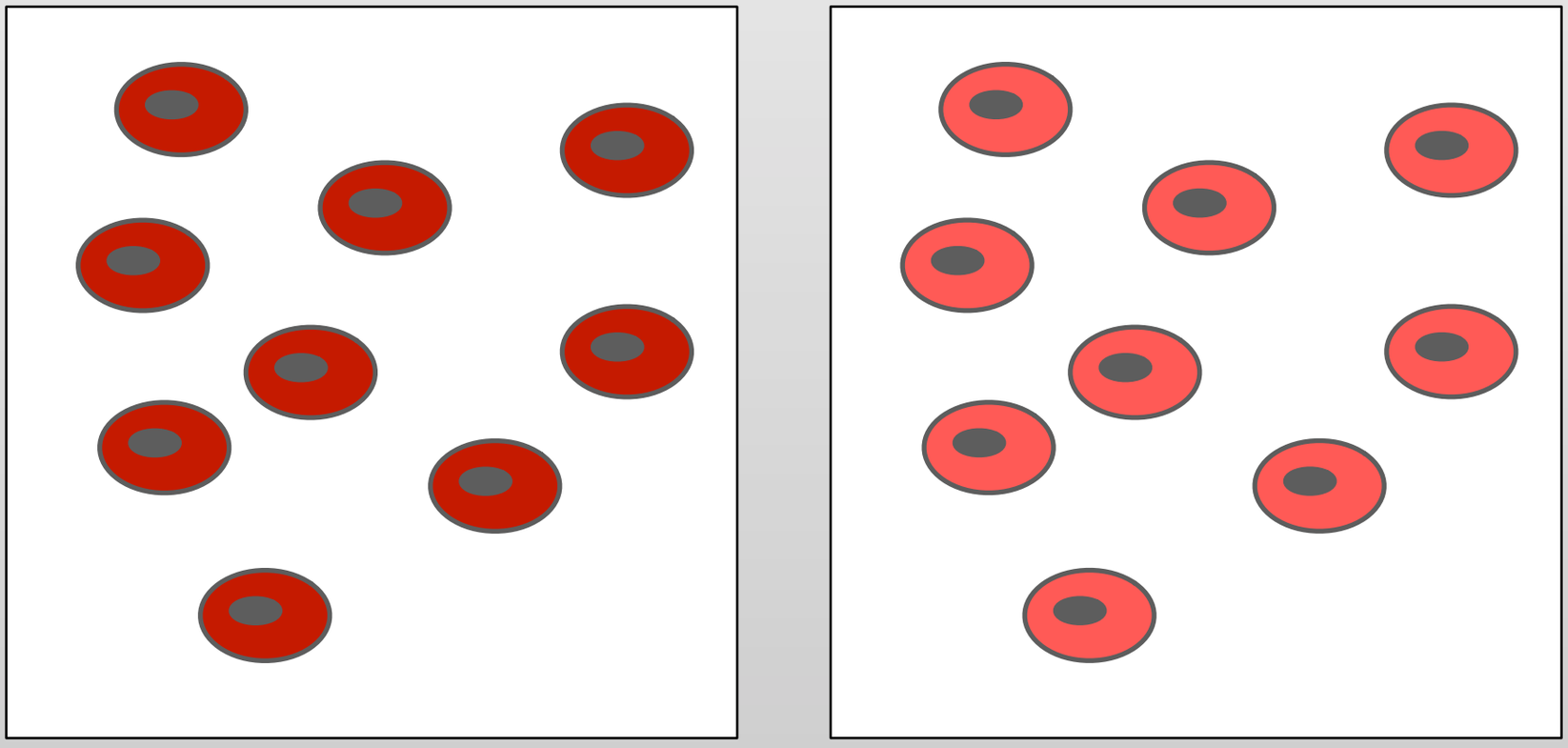
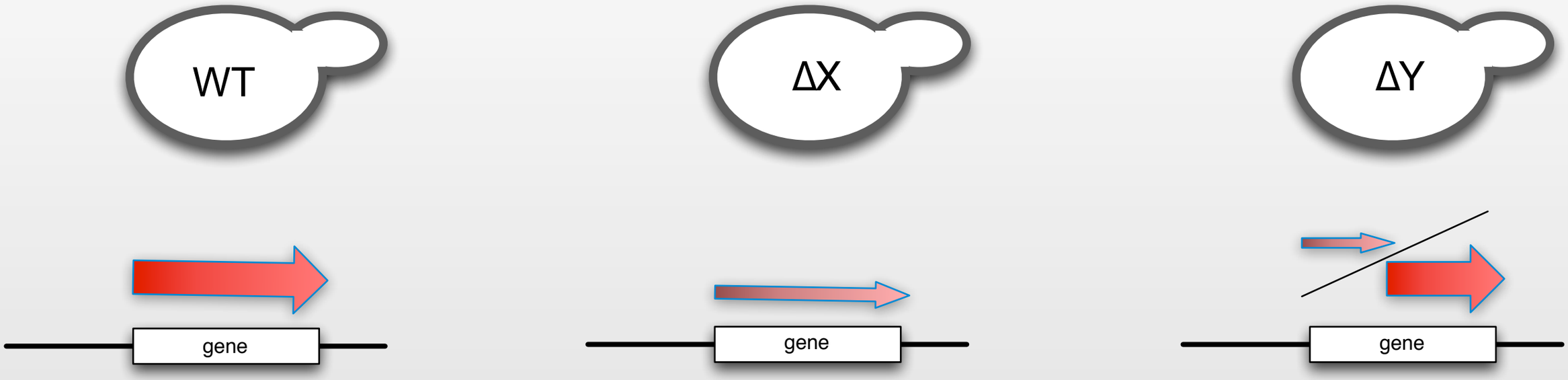
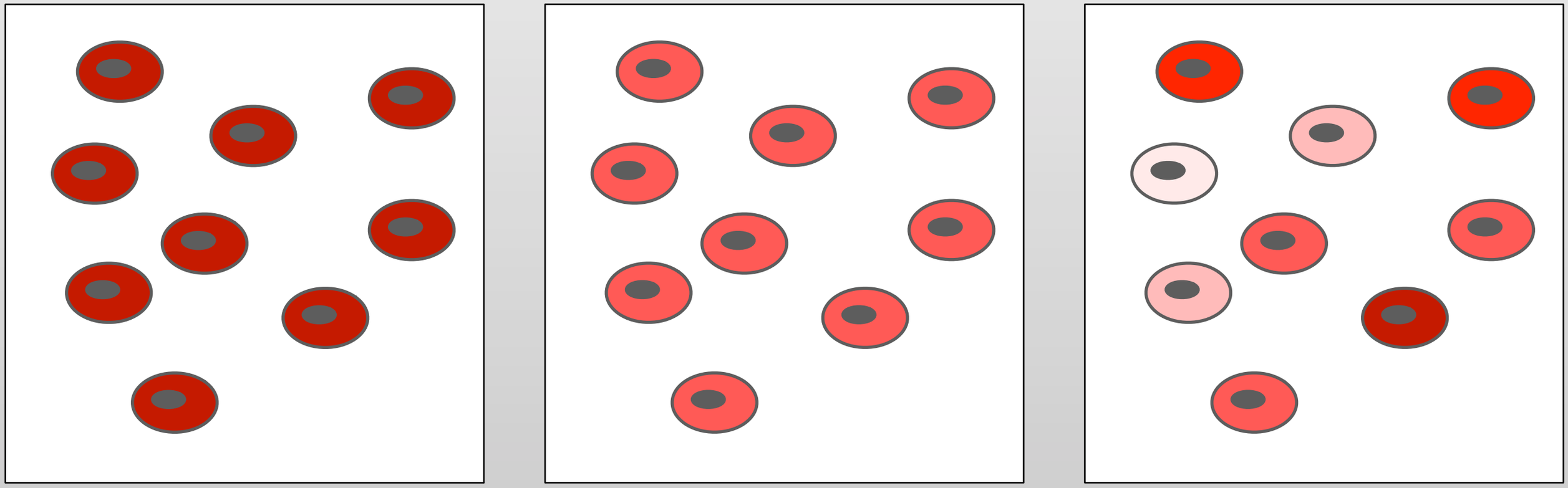


# Thought Experiment



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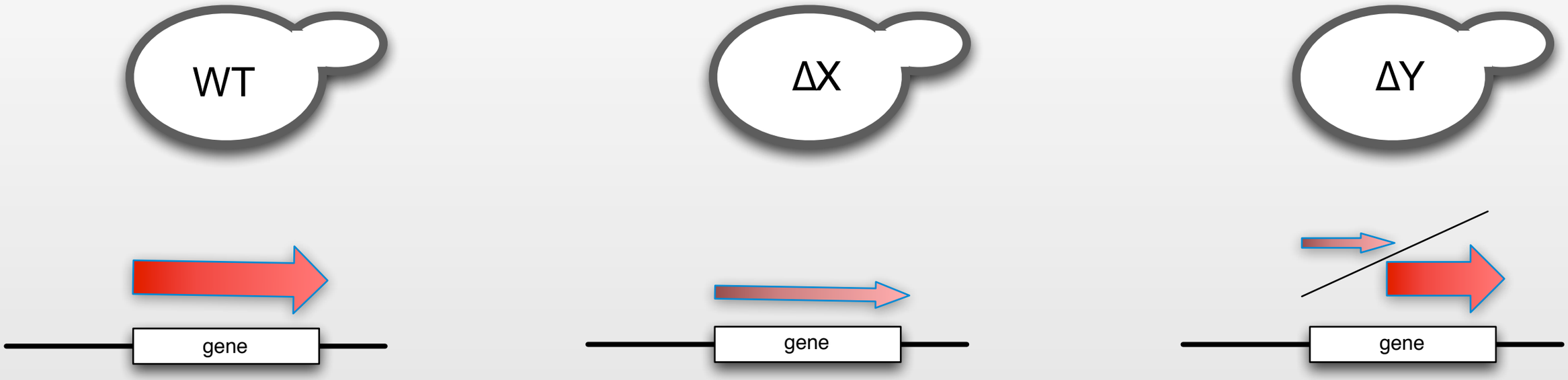
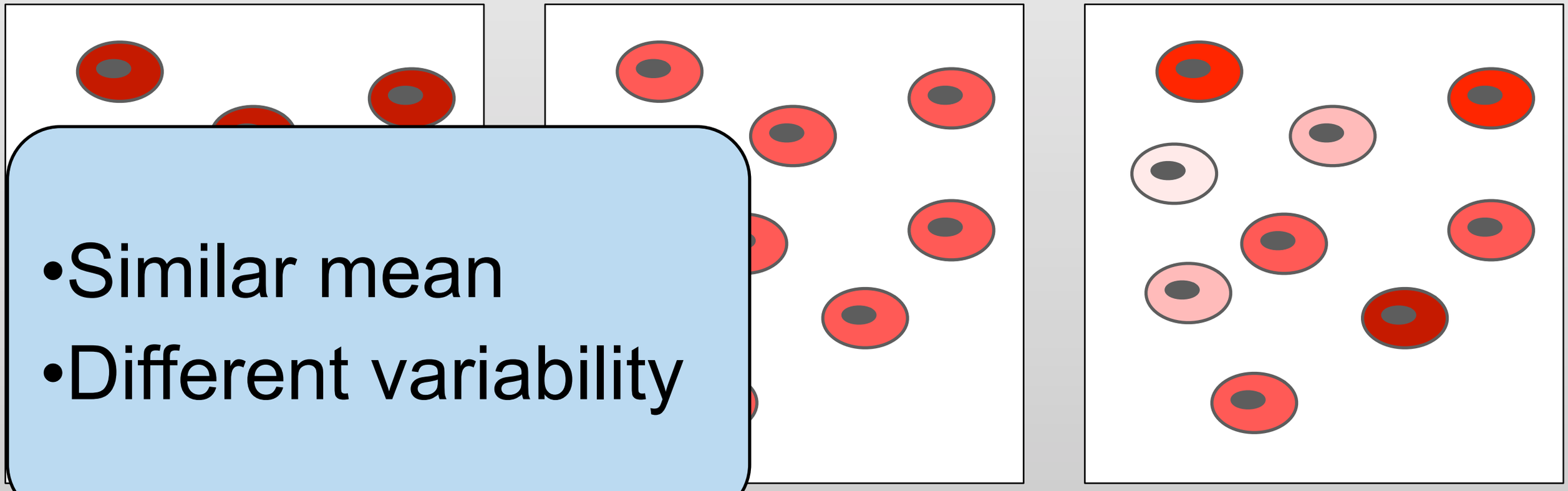
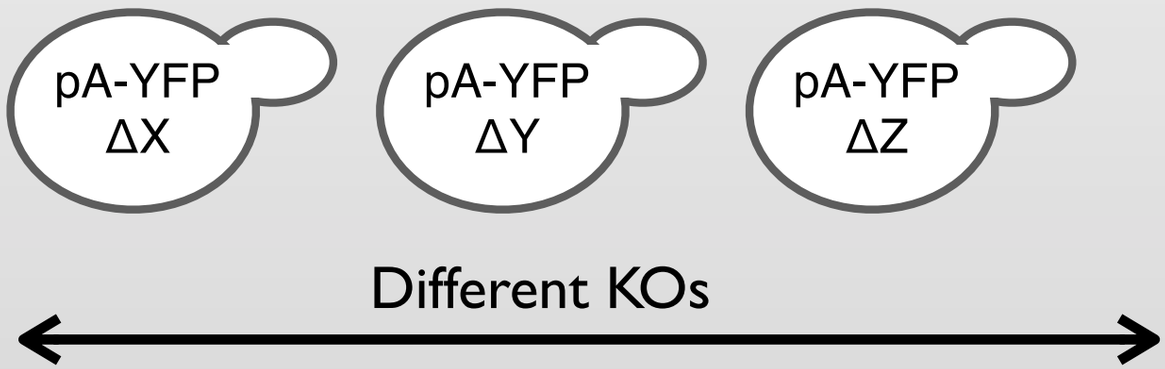


# Screening for Variability

Experiment design

- Build fluorescent protein reporter for target promoter
- Cross with KO library
- Scan fluorescence levels in cells from each KO



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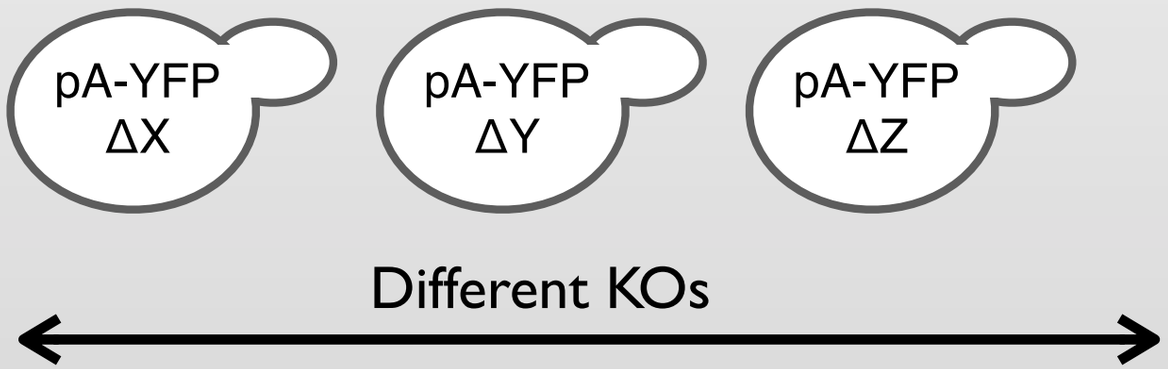


Luckily, the data was already collected

# UPRE Screen

**Jonikas *et al*, *Science* 2009**

# UPRE Screen

## Comprehensive Characterization of Genes Required for Protein Folding in the Endoplasmic Reticulum

Martin C. Jonikas,[1,2,3,4] Sean R. Collins,[1,3,4] Vladimir Denic,[1,3,4*] Eugene Oh,[1,3,4] Erin M. Quan,[1,3,4] Volker Schmid,[5] Jimena Weibezahn,[1,3,4] Blanche Schwappach,[5] Peter Walter,[2,3] Jonathan S. Weissman,[1,3,4†] Maya Schuldiner[1,3,4‡]

**Jonikas *et al*, *Science* 2009**

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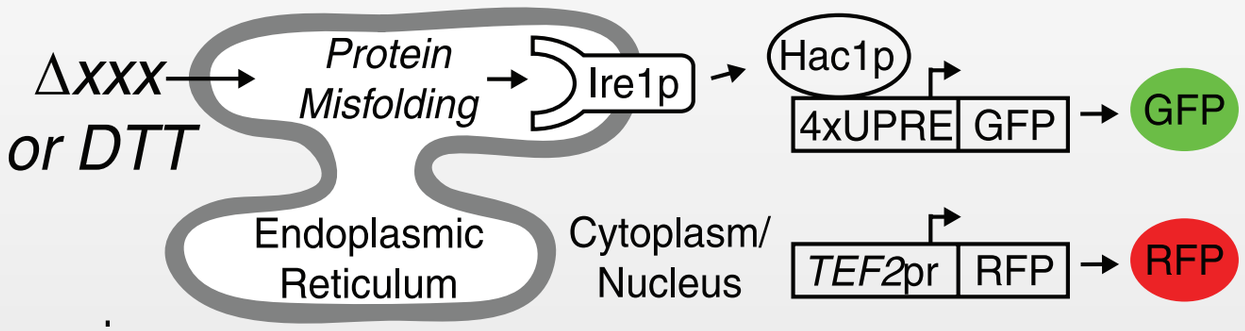


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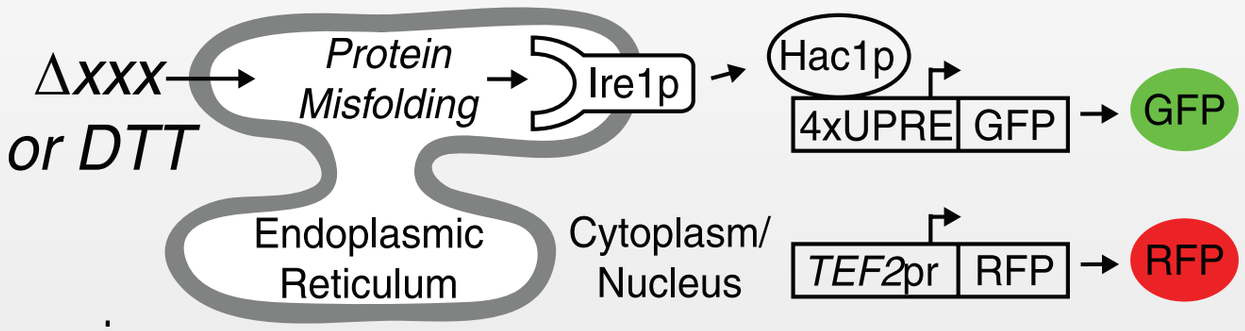


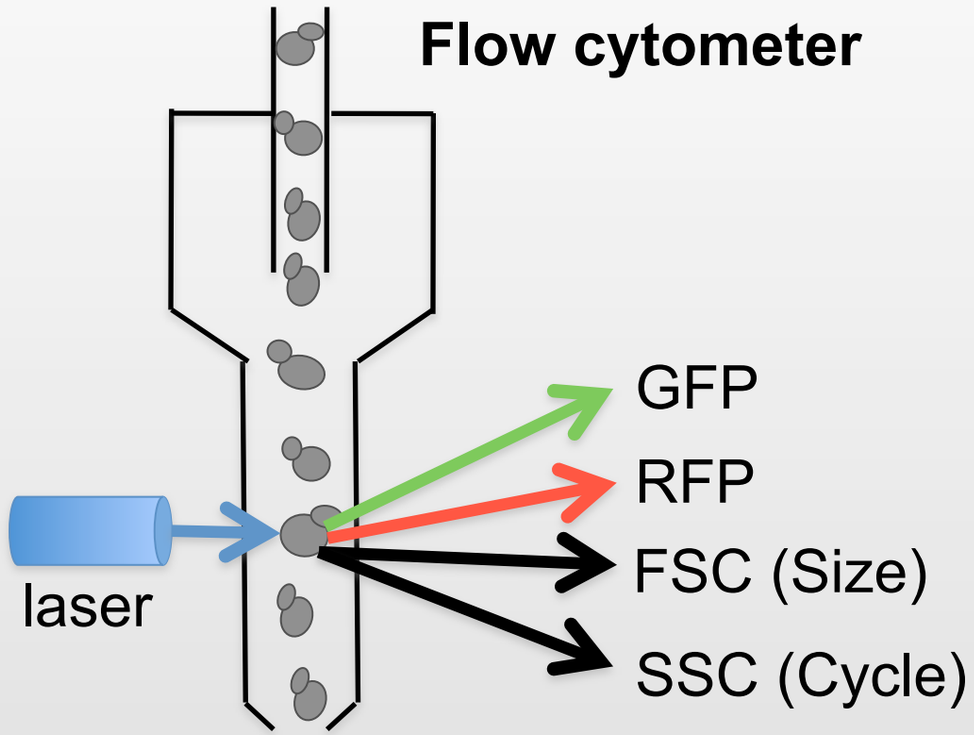


**Jonikas *et al*, *Science* 2009**

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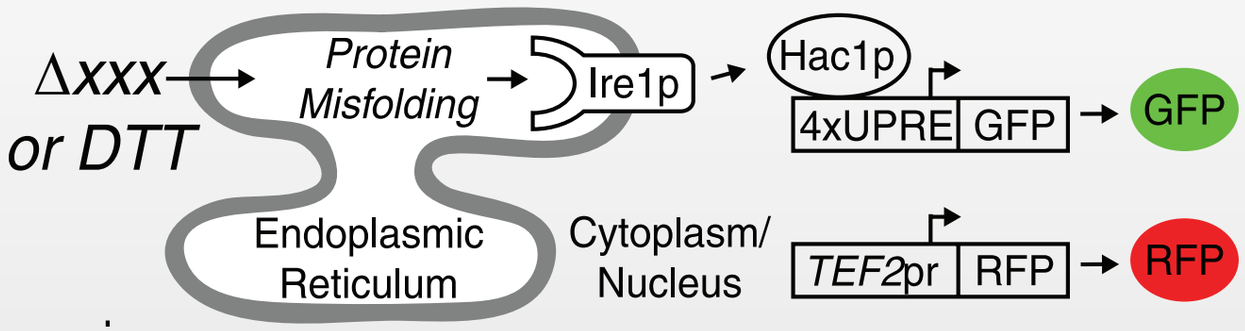


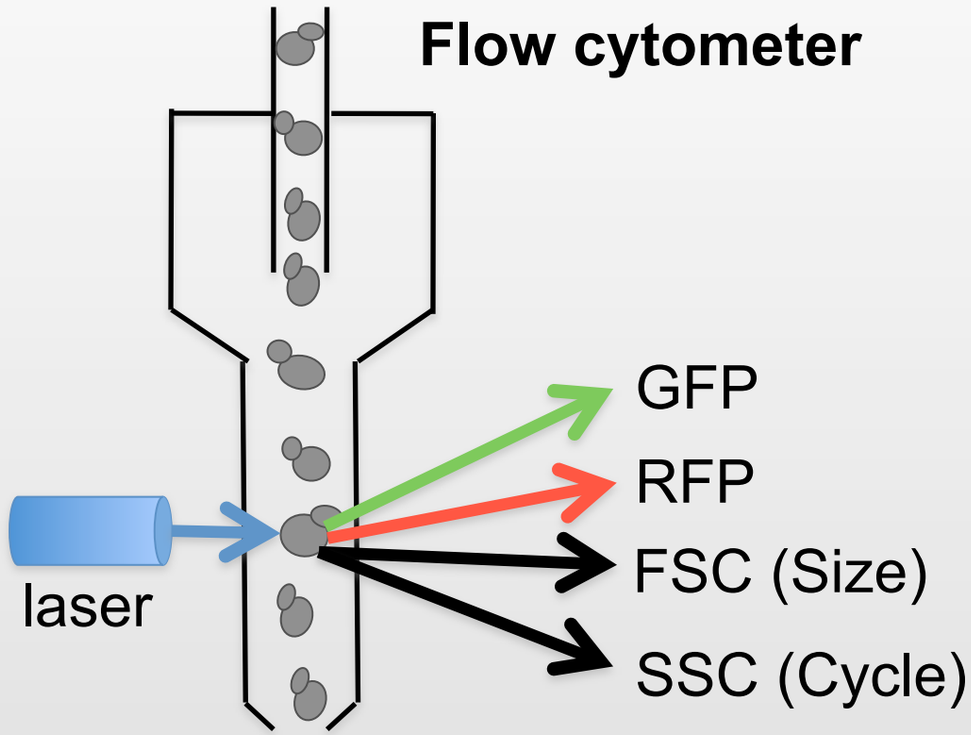


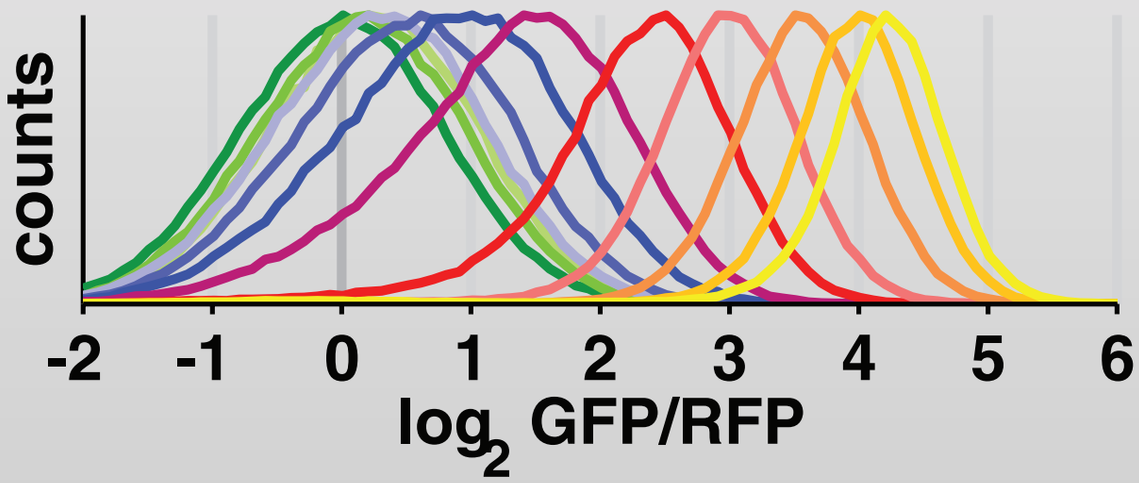


Jonikas *et al*, *Science* 2009

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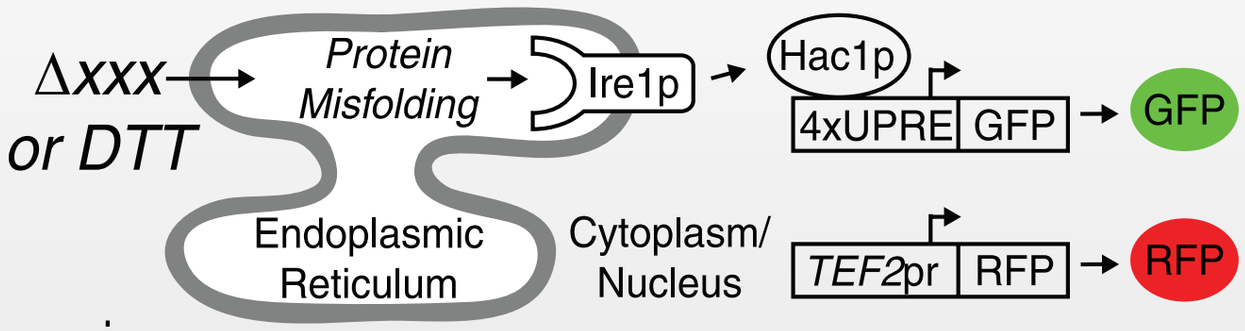


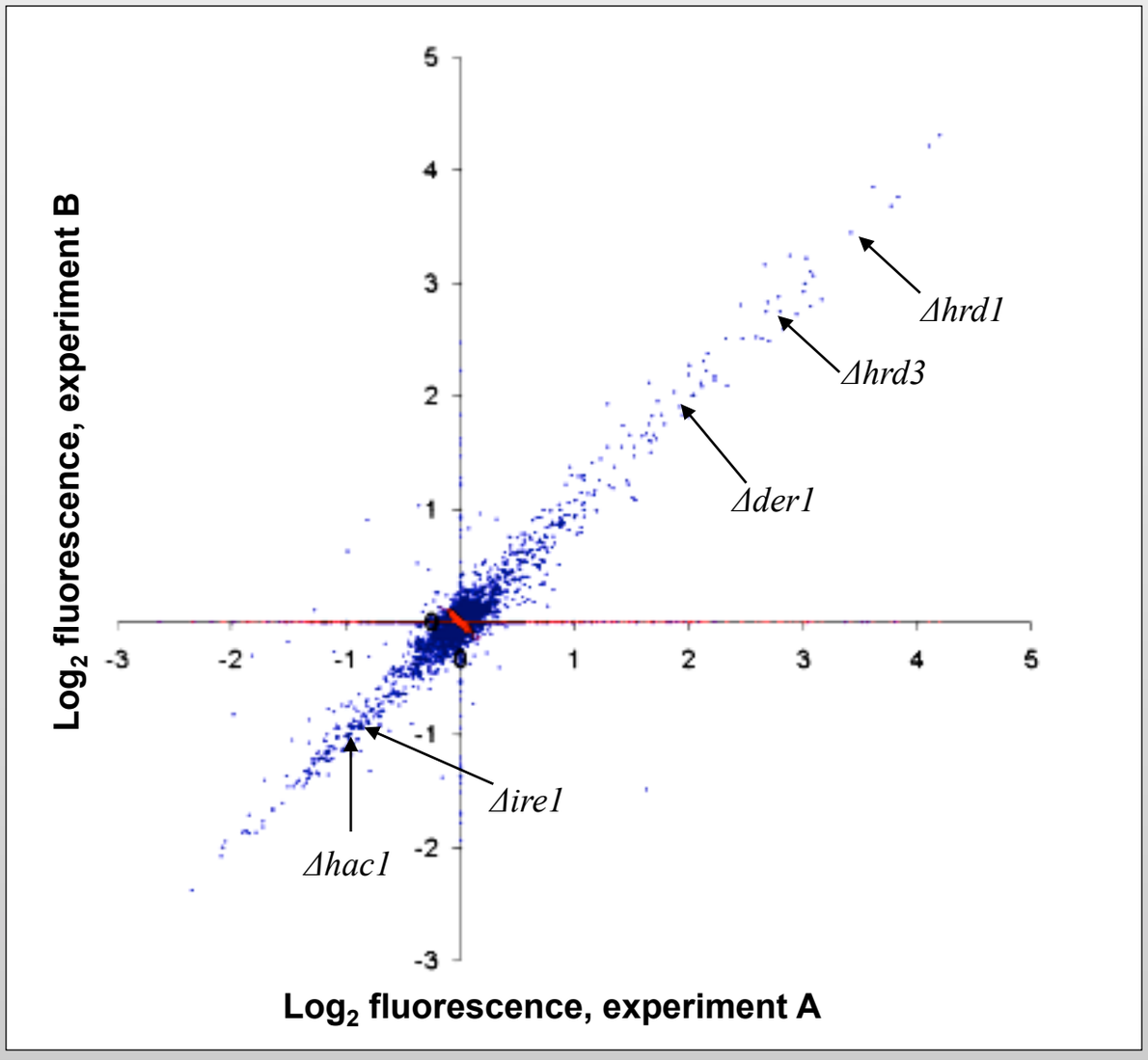


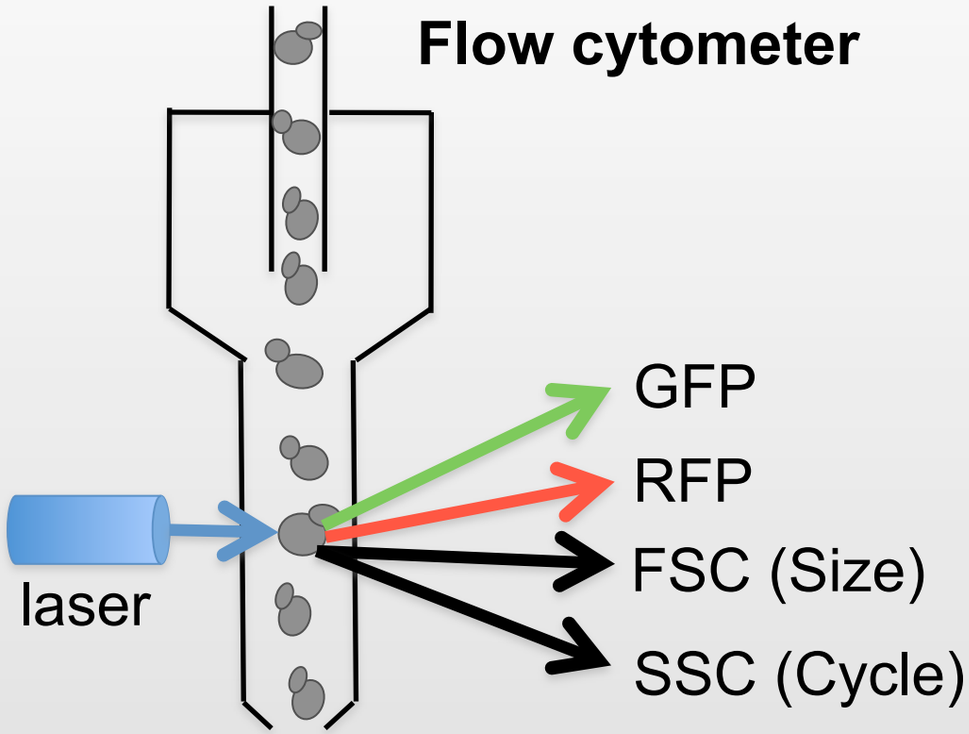


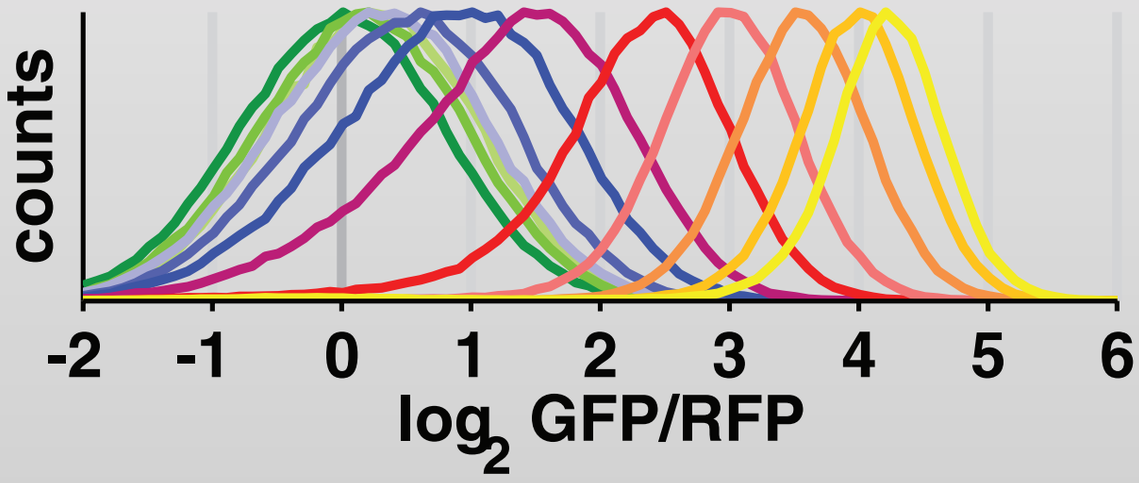


Jonikas *et al*, *Science* 2009

# Estimating Variability

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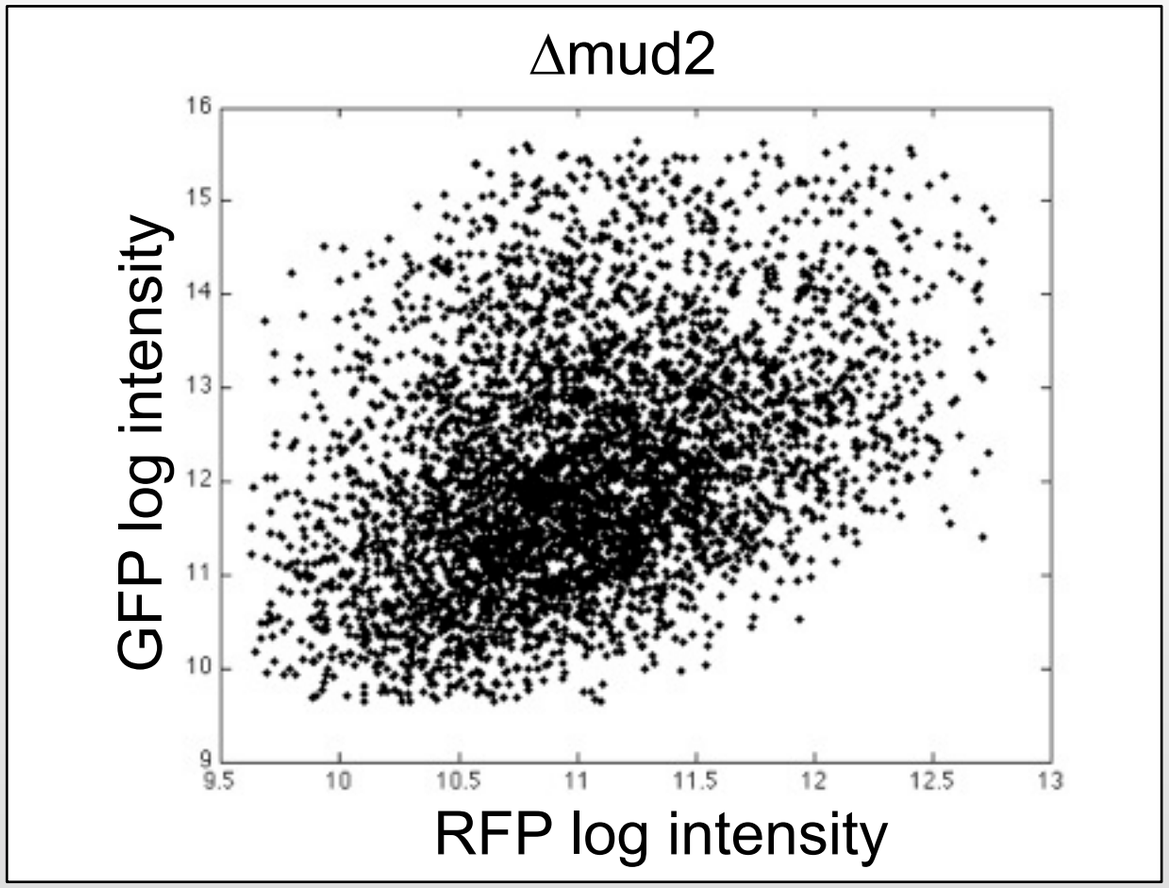

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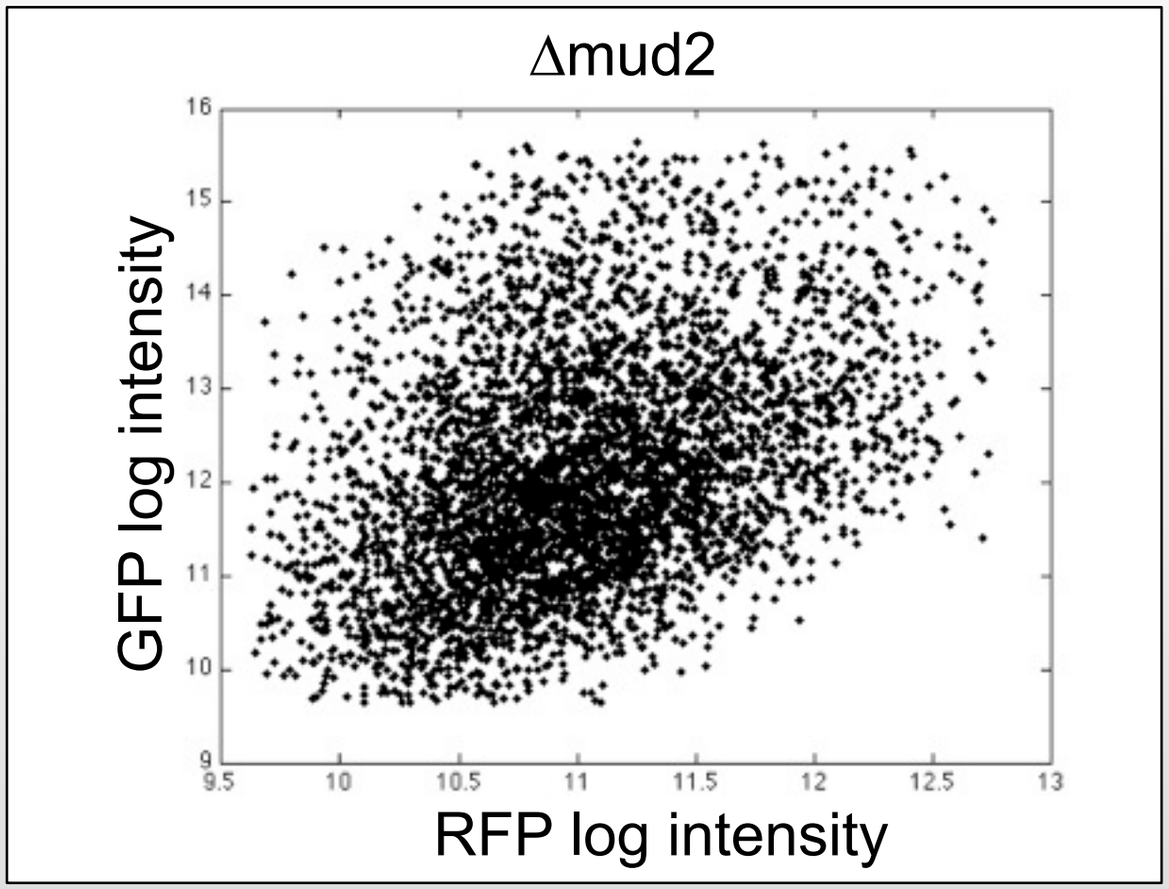
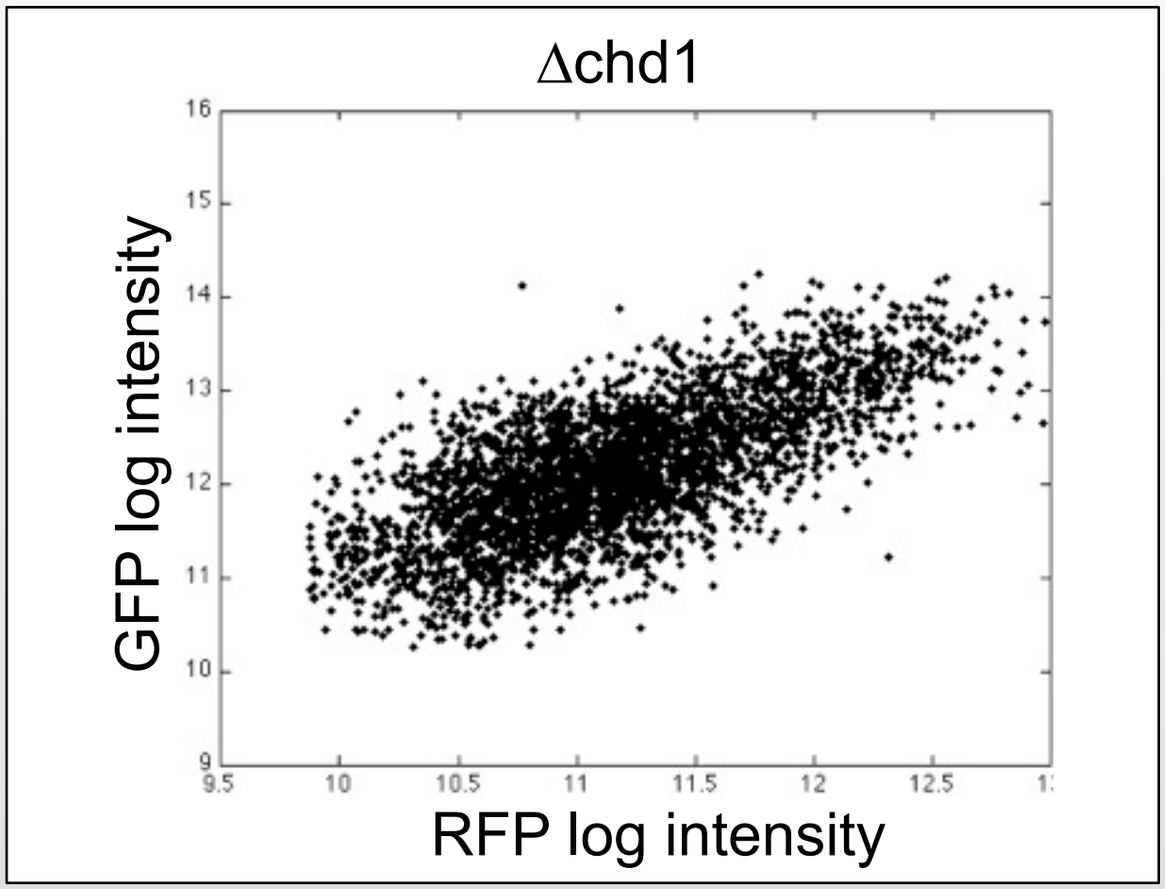
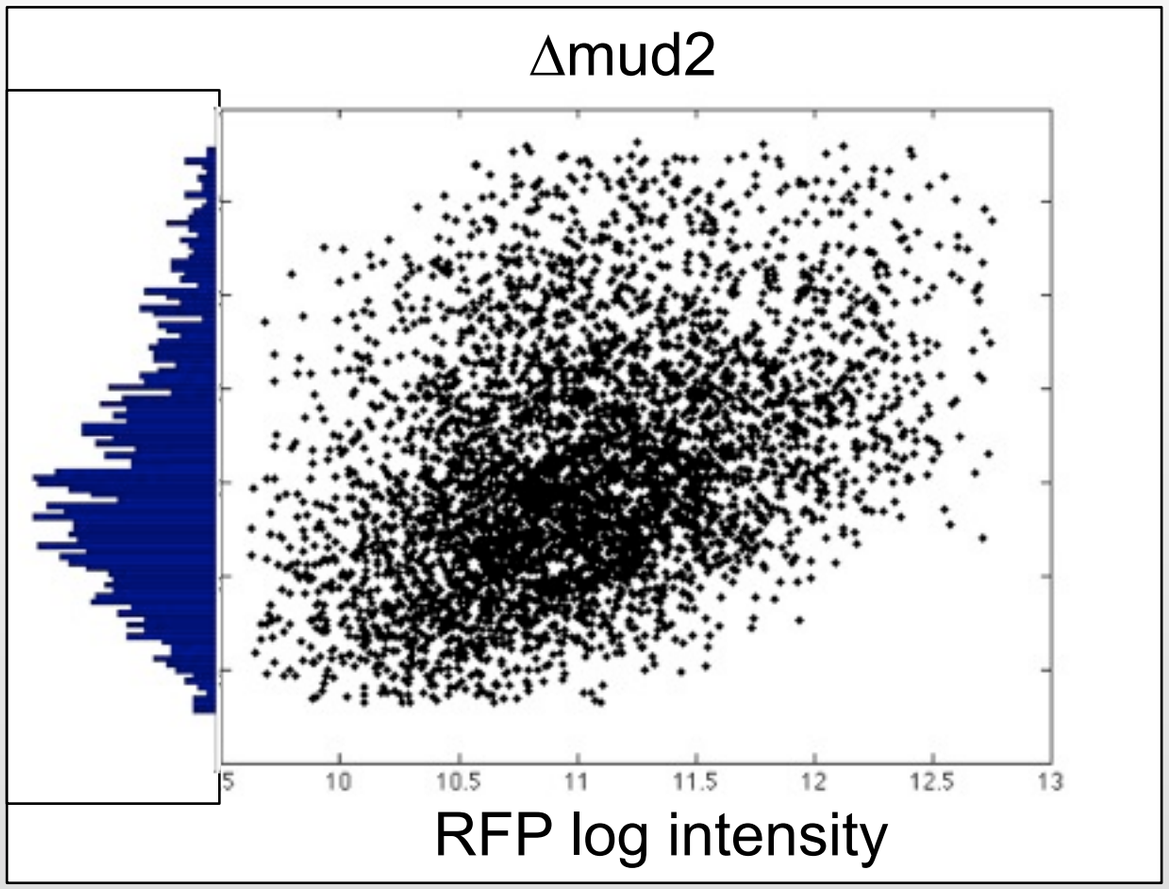
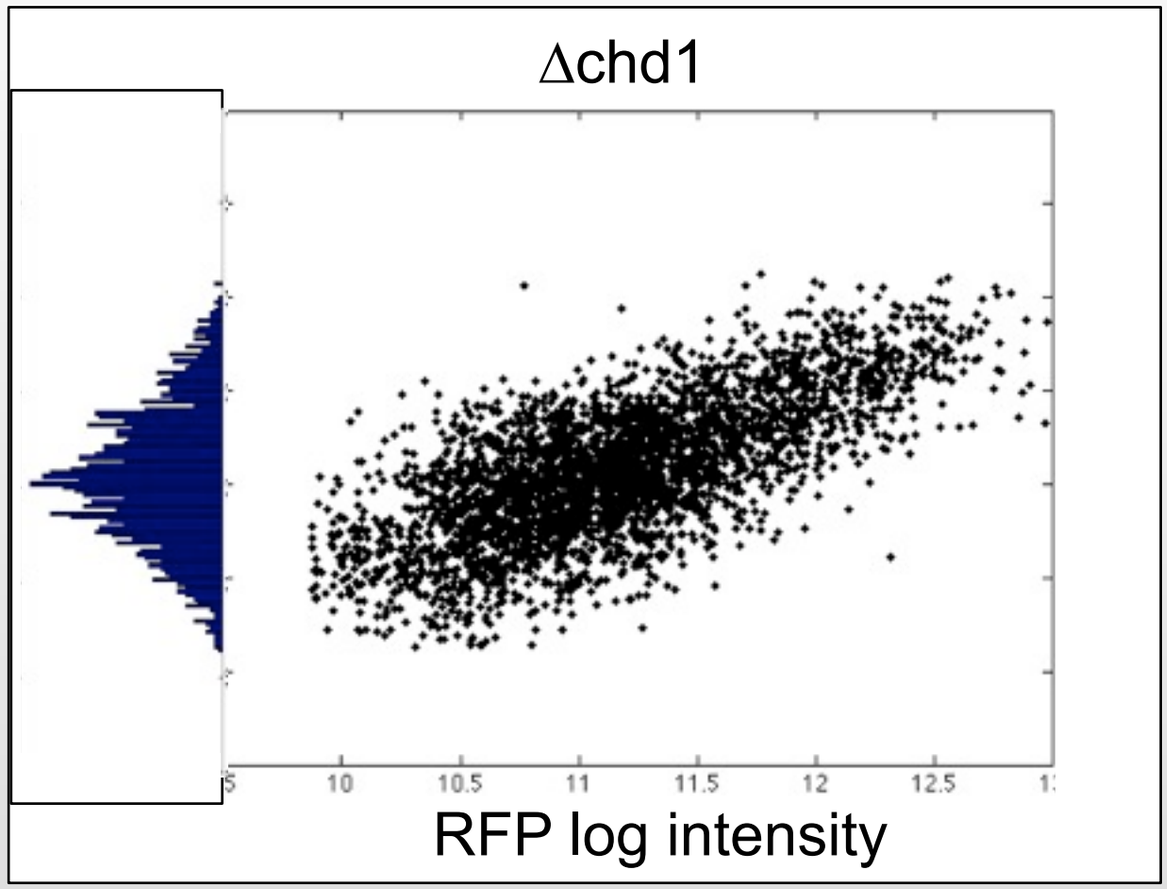


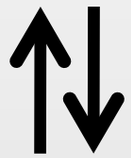
# Estimating Variability



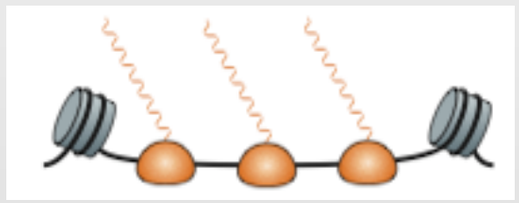



# Sources of Variability

Closed chromatin



Open chromatin



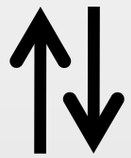
mRNA

Protein

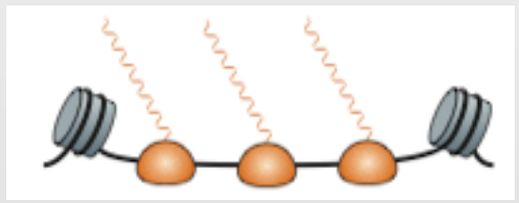
# Sources of Variability

## Local

Closed chromatin



Open chromatin



mRNA

Protein

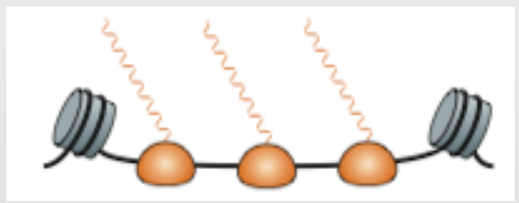
# Sources of Variability

## Local

## Global

Closed chromatin

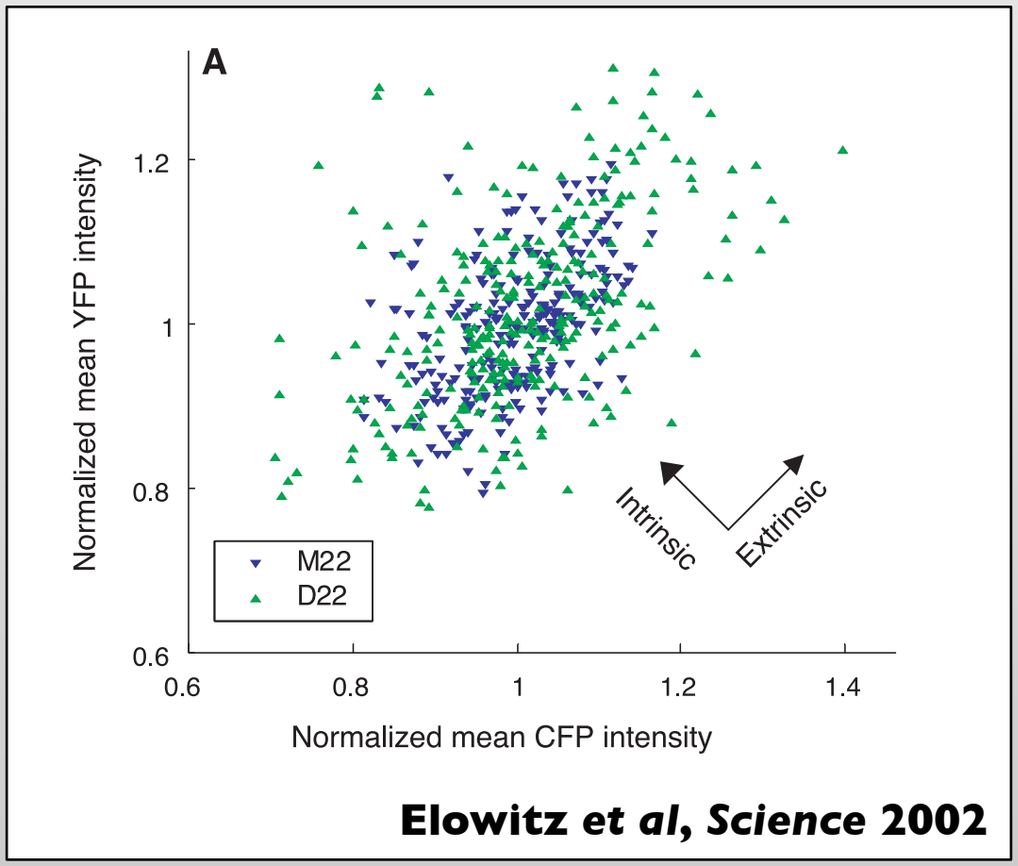
Open chromatin



mRNA

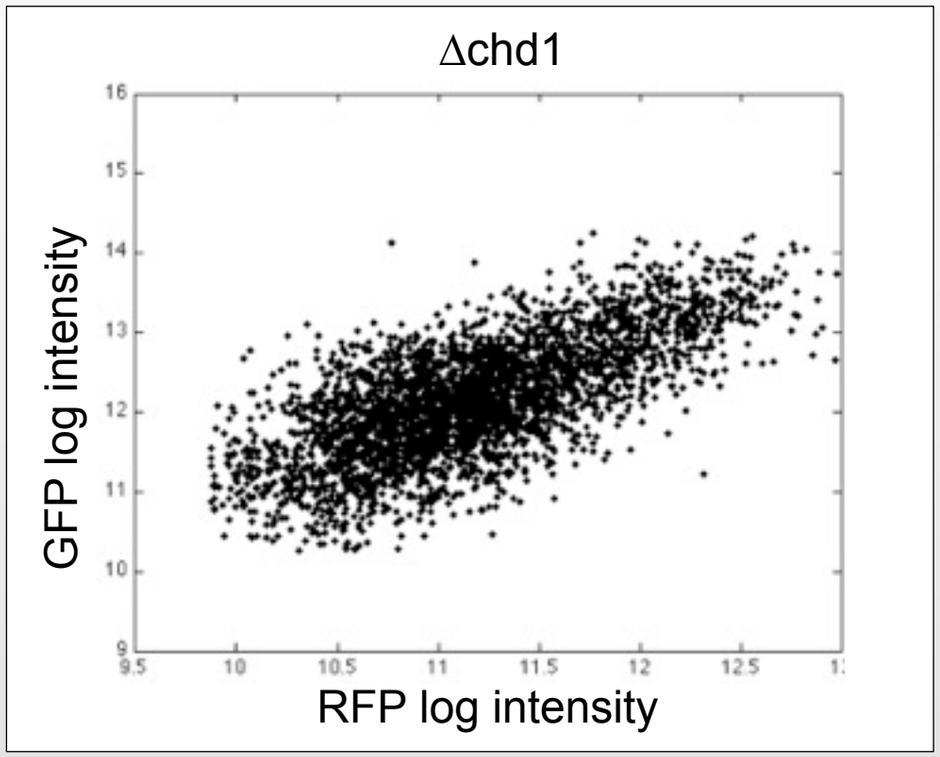
Protein

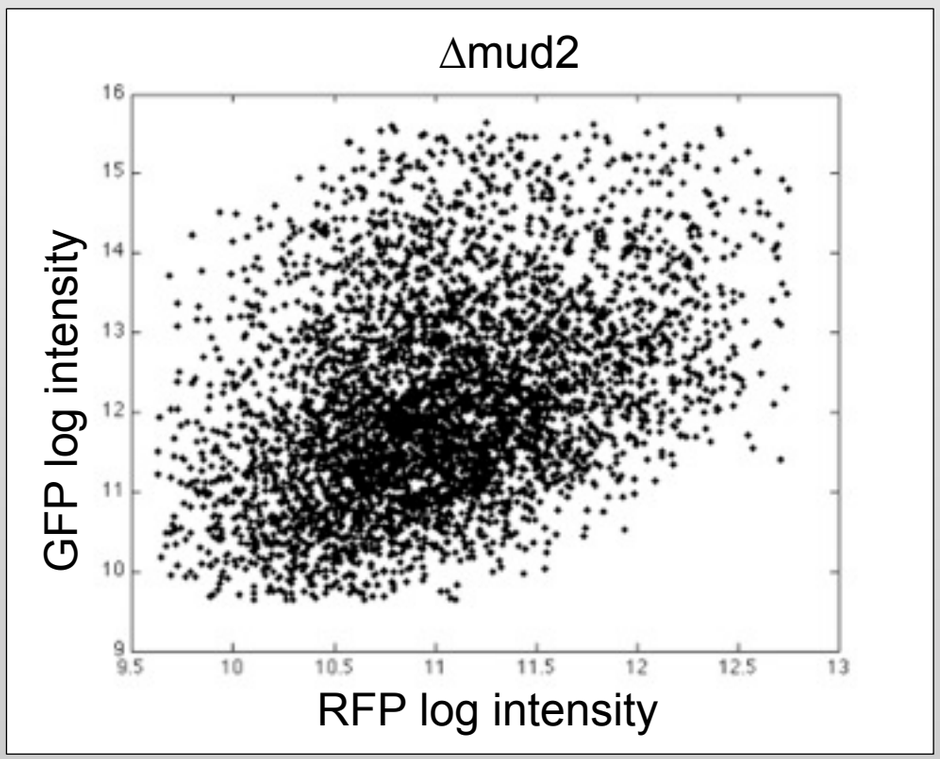
- Sensory input
- Metabolites/energy
- Cell cycle
- Cell size



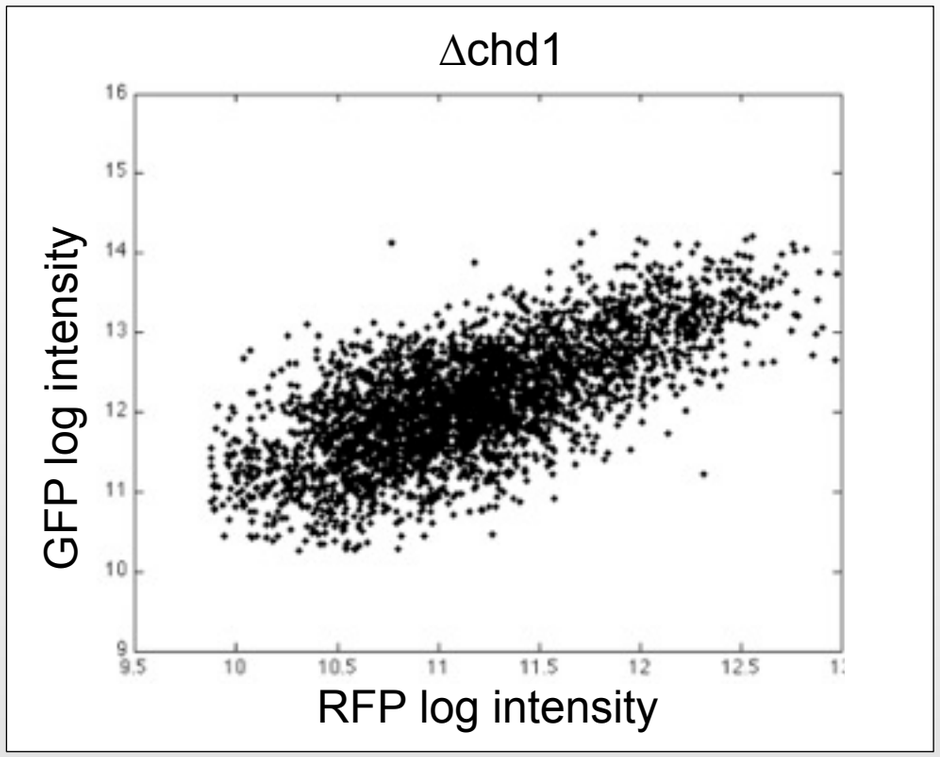

**Elowitz *et al*, *Science* 2002**

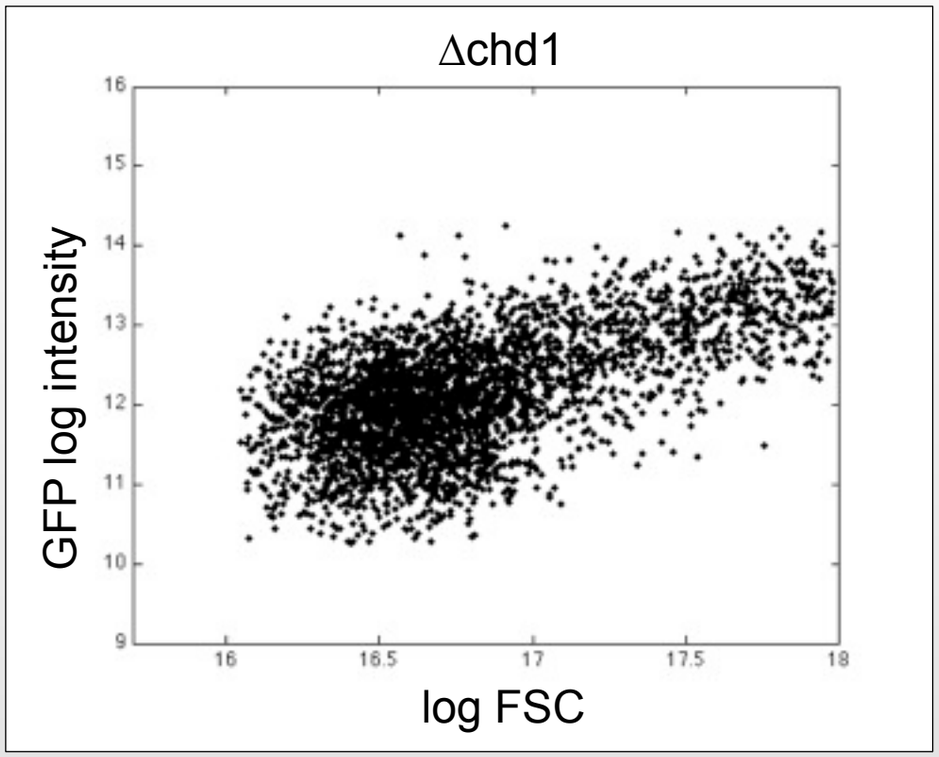
# Global Noise and FSC/SSC

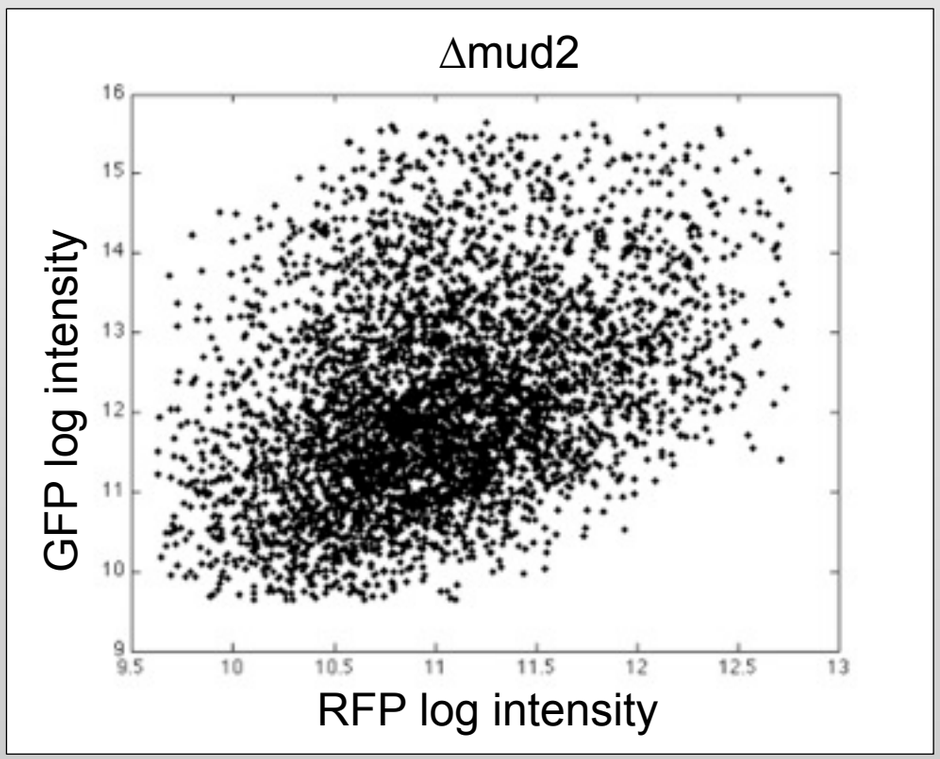


# Global Noise and FSC/SSC



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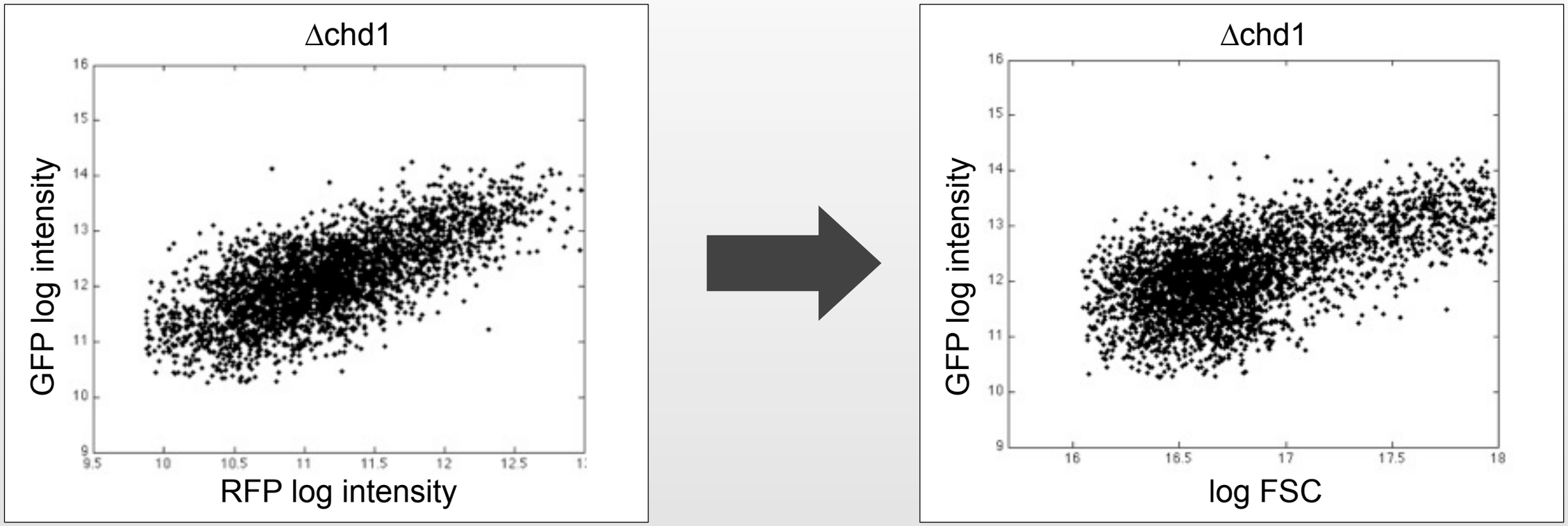
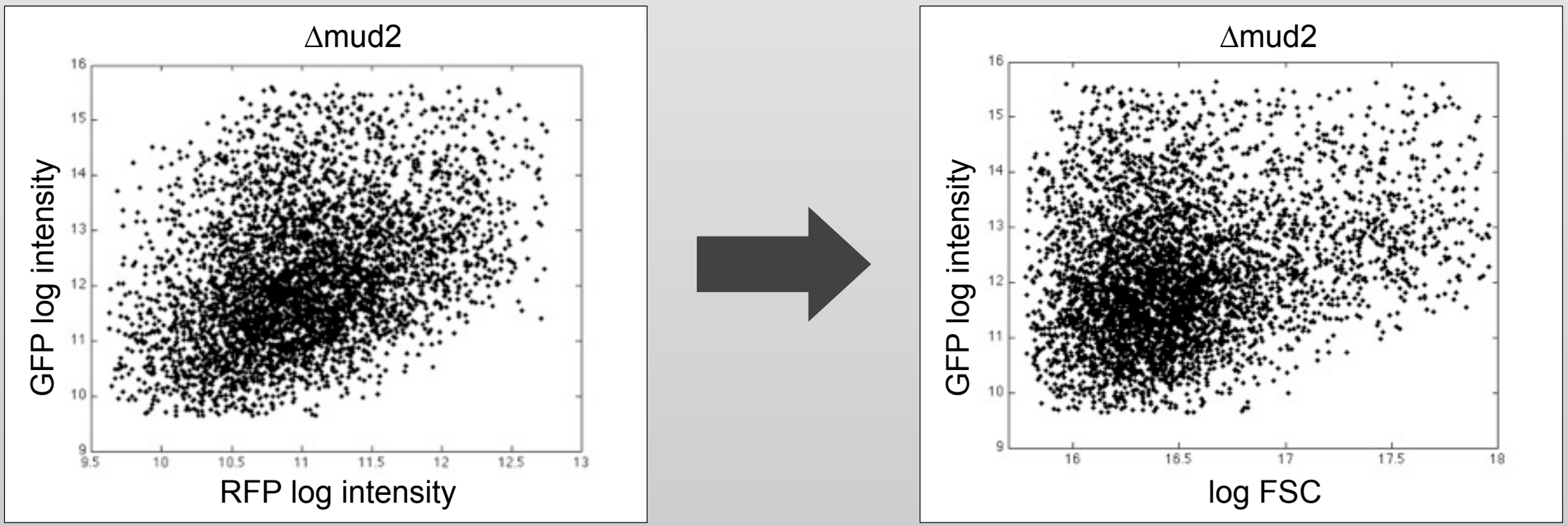
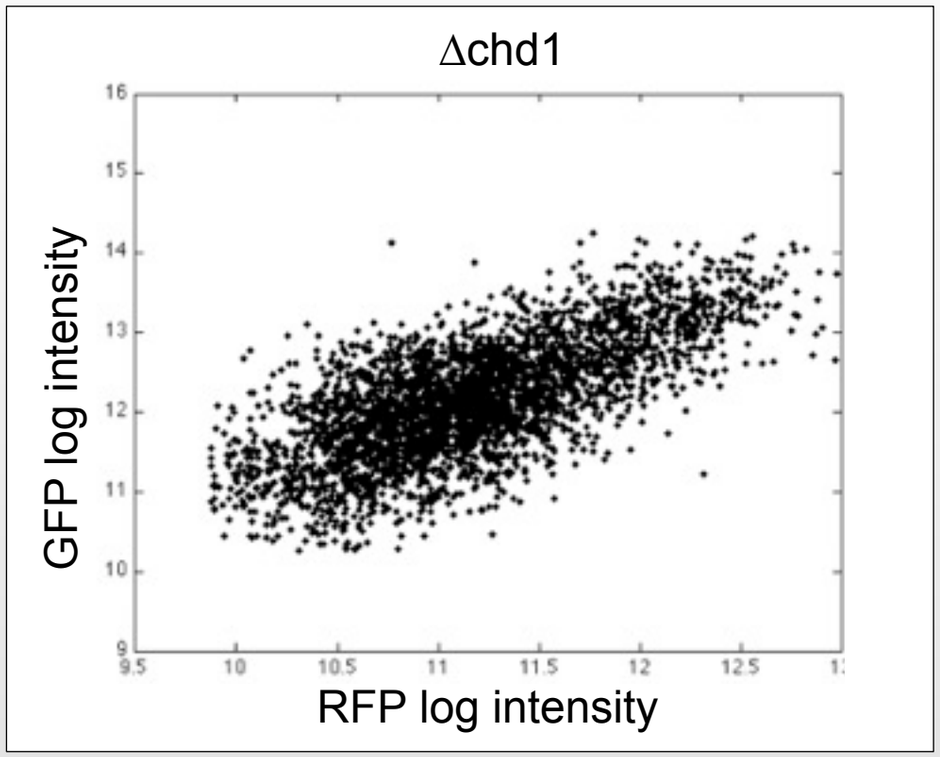
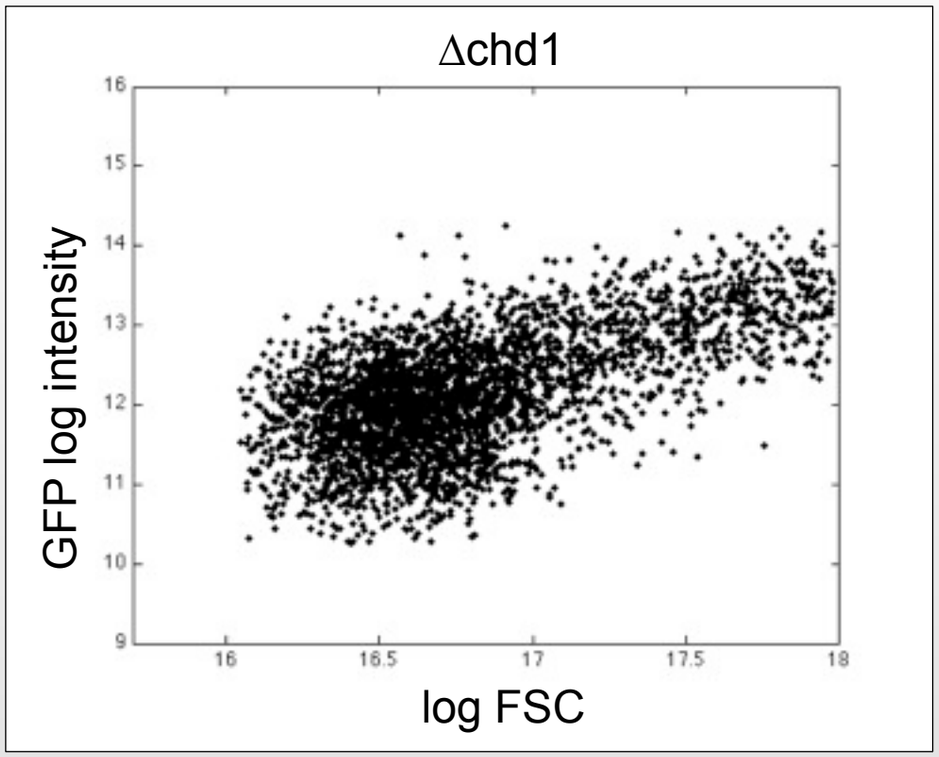
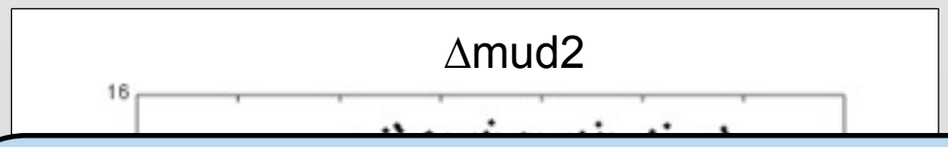


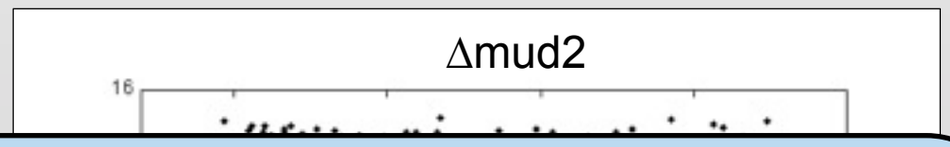



# Global Noise and FSC/SSC



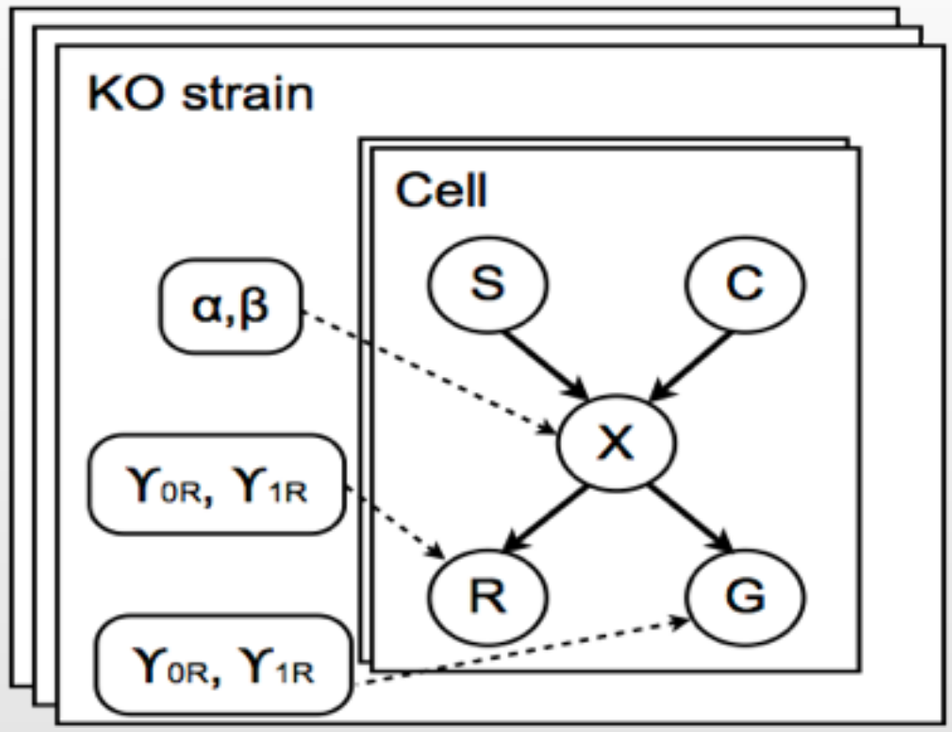




Cell size and granularity help differentiating  global & local variability

# Estimating Variability

- Linear regression model to estimate (co)-variability
- Take into account FSC/SSC




$$X = \alpha \cdot S + \beta \cdot C + \epsilon_X$$

$$G = \gamma_{0,G} + \gamma_{1,G} \cdot X + \epsilon_G \qquad \epsilon_G \sim N(0, \sigma_G^2)$$

$$R = \gamma_{0,R} + \gamma_{1,R} \cdot X + \epsilon_R \qquad \epsilon_R \sim N(0, \sigma_R^2)$$

## Local Variability

$$\ell_G = \sigma_G / \mu_G$$

$$\ell_R = \sigma_R / \mu_R$$

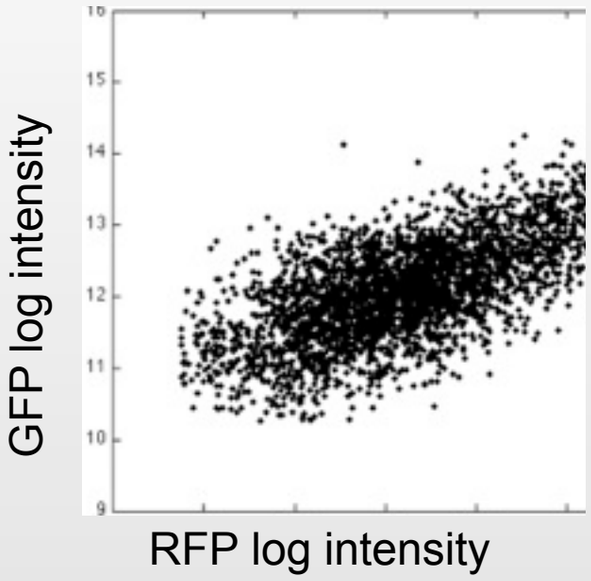
## Global Variability

$$g_G = \sqrt{\mathrm{Var}[G] - \sigma_G^2} / \mu_G$$

$$g_R = \sqrt{\mathrm{Var}[R] - \sigma_R^2} / \mu_R$$

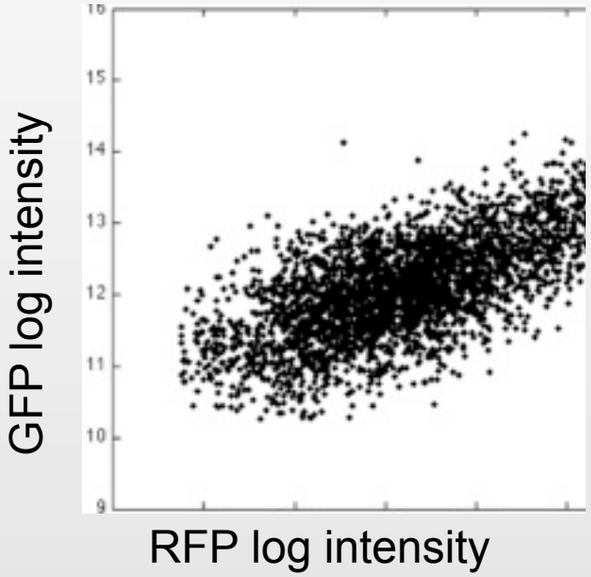
# Example

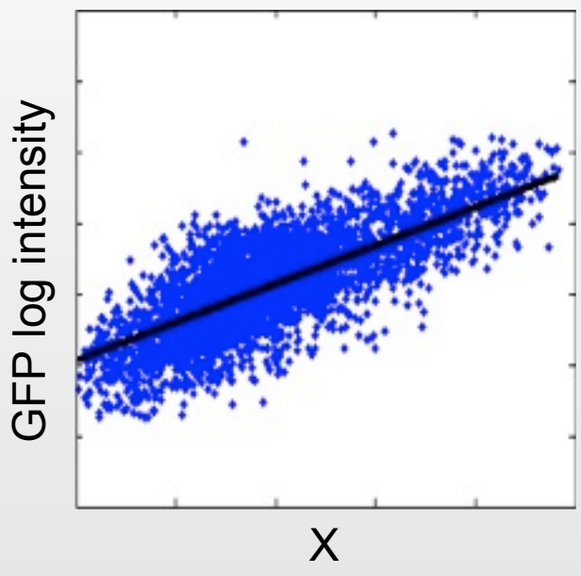
## Δchd1

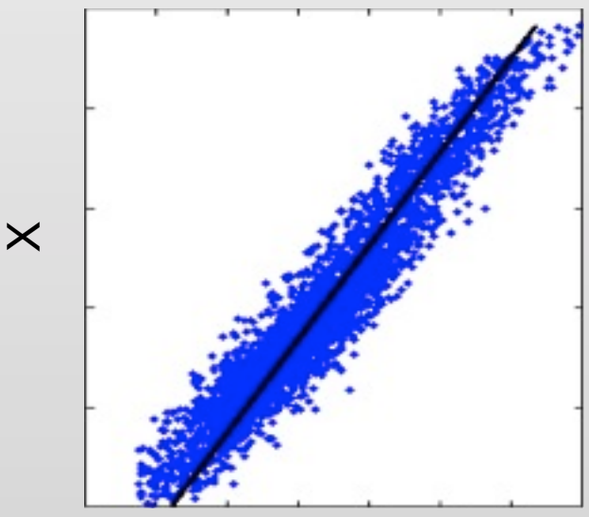


# Example

## Δchd1

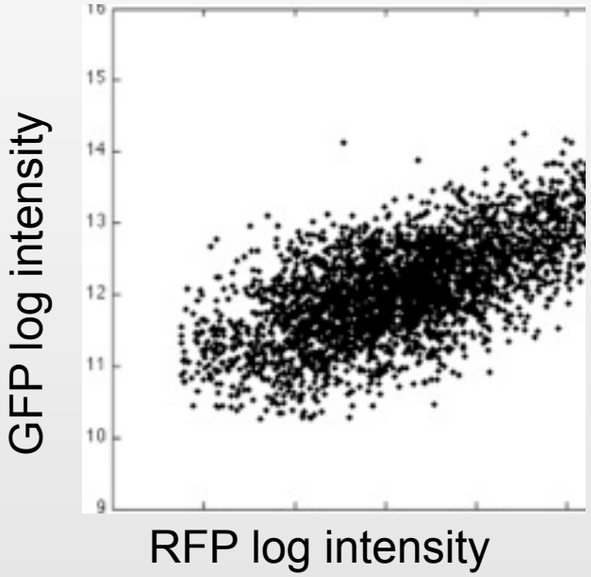


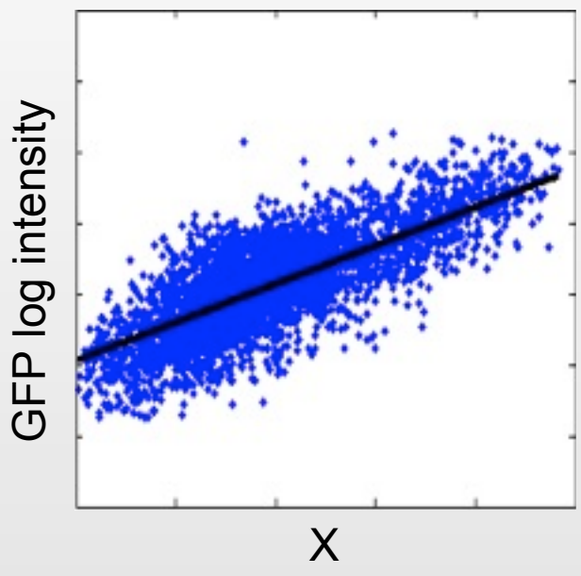



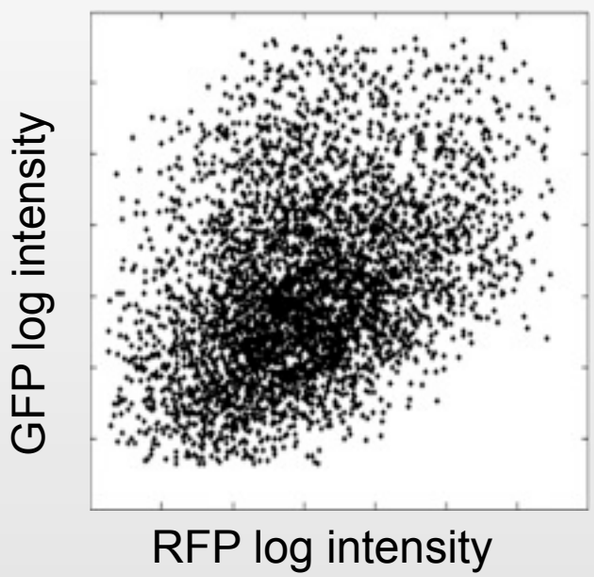
# Example

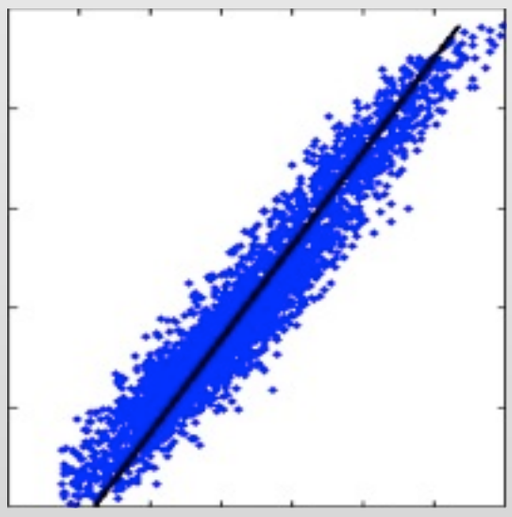
## Δchd1



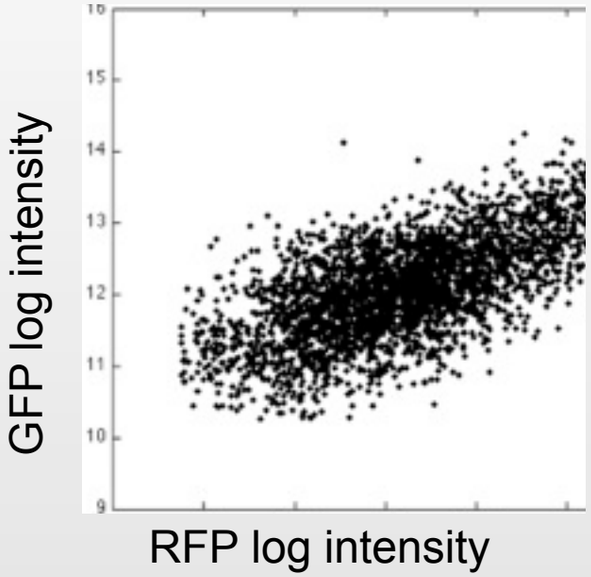

## Δmud2

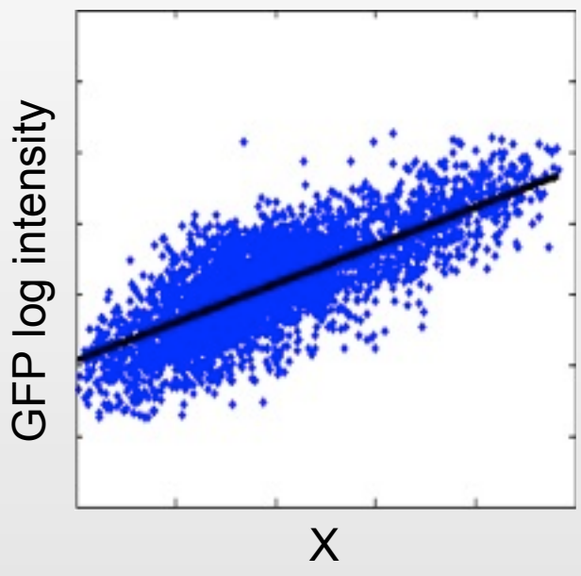


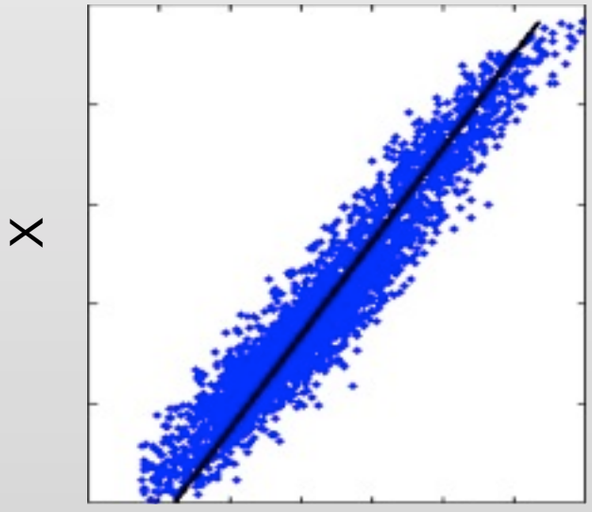



# Example

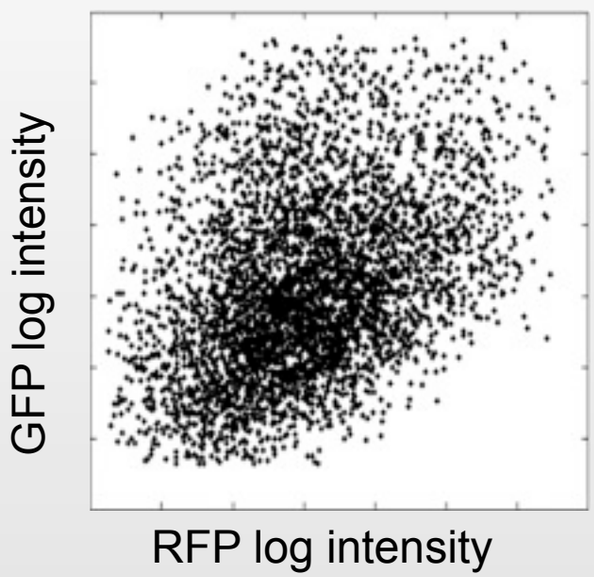
## Δchd1

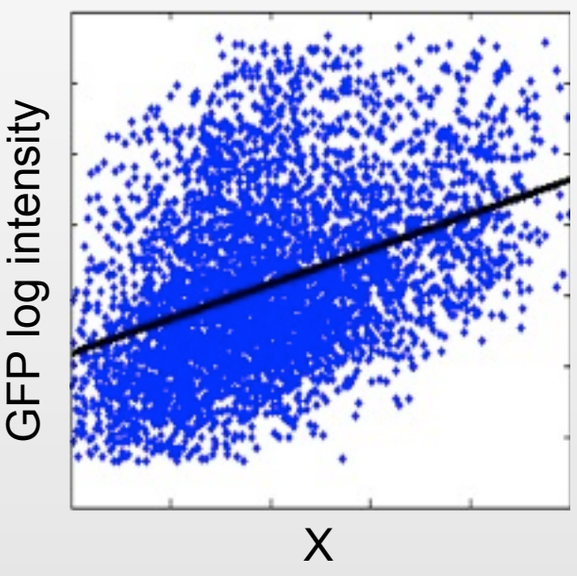


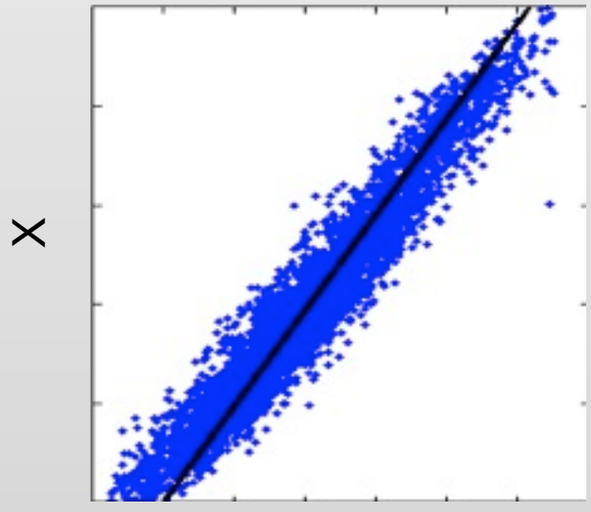




## Δmud2

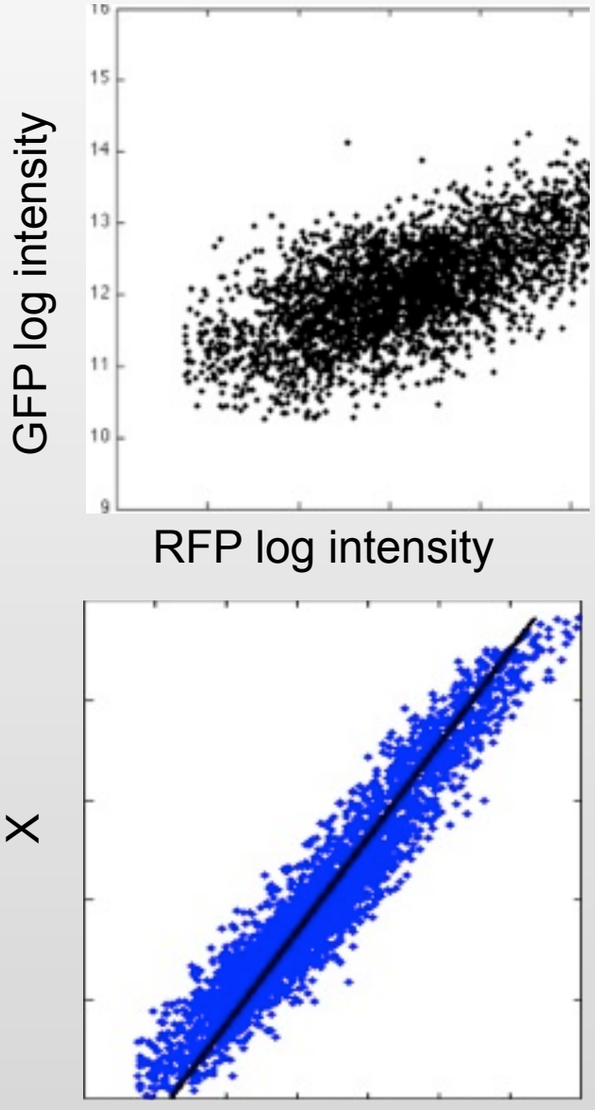


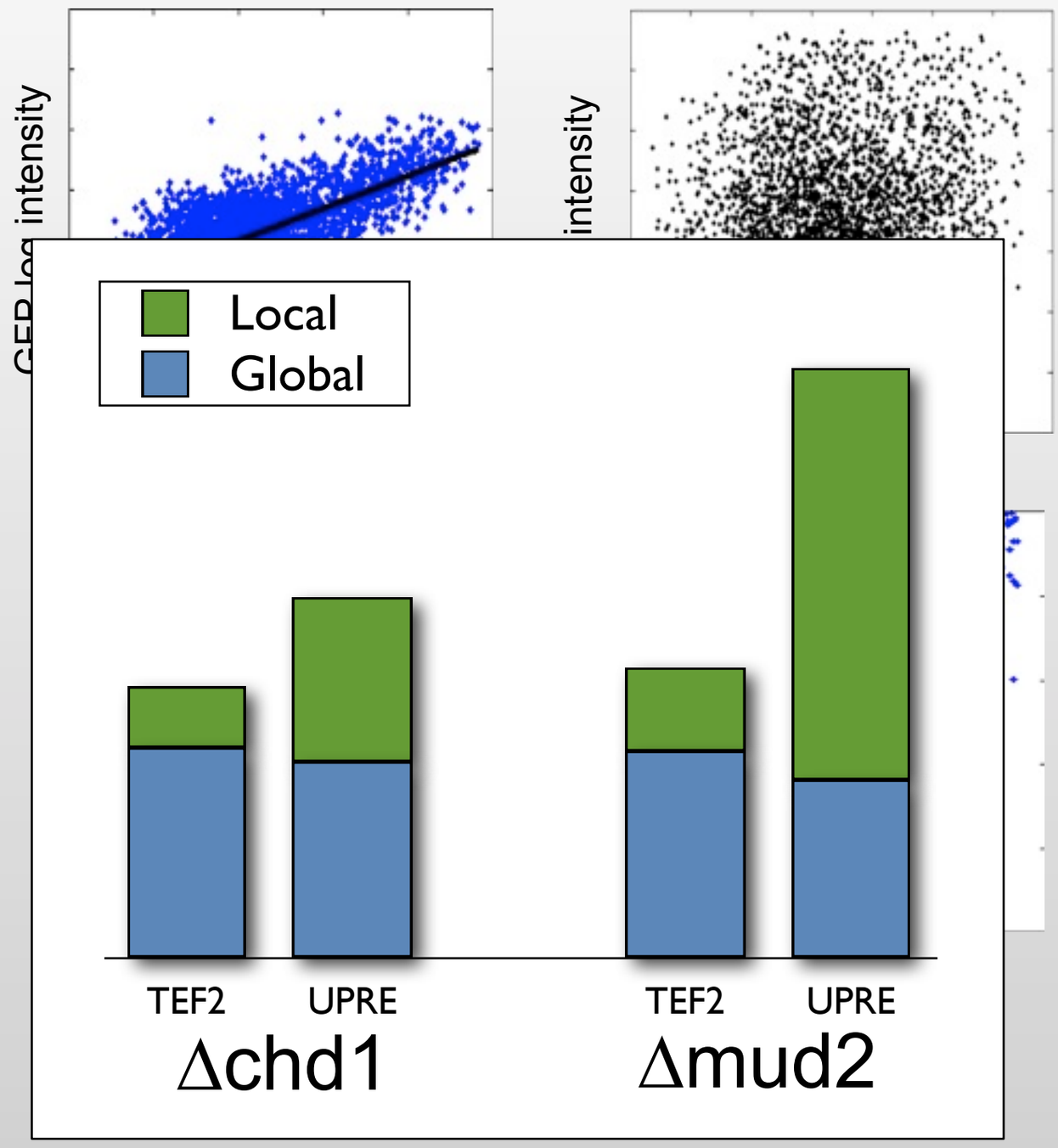



# Example

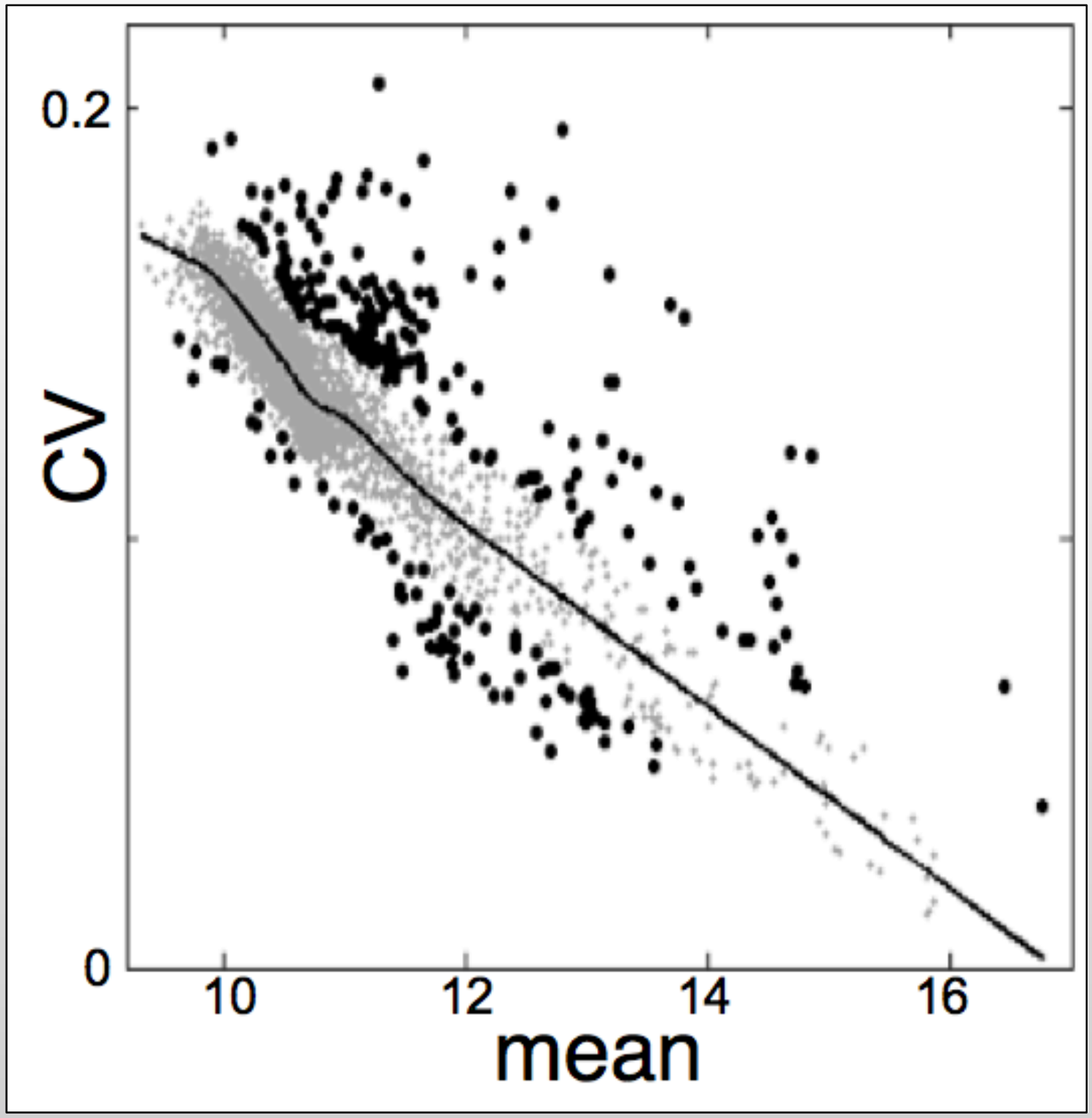
## Δchd1




## Δmud2



# Detecting Variability Effects



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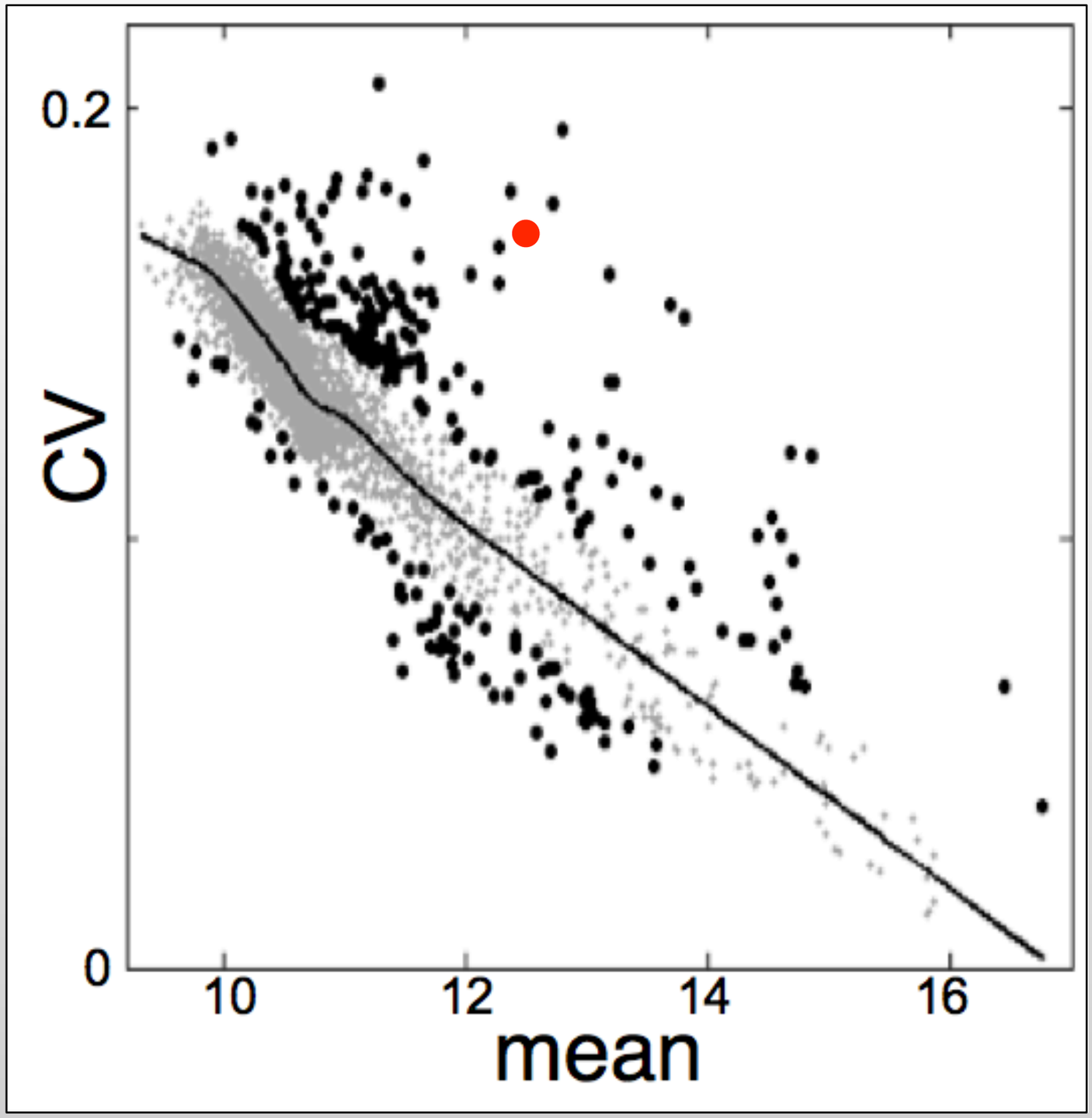
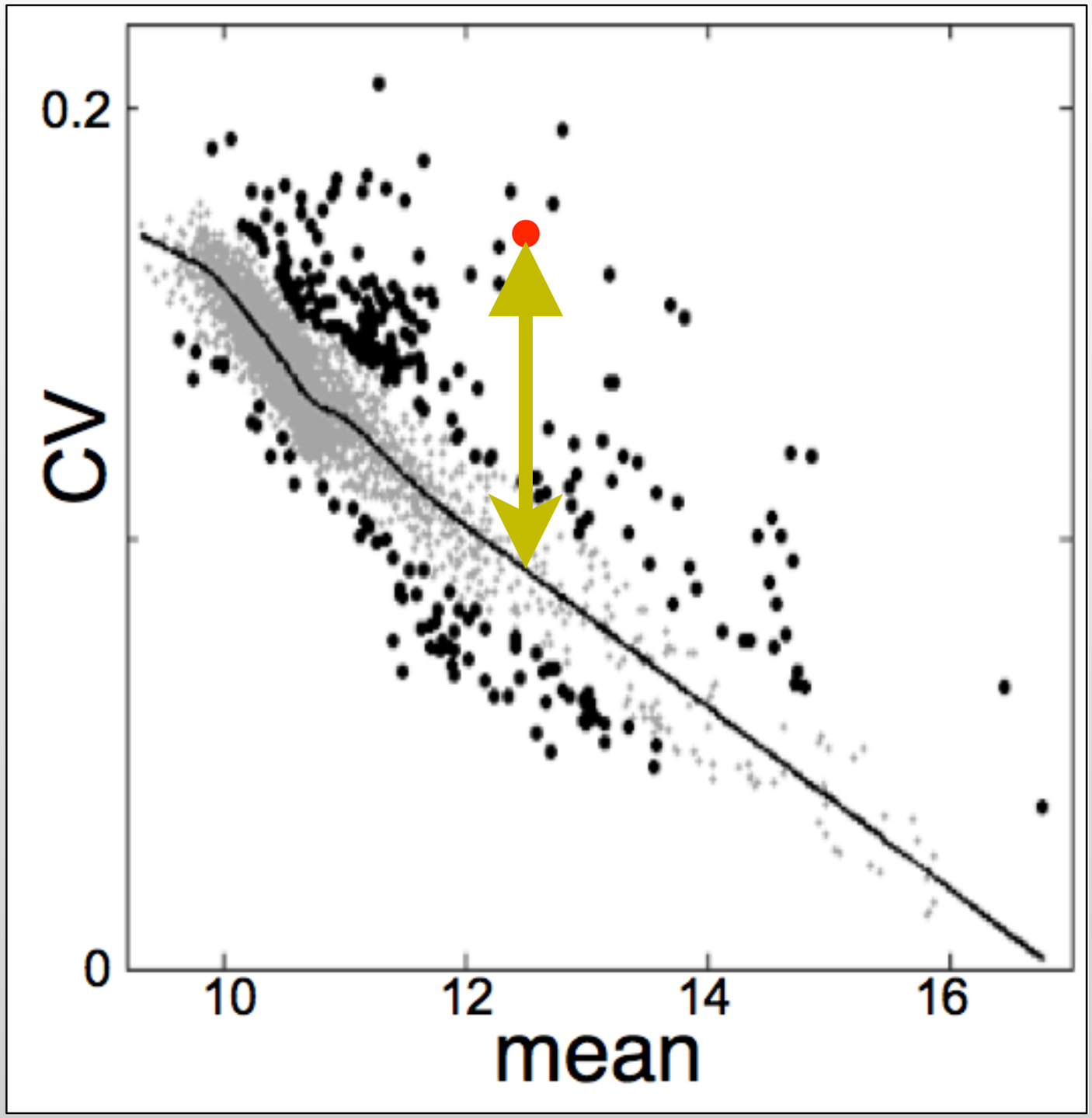
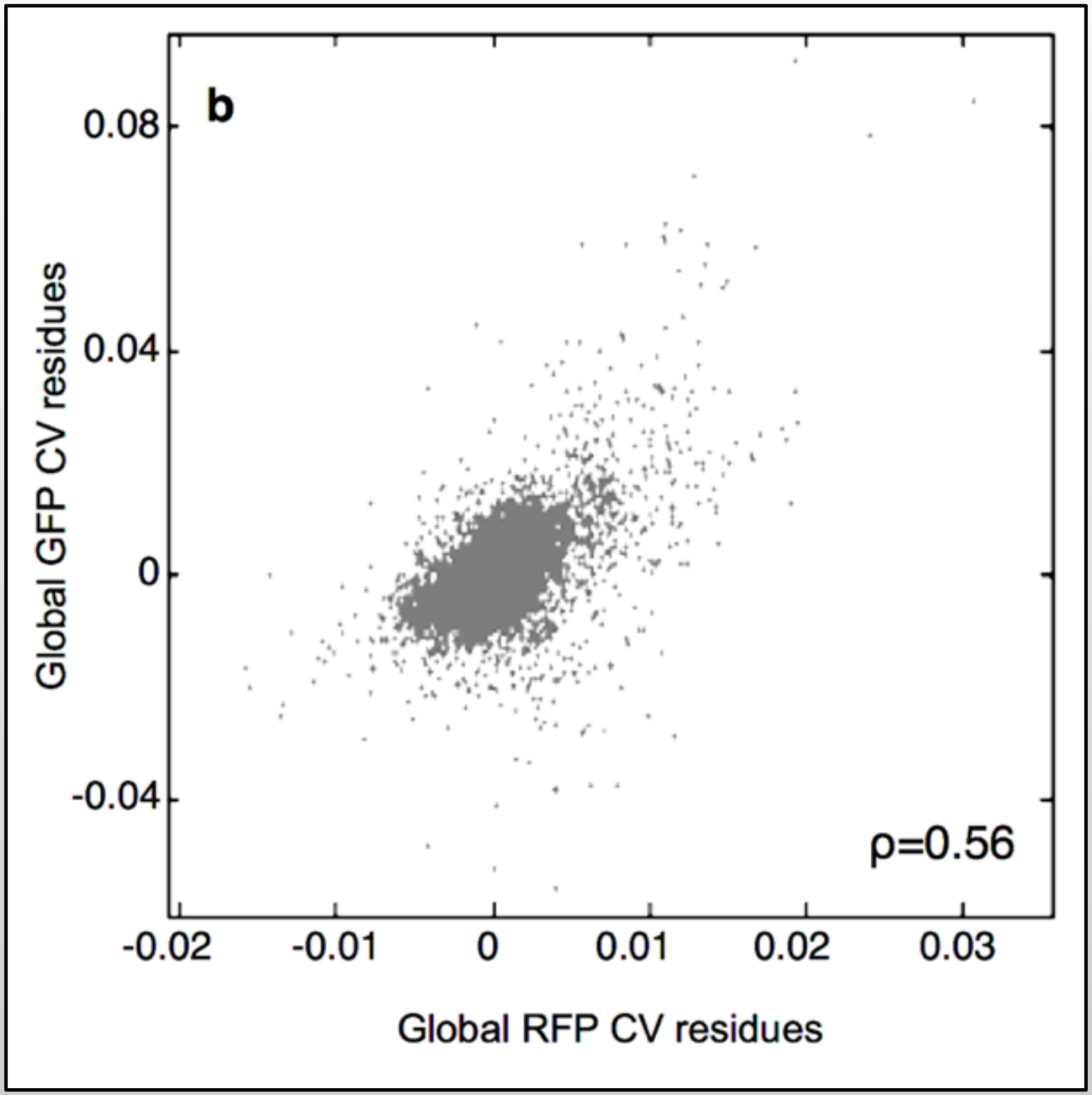


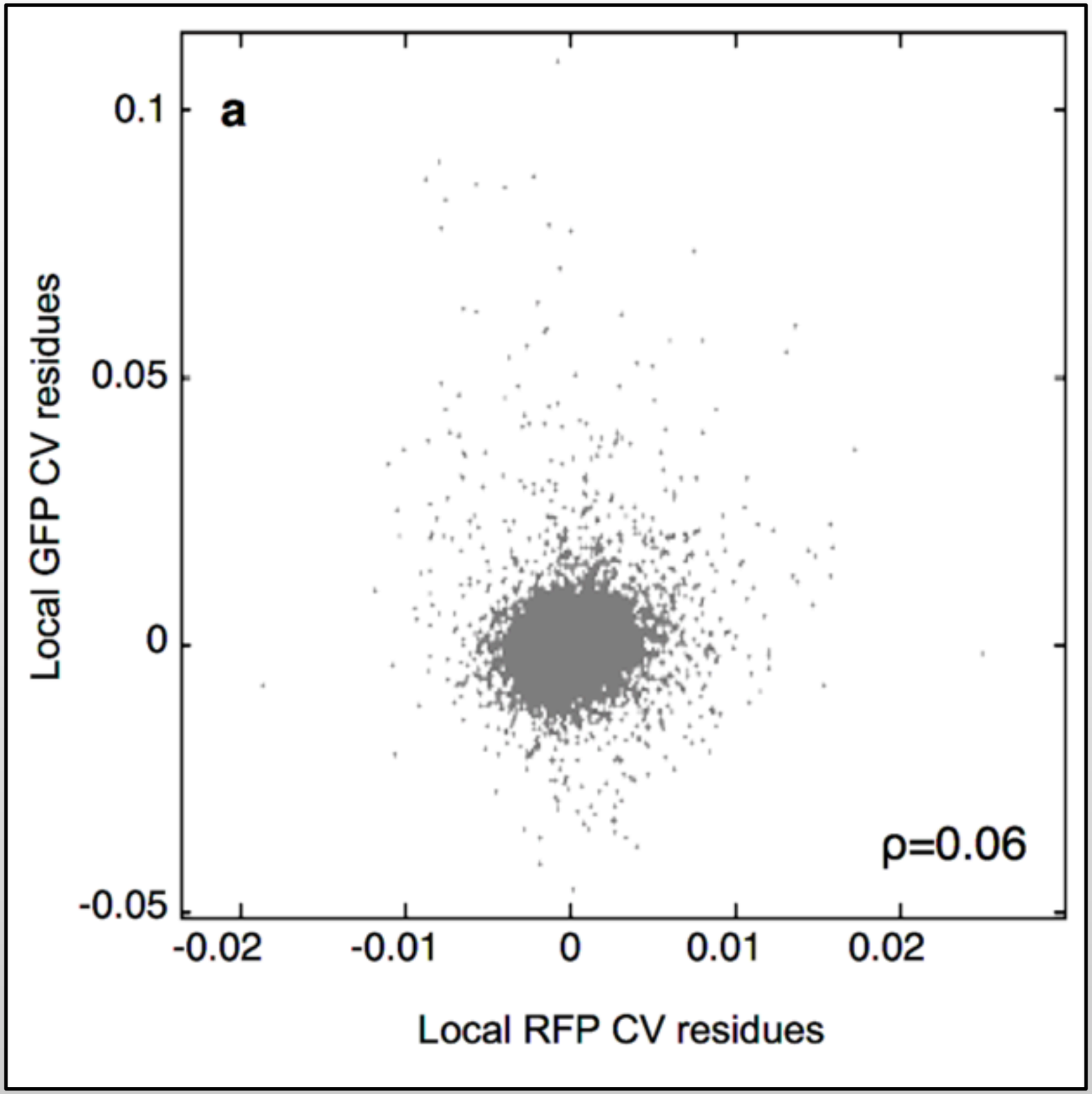
# Detecting Variability Effects



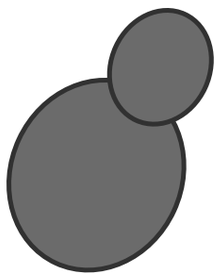
# Correlated Global Variability

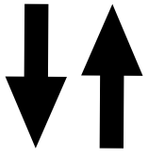


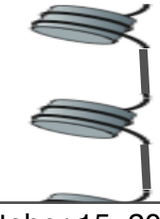
# Uncorrelated Local Variability



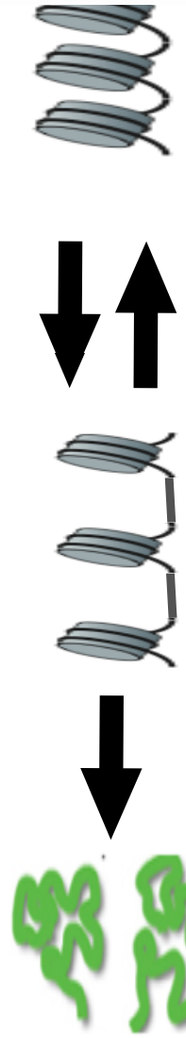
# Multiple Functions Affect Variability







| GO category | Local GFP | Local RFP | Global GFP | Global RFP |
|---|---|---|---|---|
| cellular bud neck | | | | ↑ |
| regulation of cell size | | | | ↑ |
| meiosis | | | | ↓ |
| purine synthesis | | | | ↑ |
| recQ helicase | | | ↓ | |
| response to DNA damage | ↑ | | | |
| Telomere maintenance | ↑ | ↑ | ↑↓ | ↑↓ |



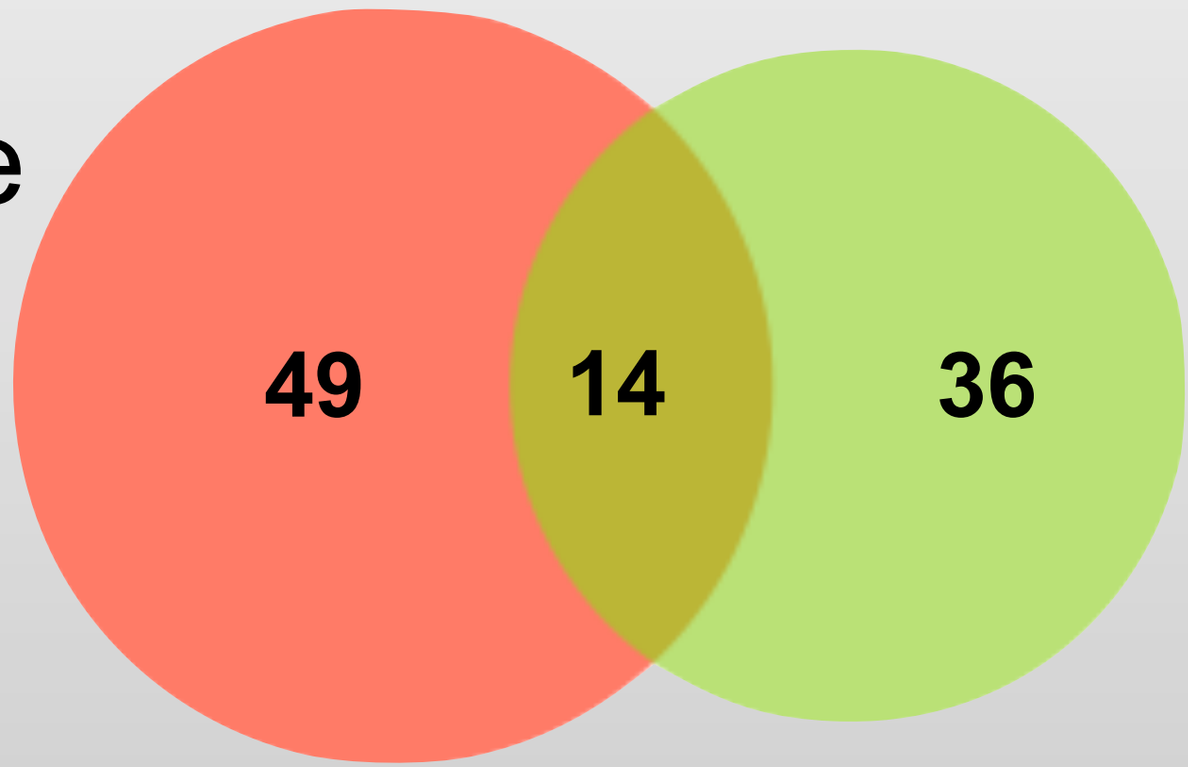
| GO category | Local GFP | Local RFP | Global GFP | Global RFP |
|---|---|---|---|---|
| Nuclear protein complex | ↓ | | | |
| Nucleosome Assembly | ↓ | | | |
| SWR1 | ↑ | | ↓ | |
| Pol II elongation Factor | ↓ | | | |
| mRNA export | ↑ | | | |
| tRNA modification | | | ↑ | ↑ |
| Protein glycosylation | | ↑ | ↑ | |
| mannosyl-transferase | | | ↑ | |
| Protein transport | | | ↑ | ↑ |
| ERAD complex | ↓ | | | |

# Phenotypes: Variability vs. Mean

- 1337 non-overlapping functional annotations
- Hypergeometric enrichment in extreme residues (FDR < 0.05)

Variability phenotype

Mean phenotype



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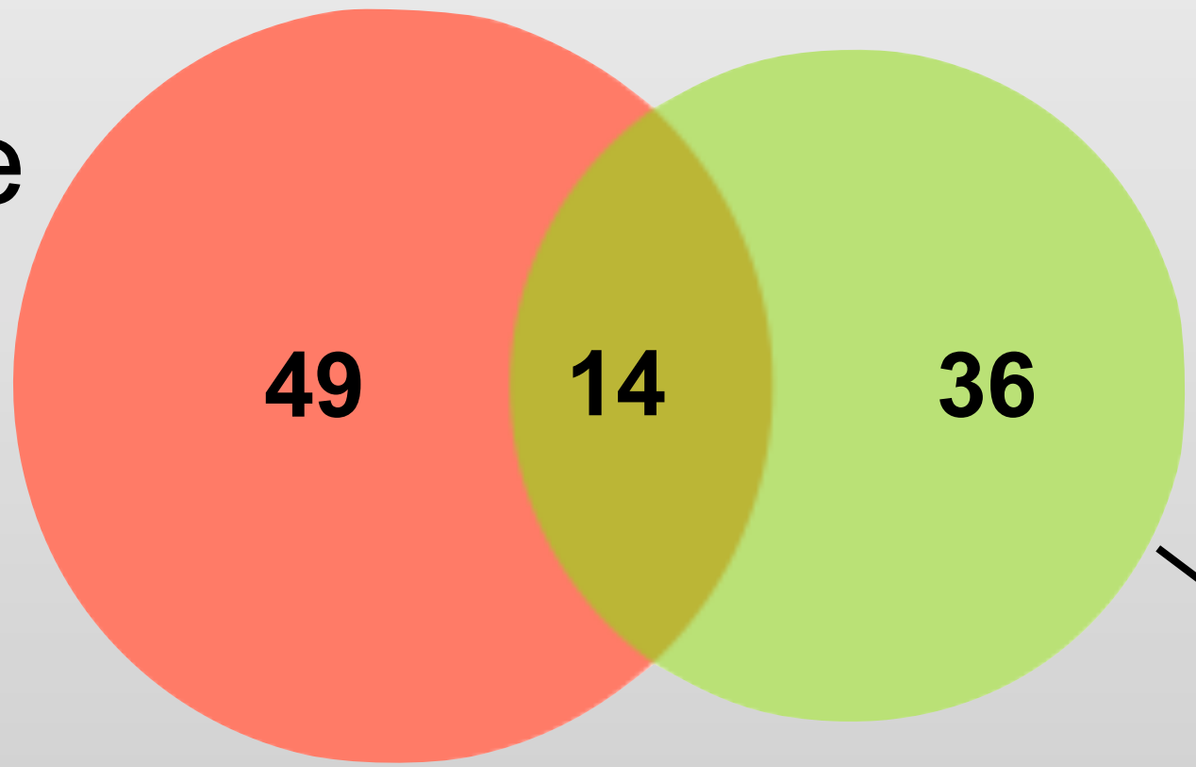


- Ribosome
- RNA catabolic process
- tRNA processing

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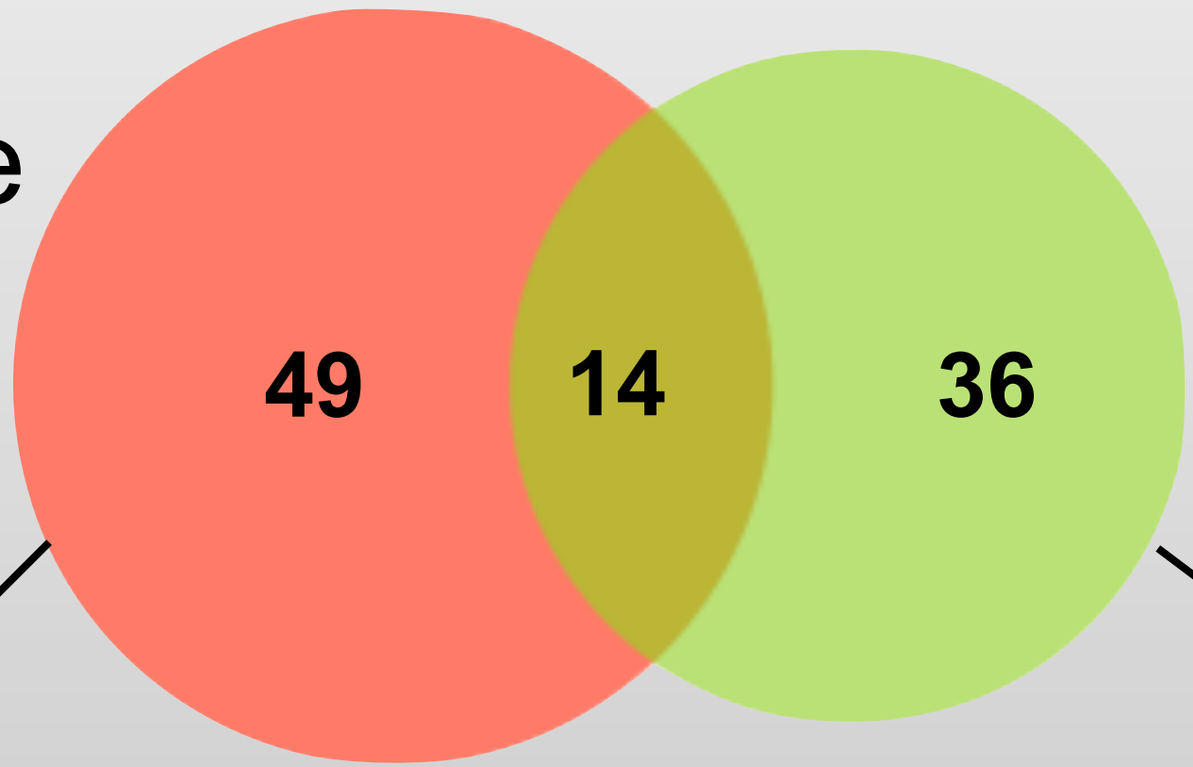
Variability phenotype

Mean phenotype




- SWR1
- Cellular bud neck

- Ribosome
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- tRNA processing

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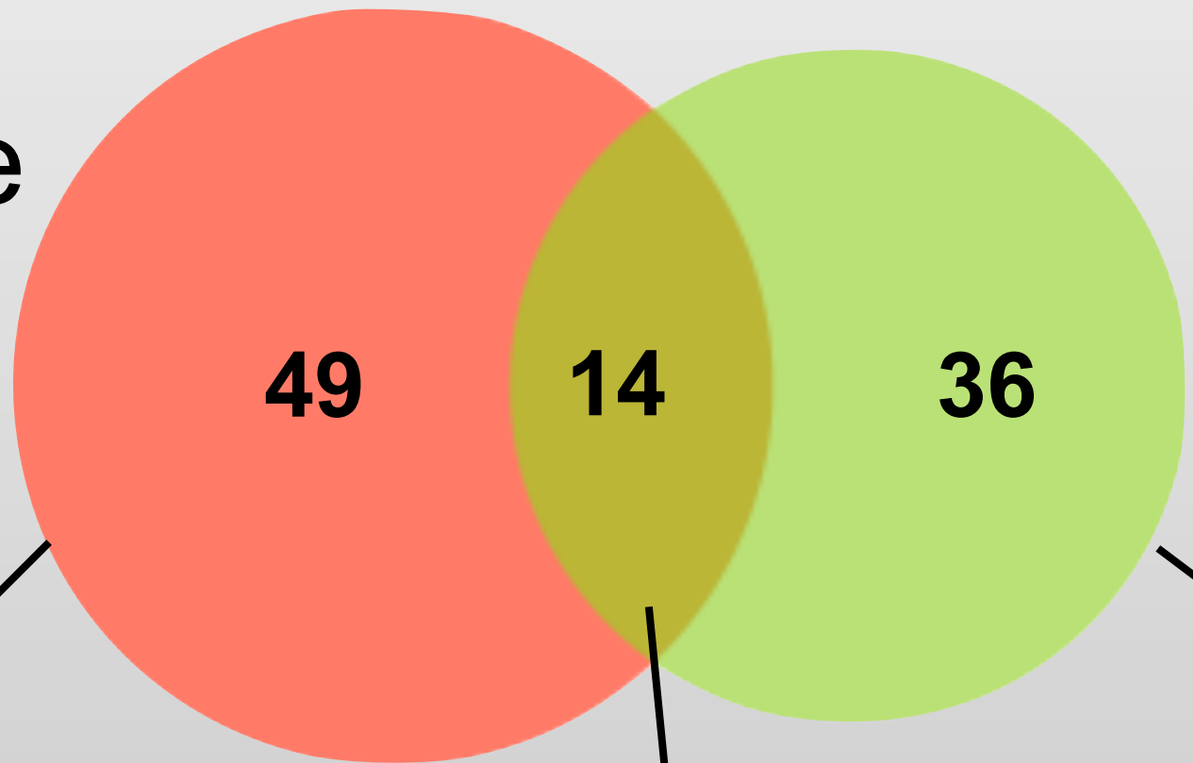
Mean phenotype



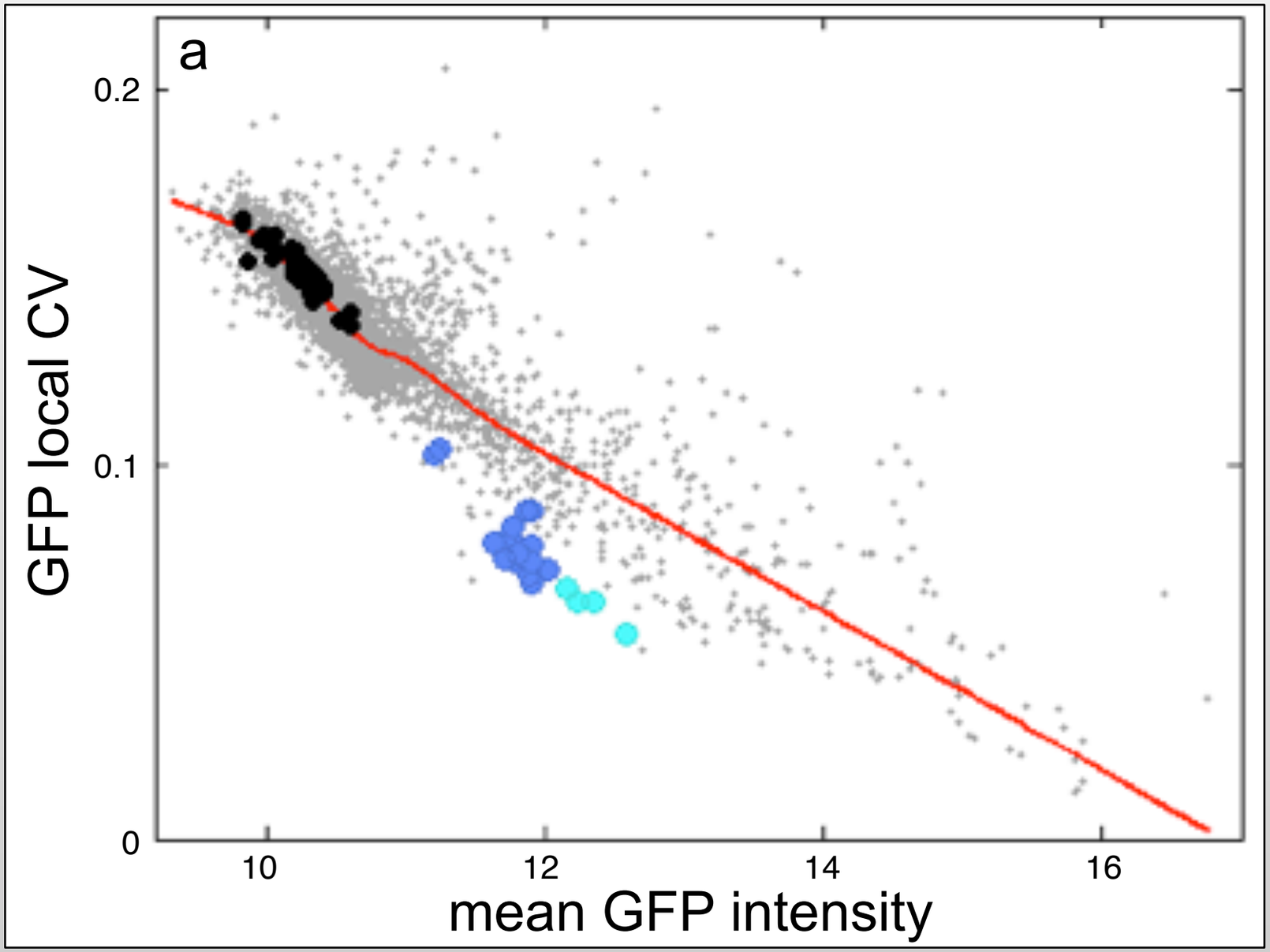

- SWR1
- Cellular bud neck

- ER-associated processes

- Ribosome
- RNA catabolic process
- tRNA processing

# Searching for Mechanisms

- Catalogue of protein complexes
- Identify complexes w/ coherent variability phenotype



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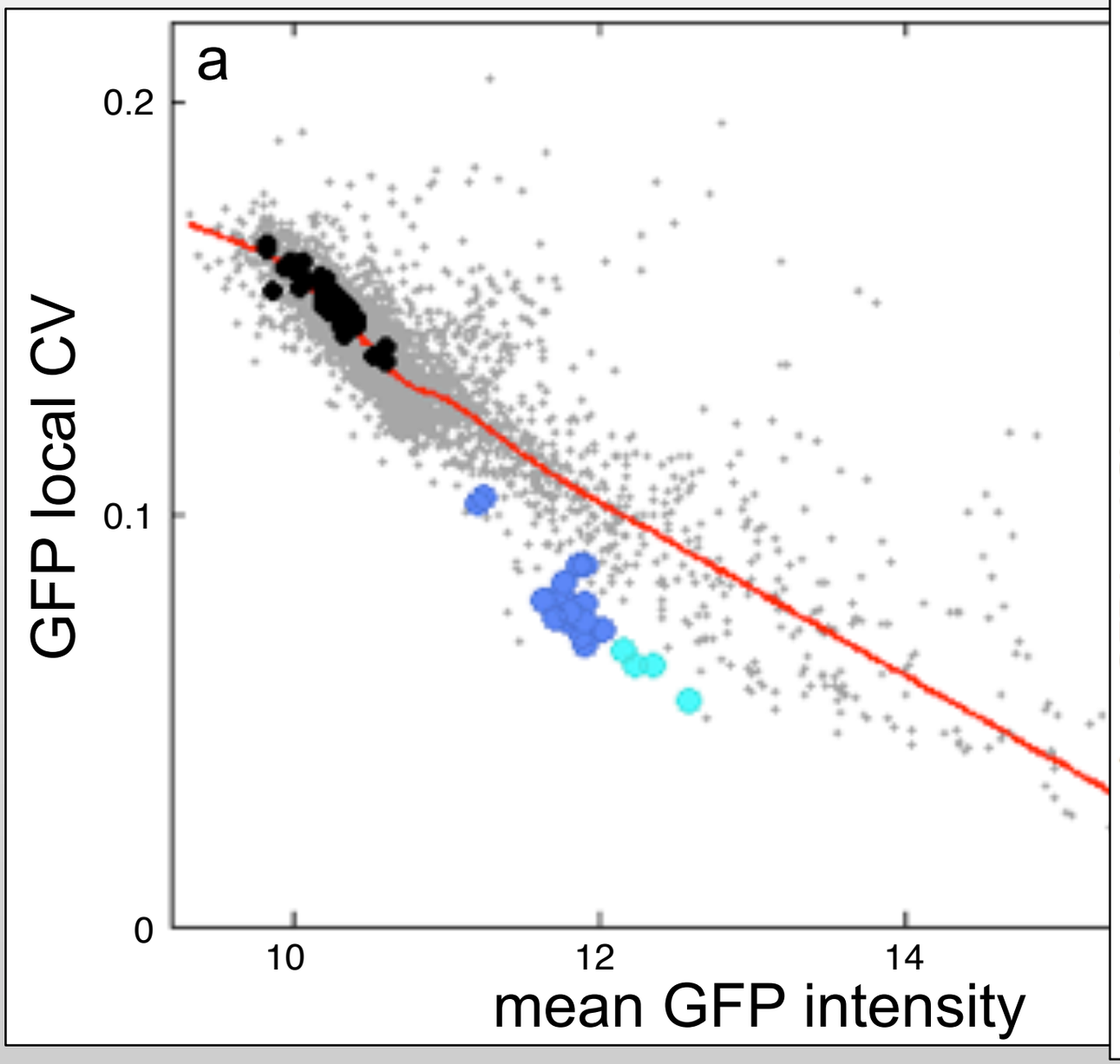


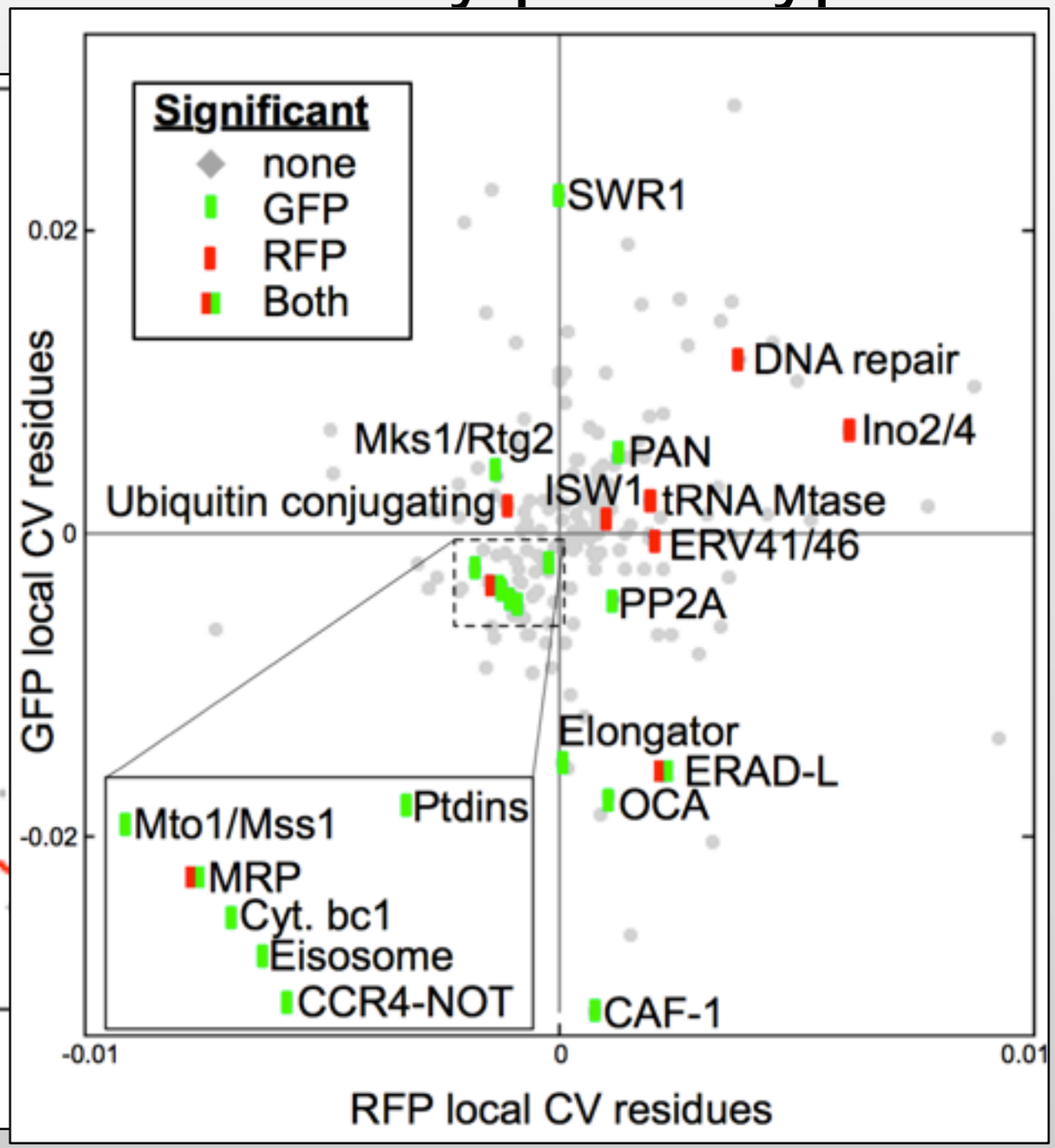

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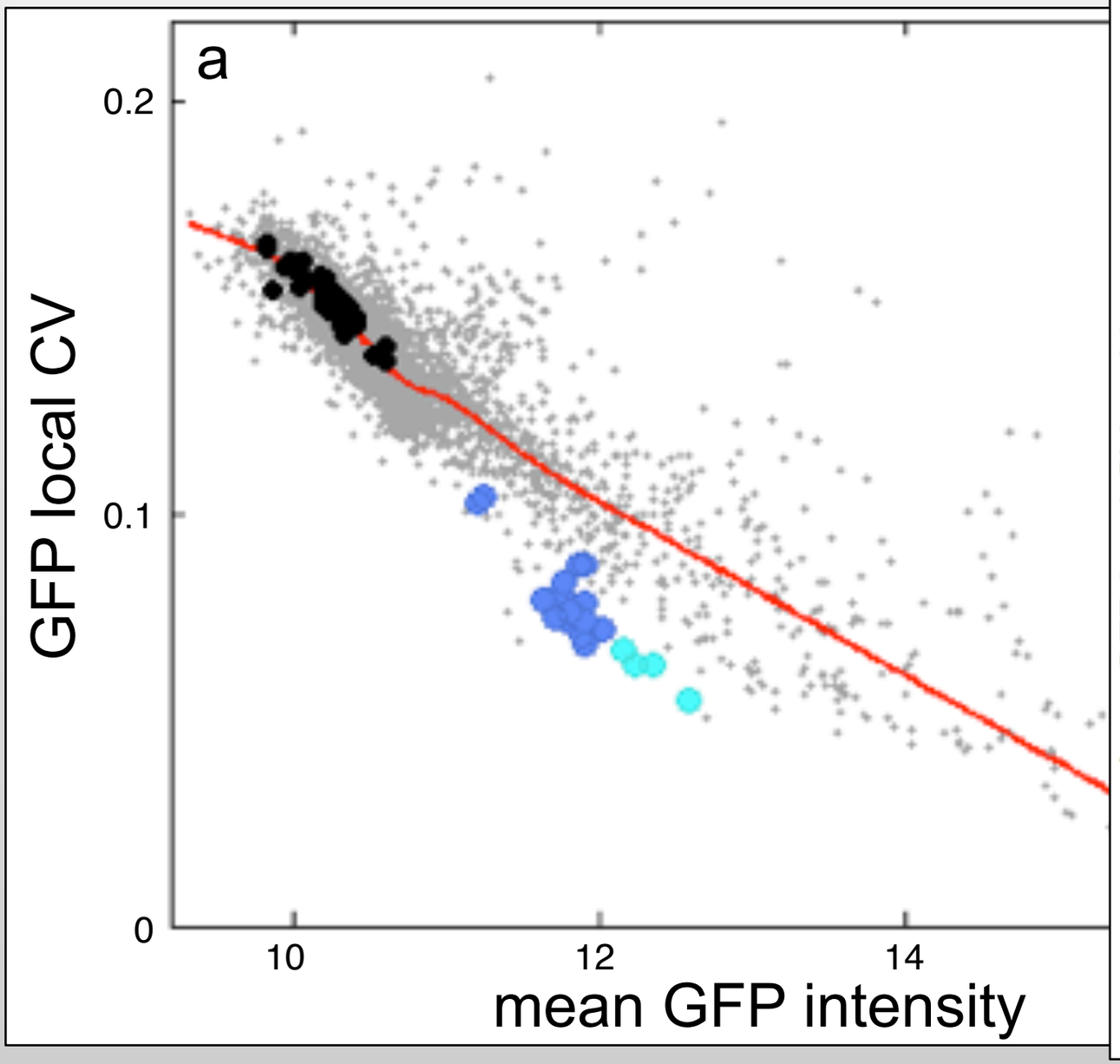
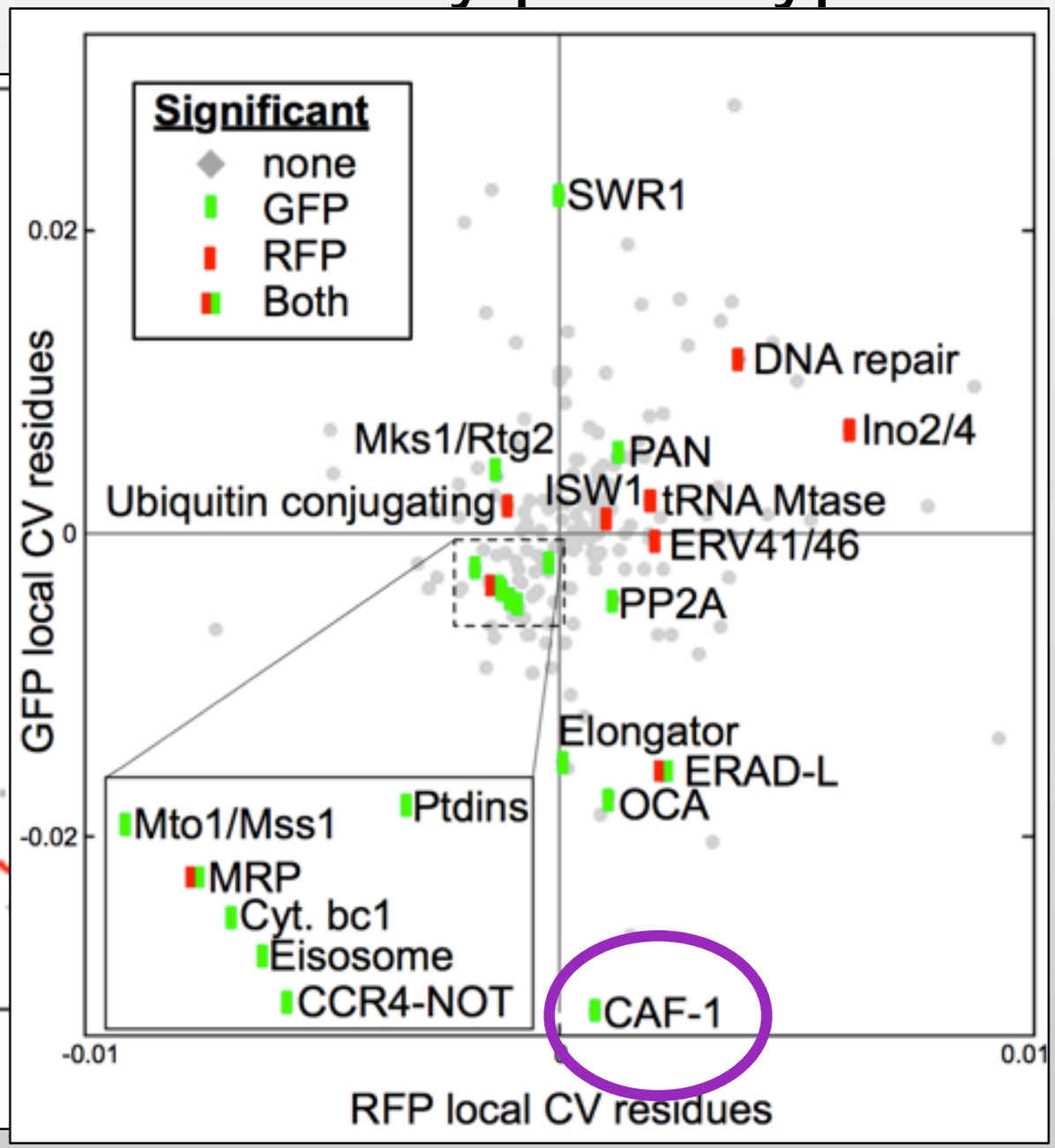
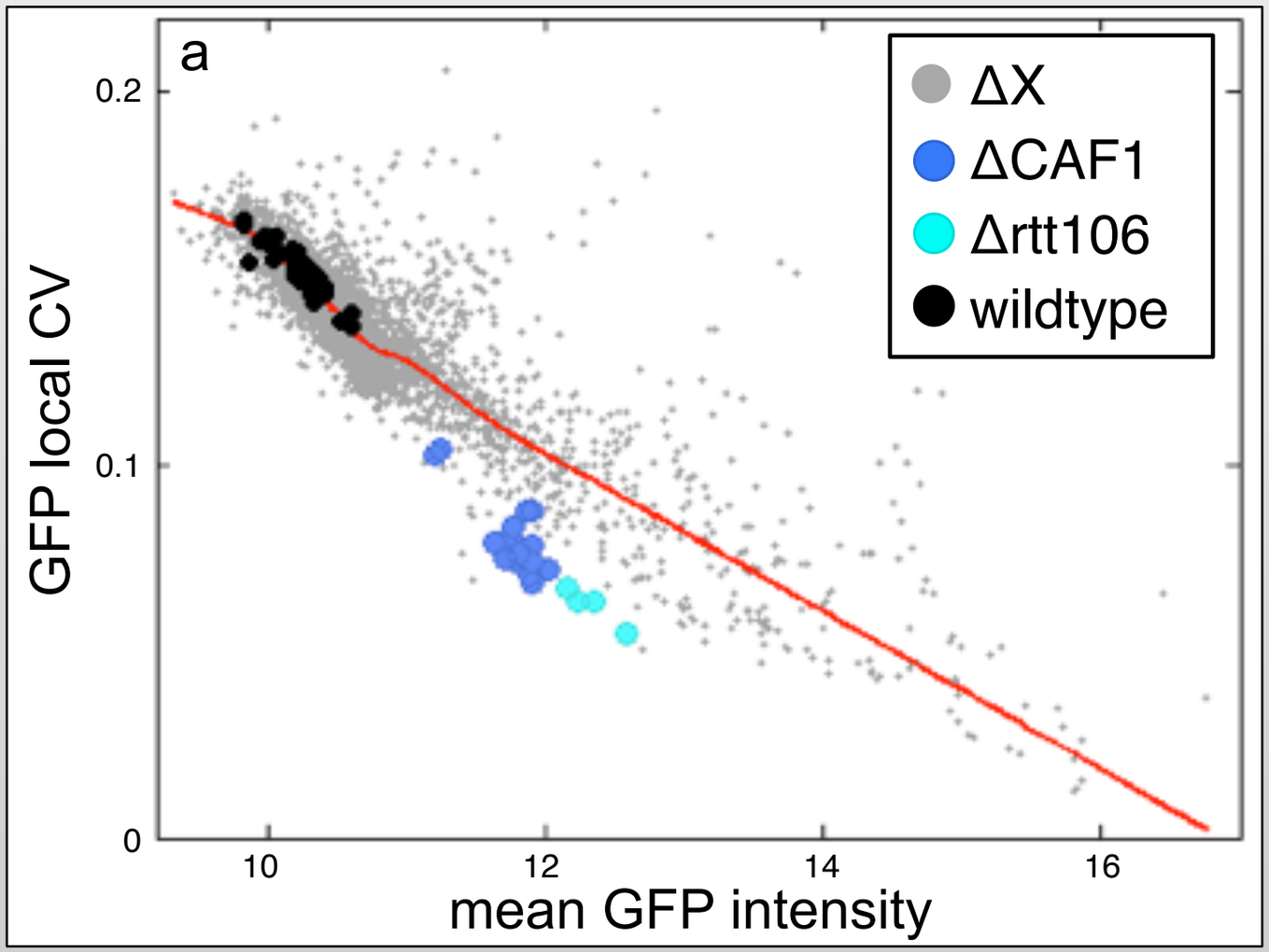
- Identify complexes w/ coherent variability phenotype

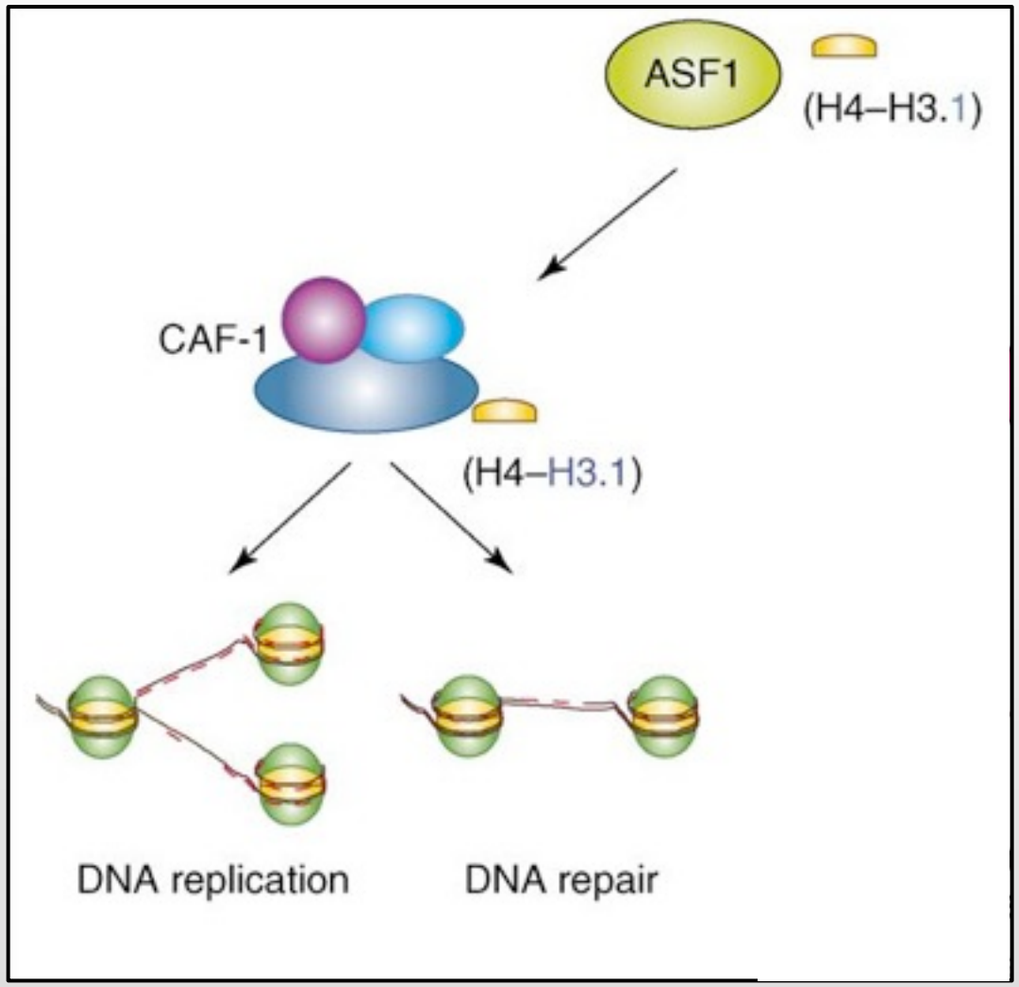


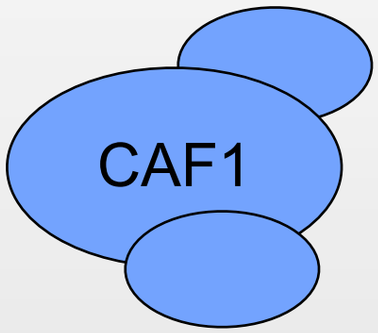
# Reducing Local Variability: CAF1

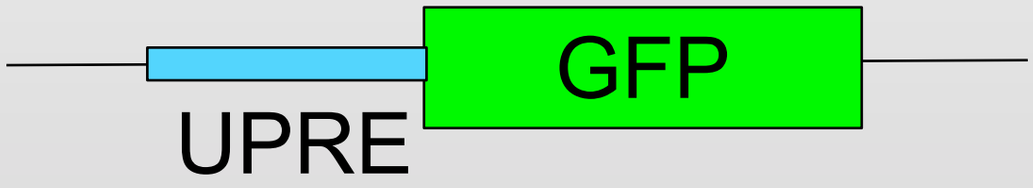
- Chromatin assembly incorporates H3/H4 dimer



# Changes in Local Variability: Direct or Indirect Effect?



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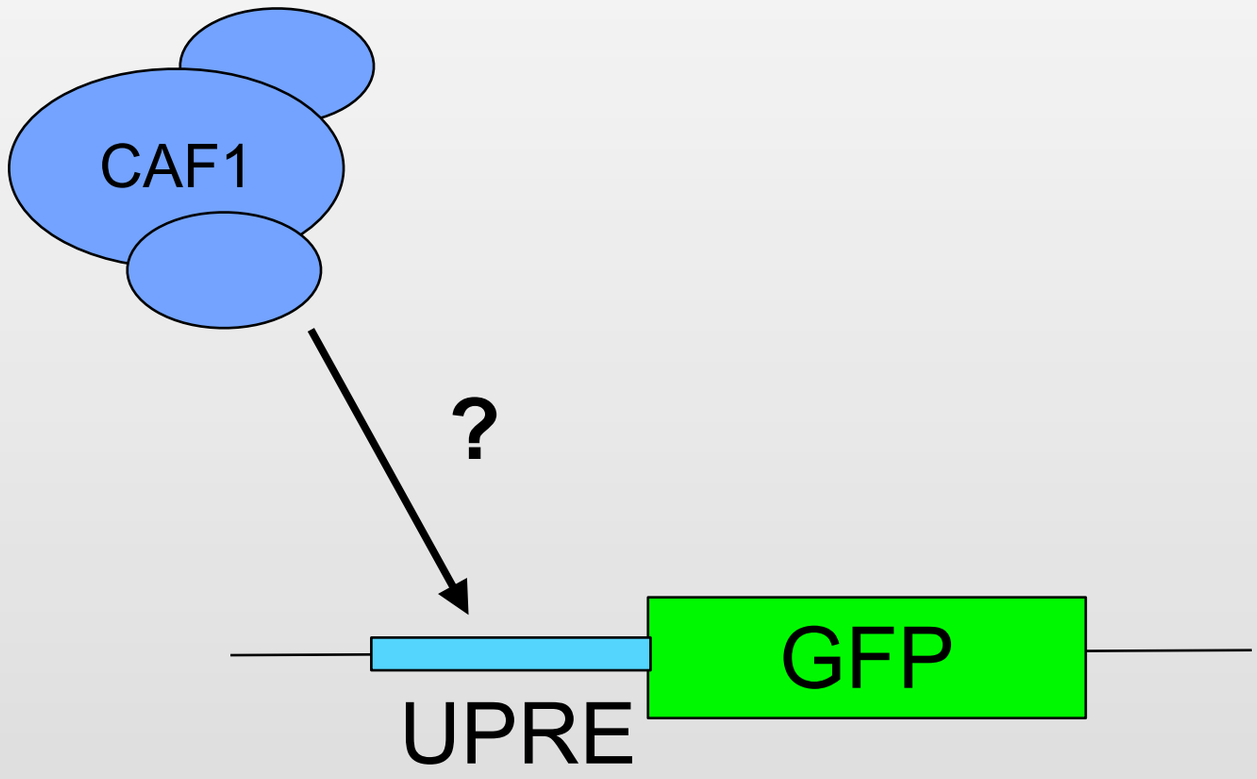

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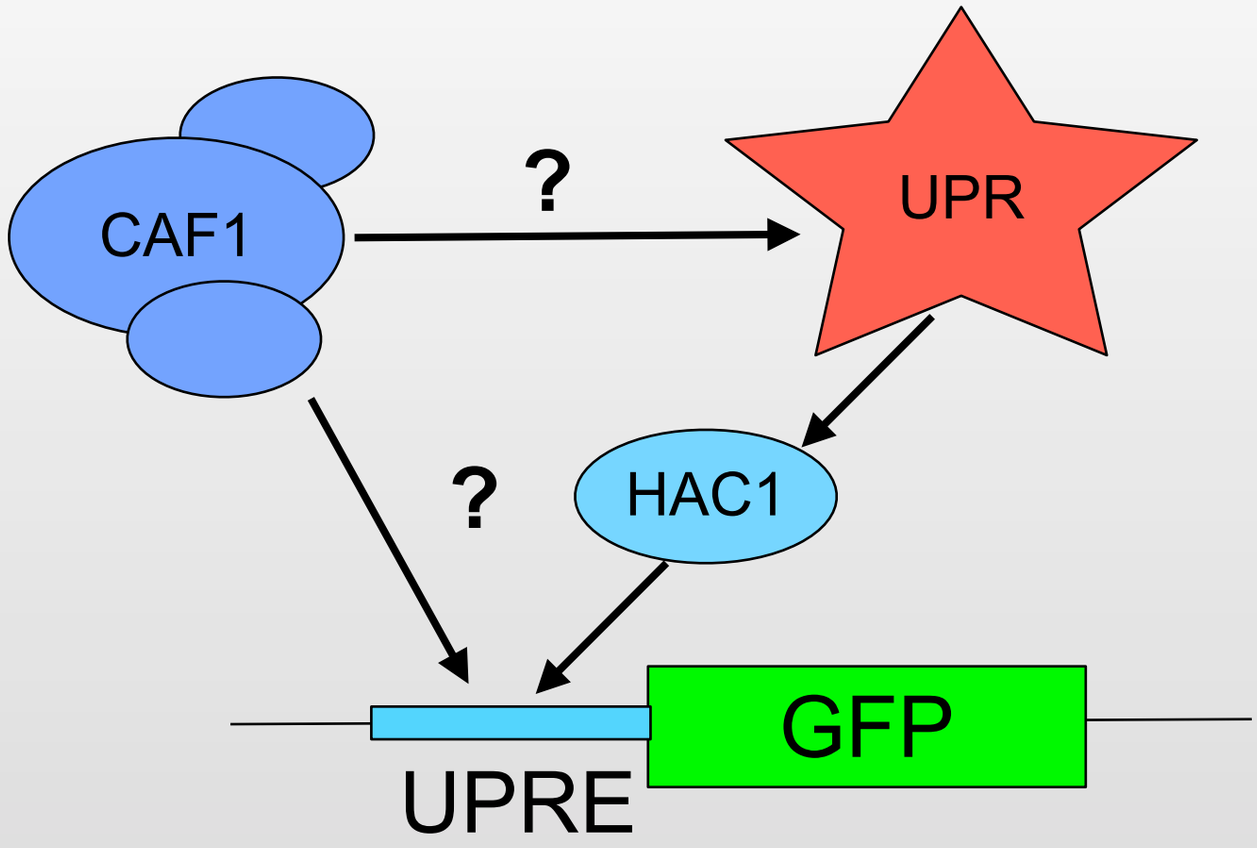

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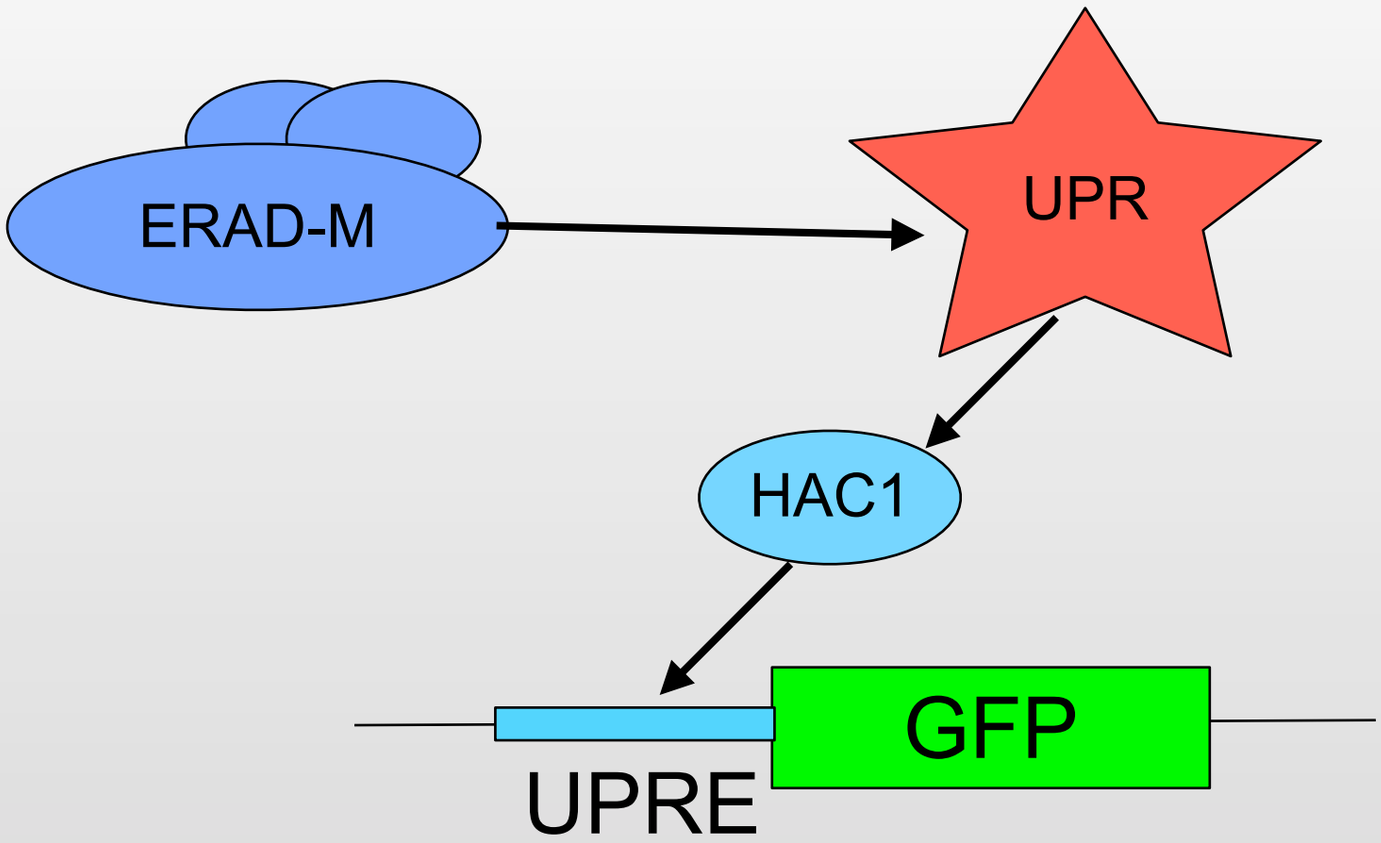

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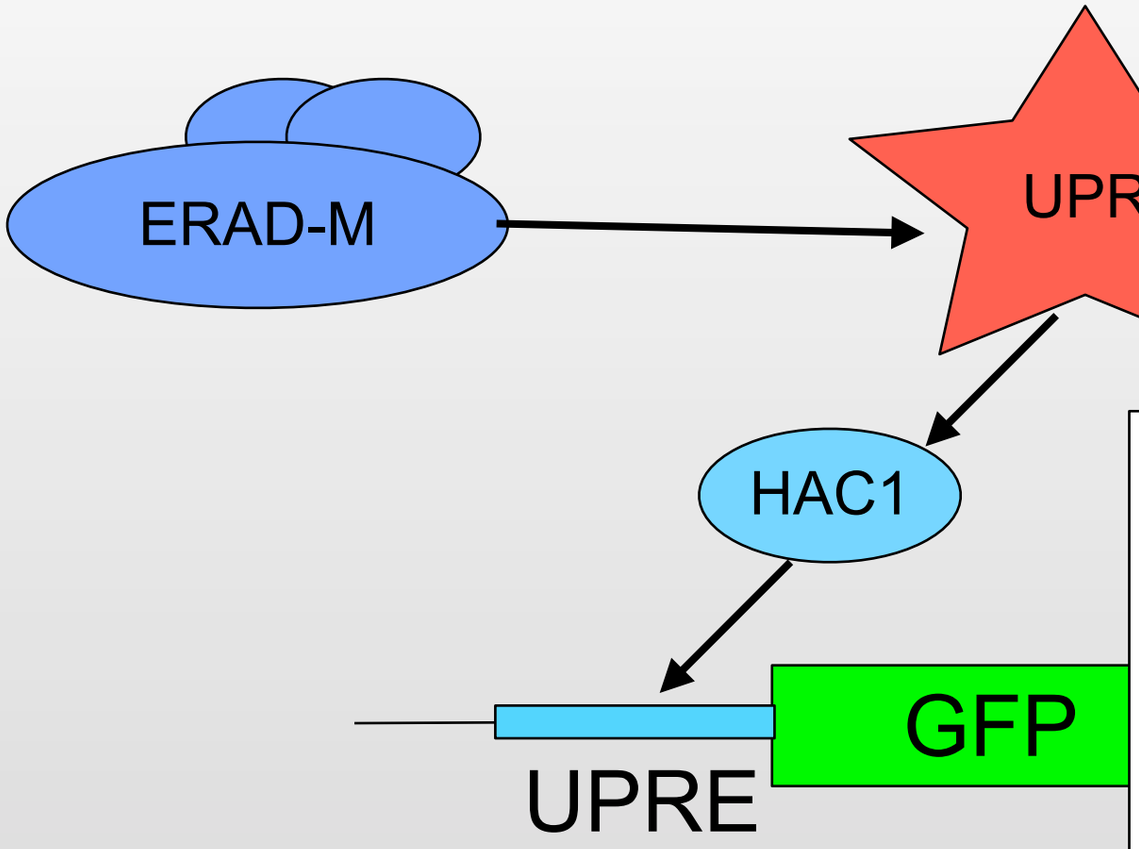


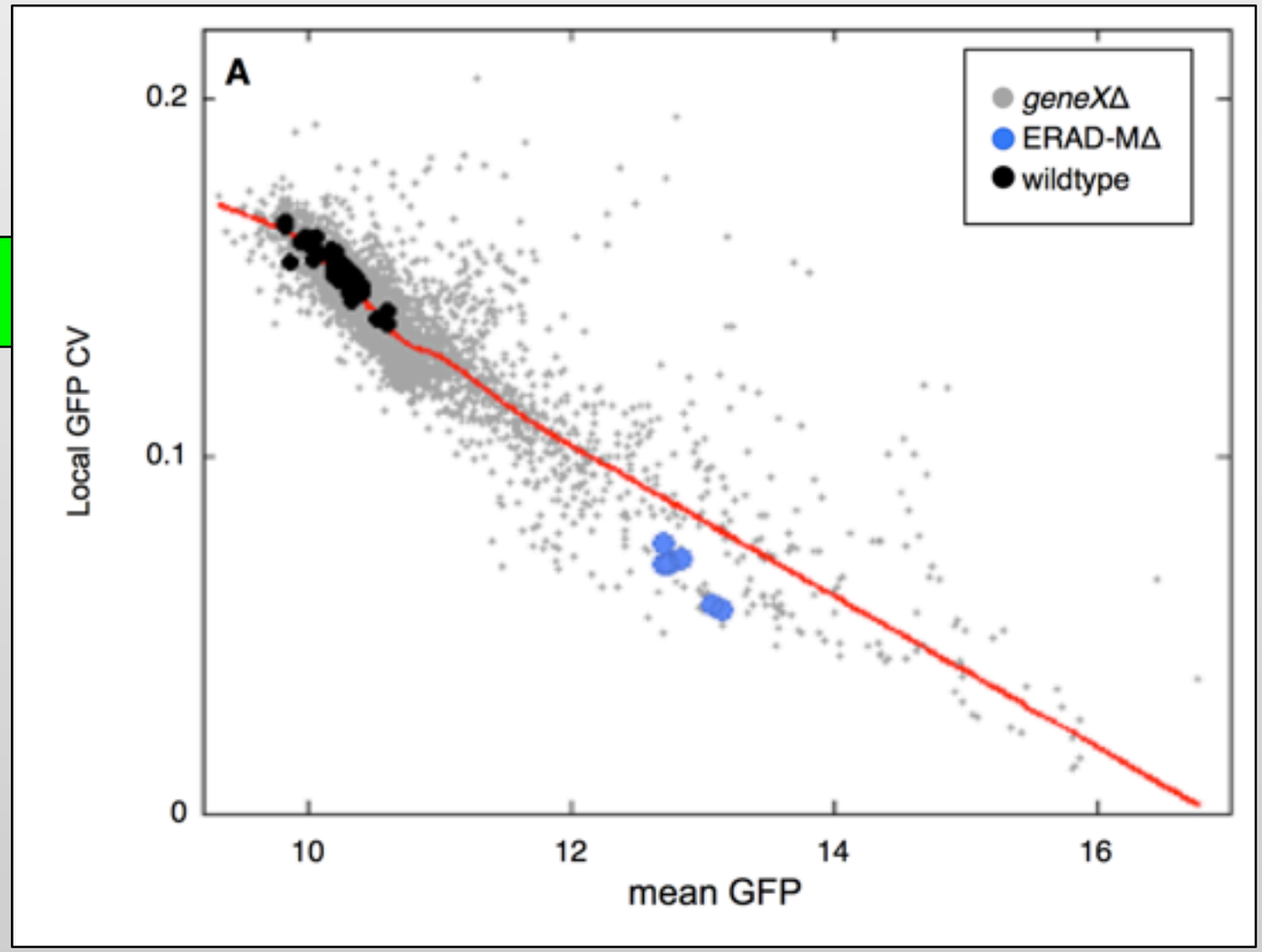

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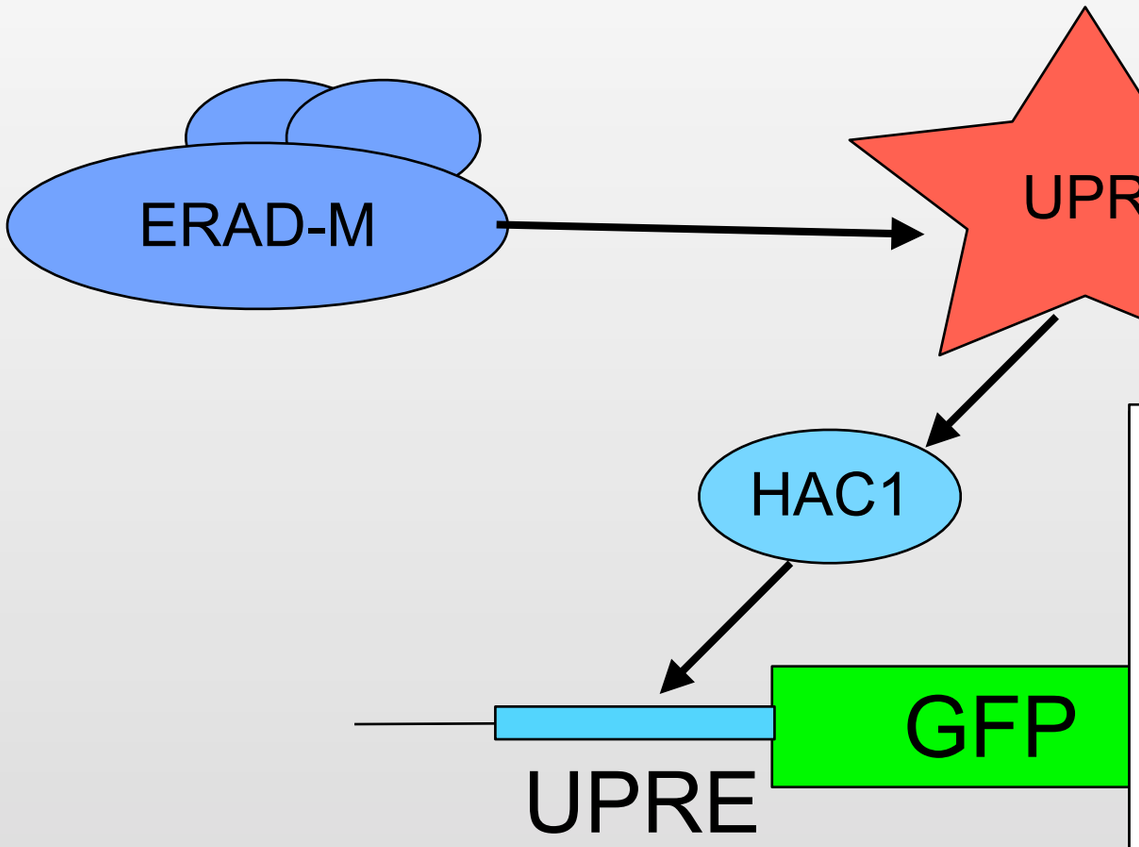


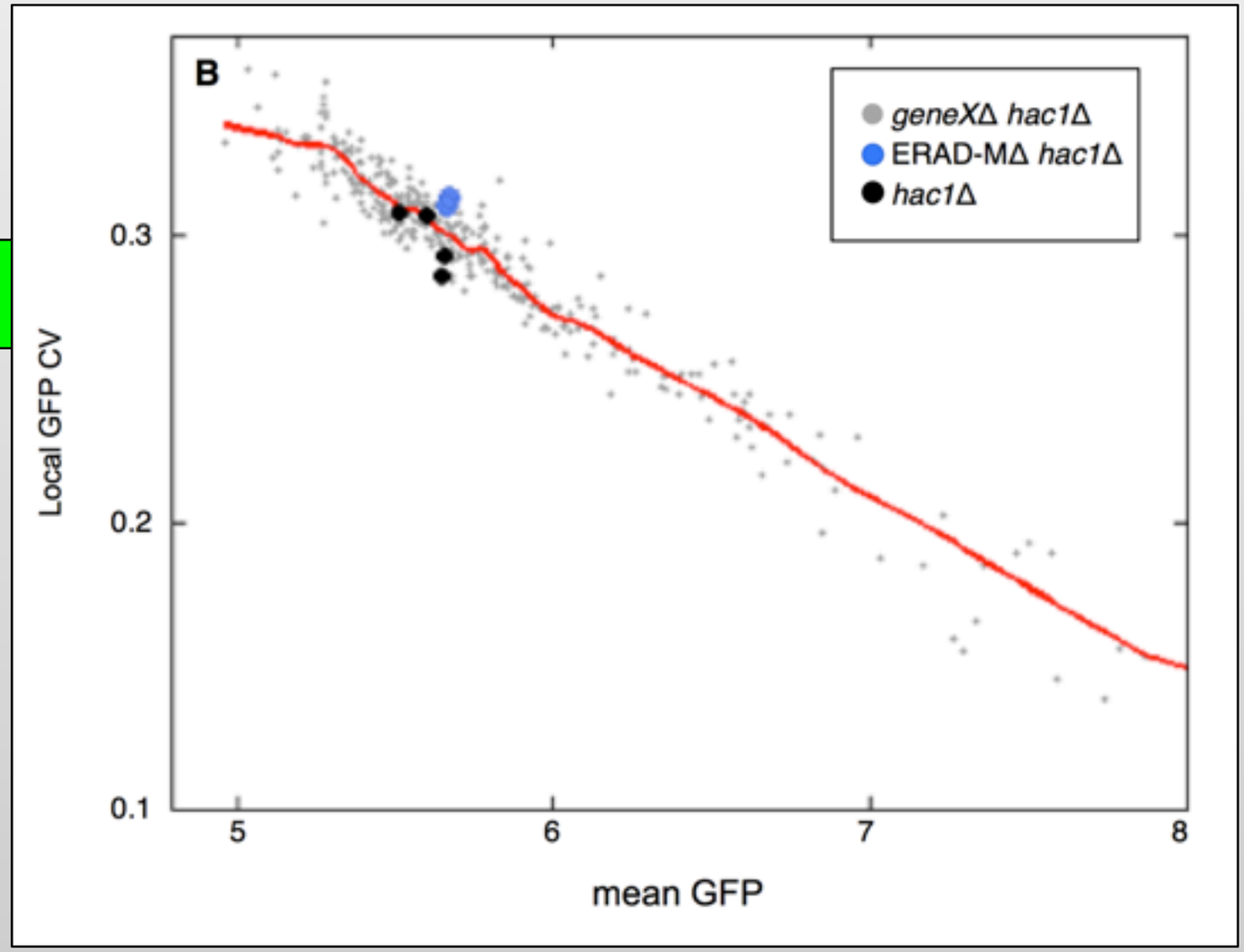

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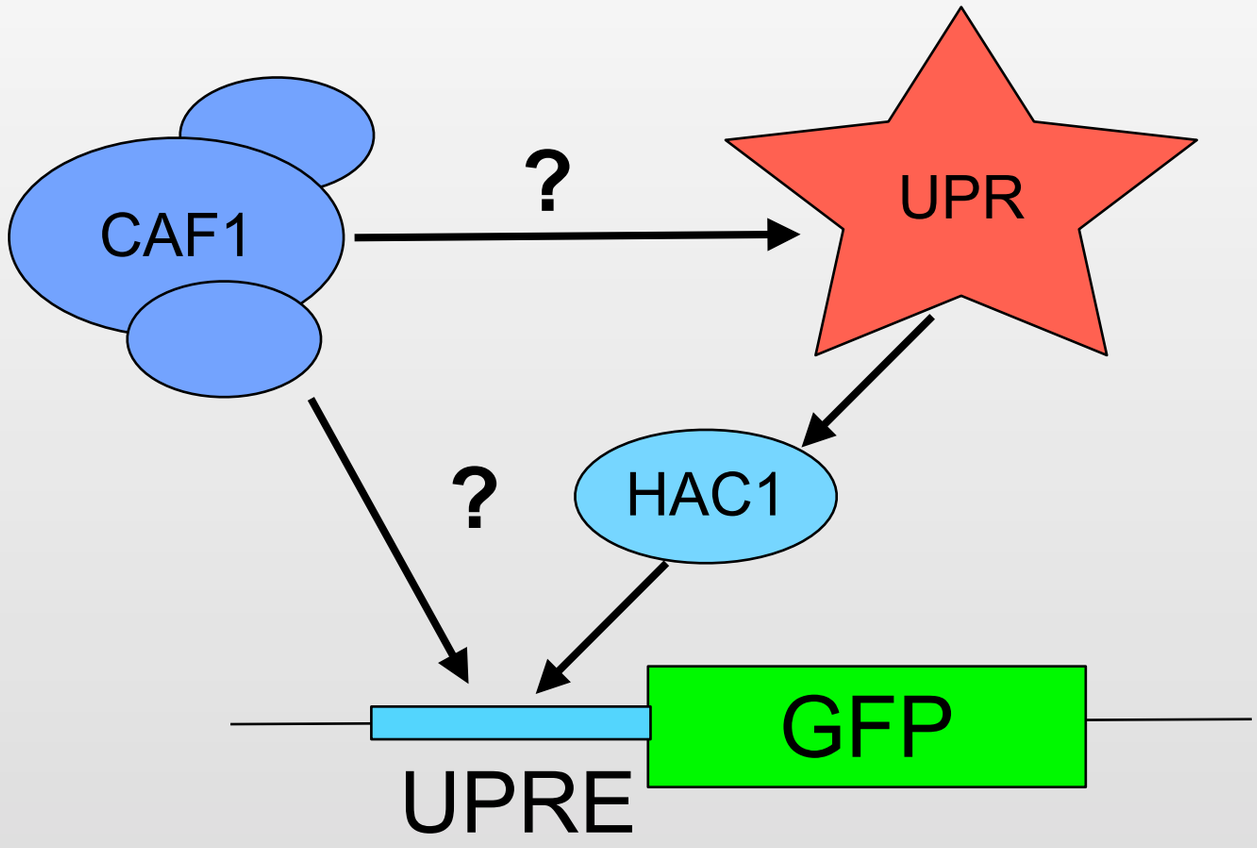

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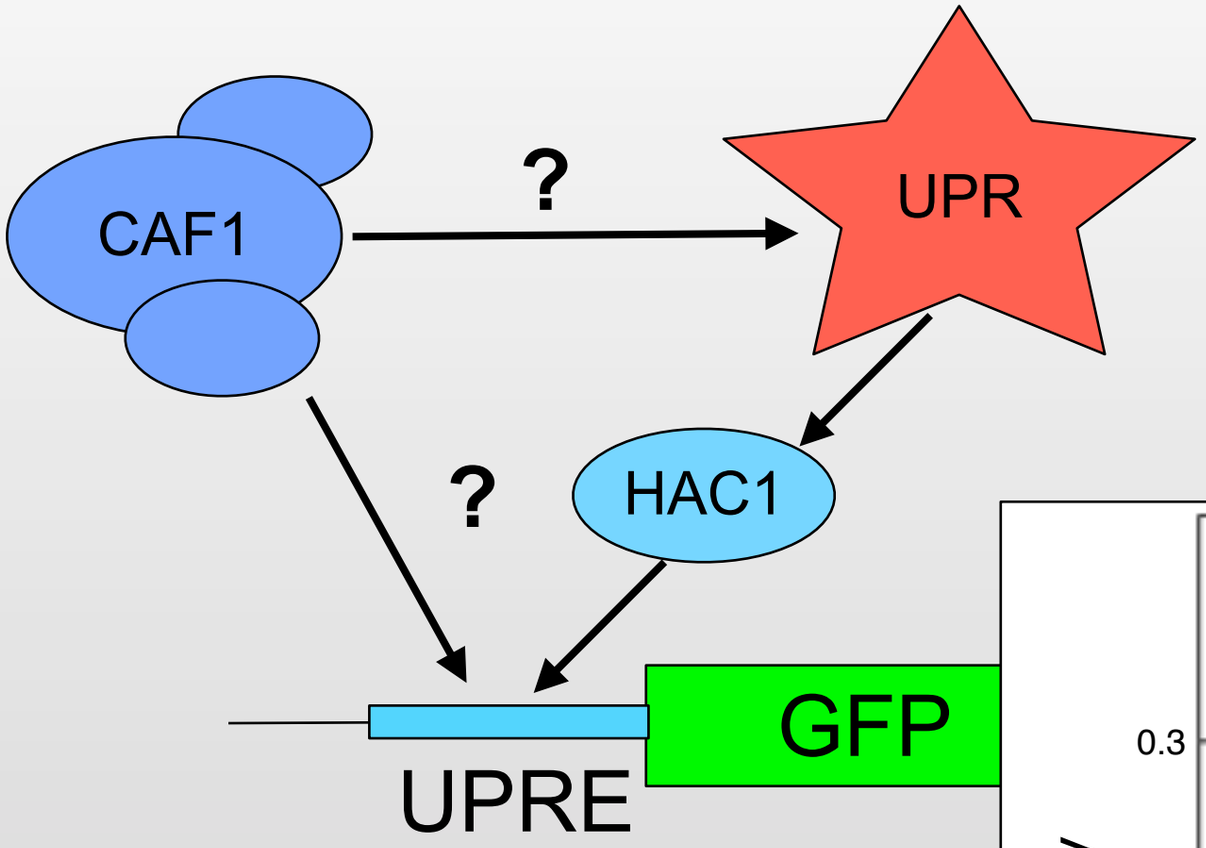


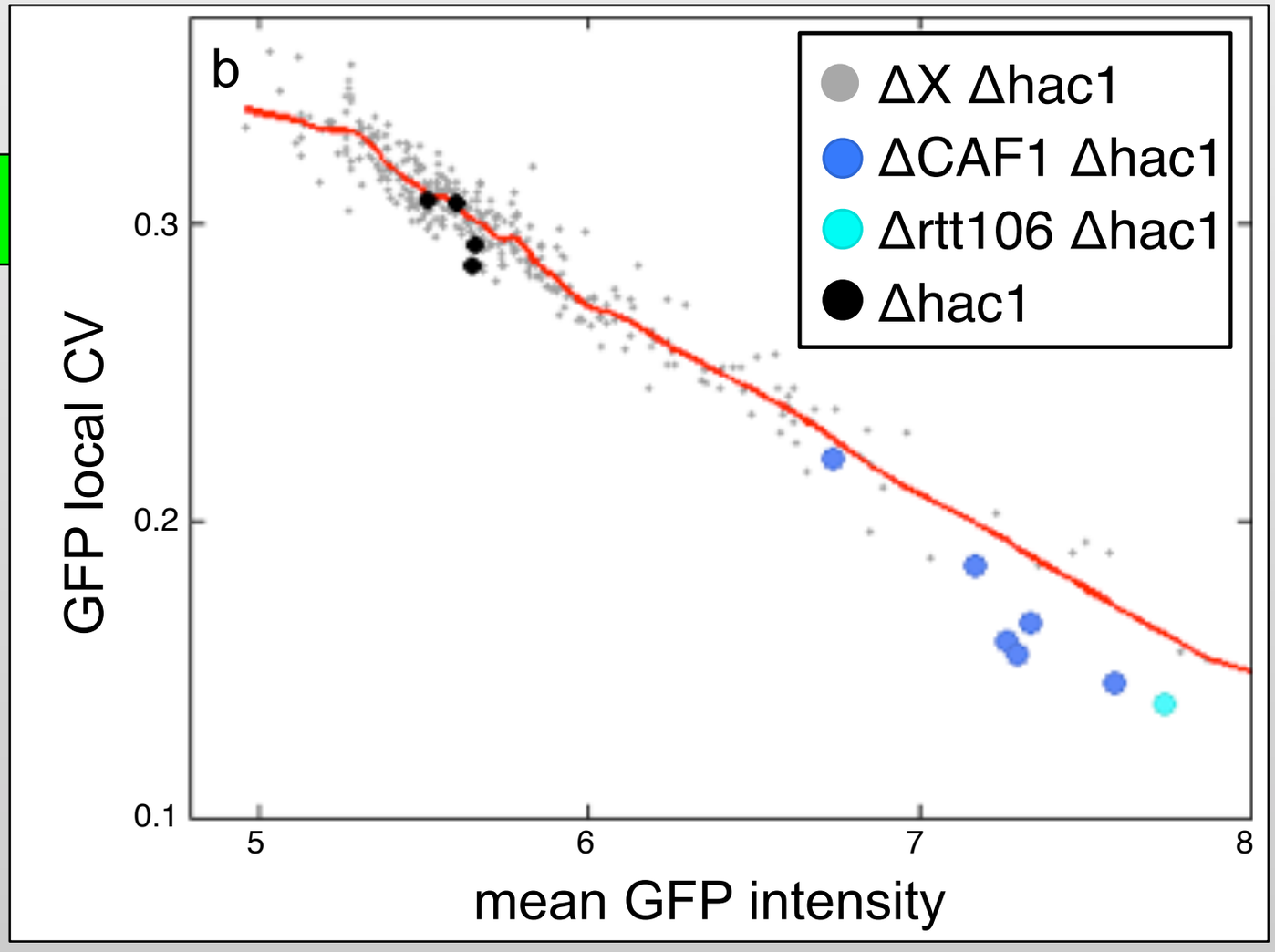

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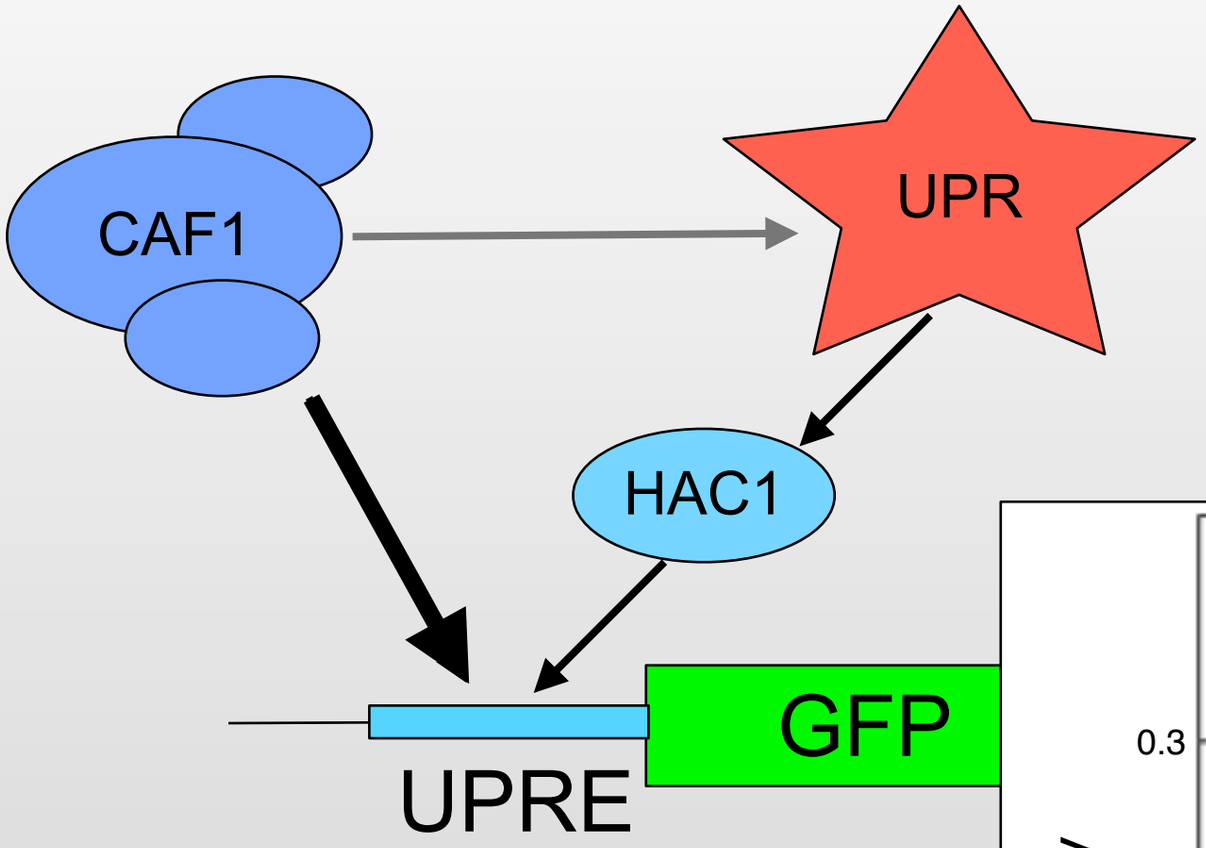
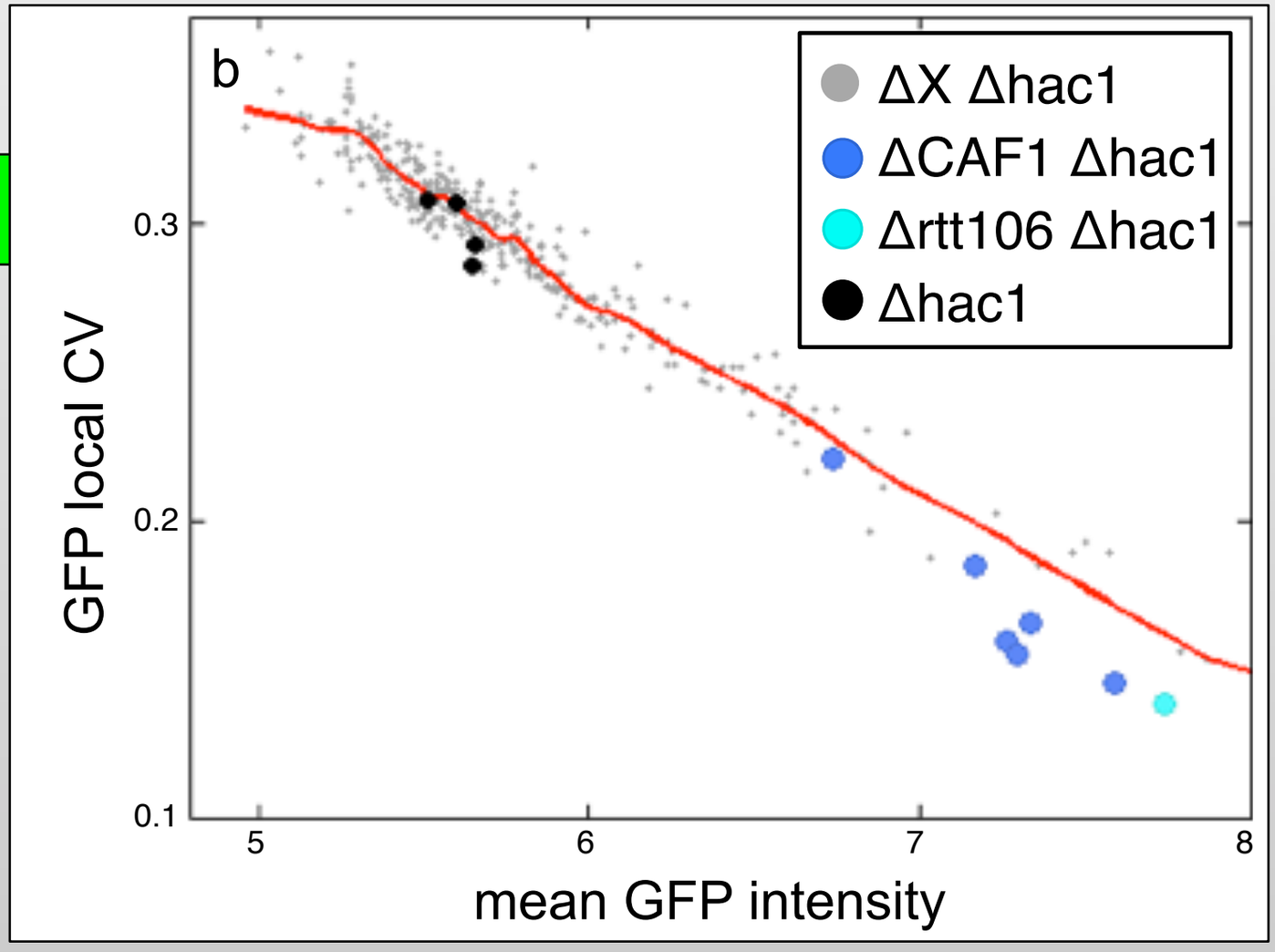
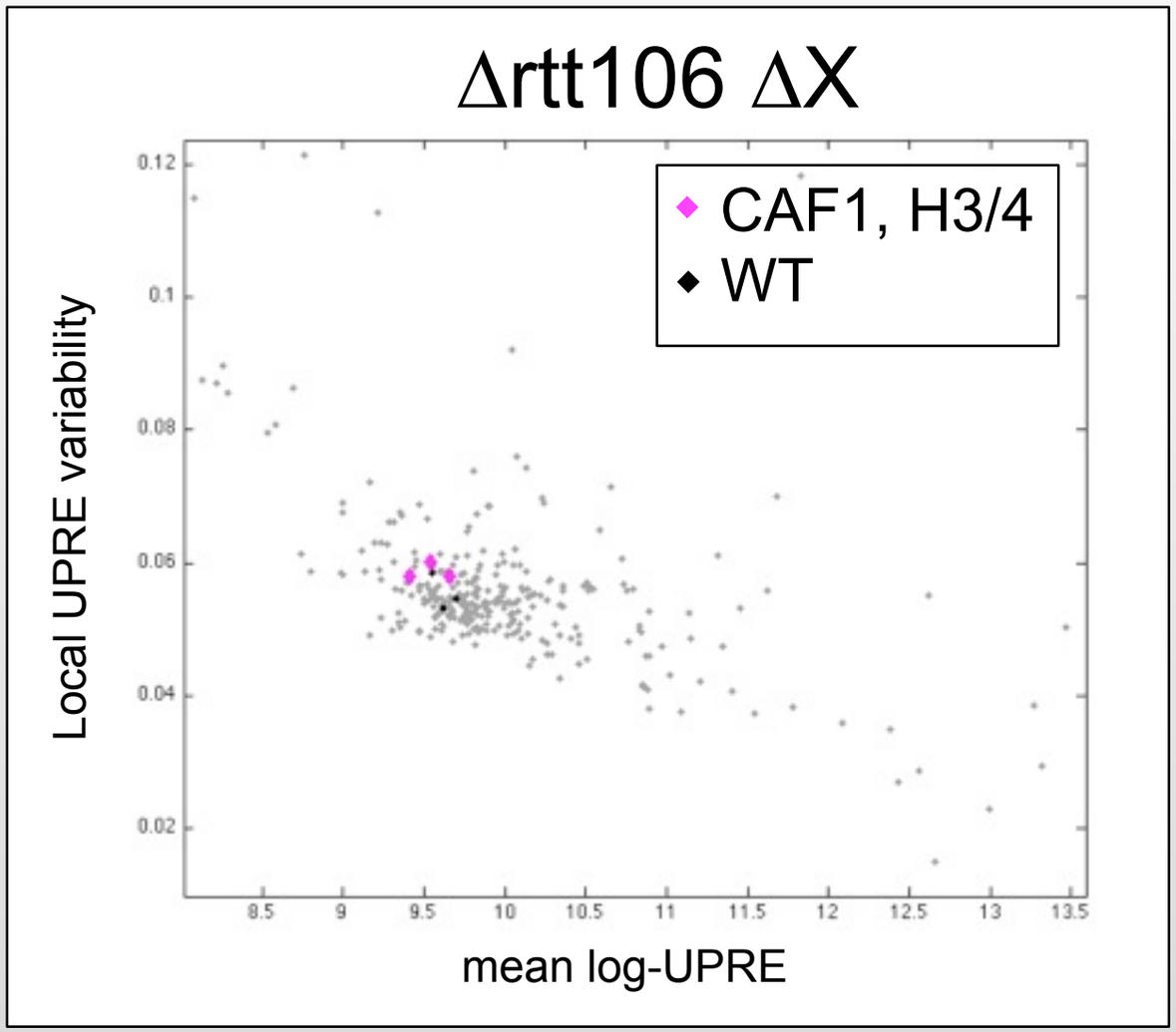


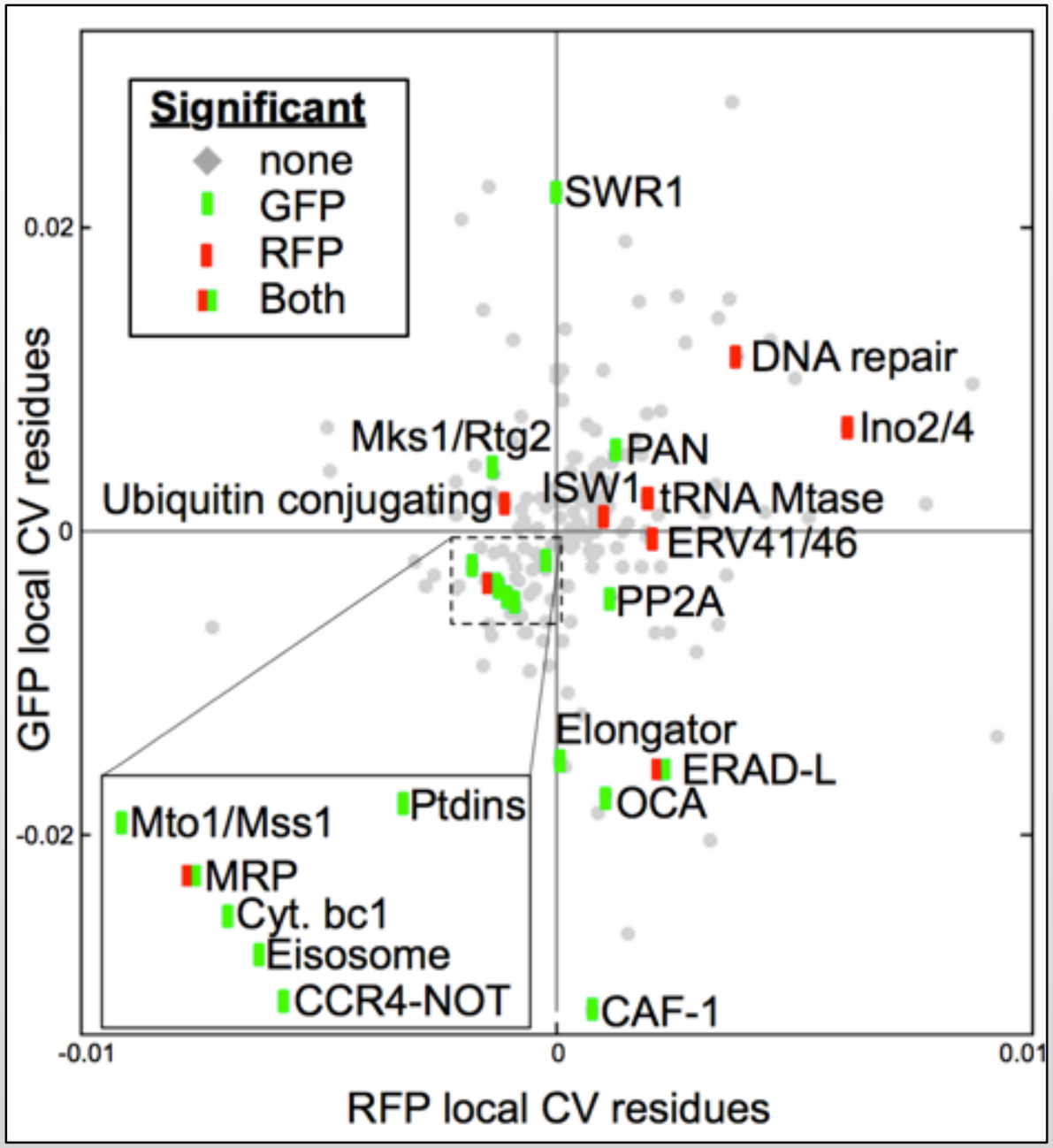
# CAF1: Full Epistasis with RTT106




**Hypothesis**:

- Defects in CAF1 reduce nucleosome density
- Synergistic activity with RTT106
- Nucleosome remodeling more crucial for UPRE

# Increasing Local Variability: SWR1



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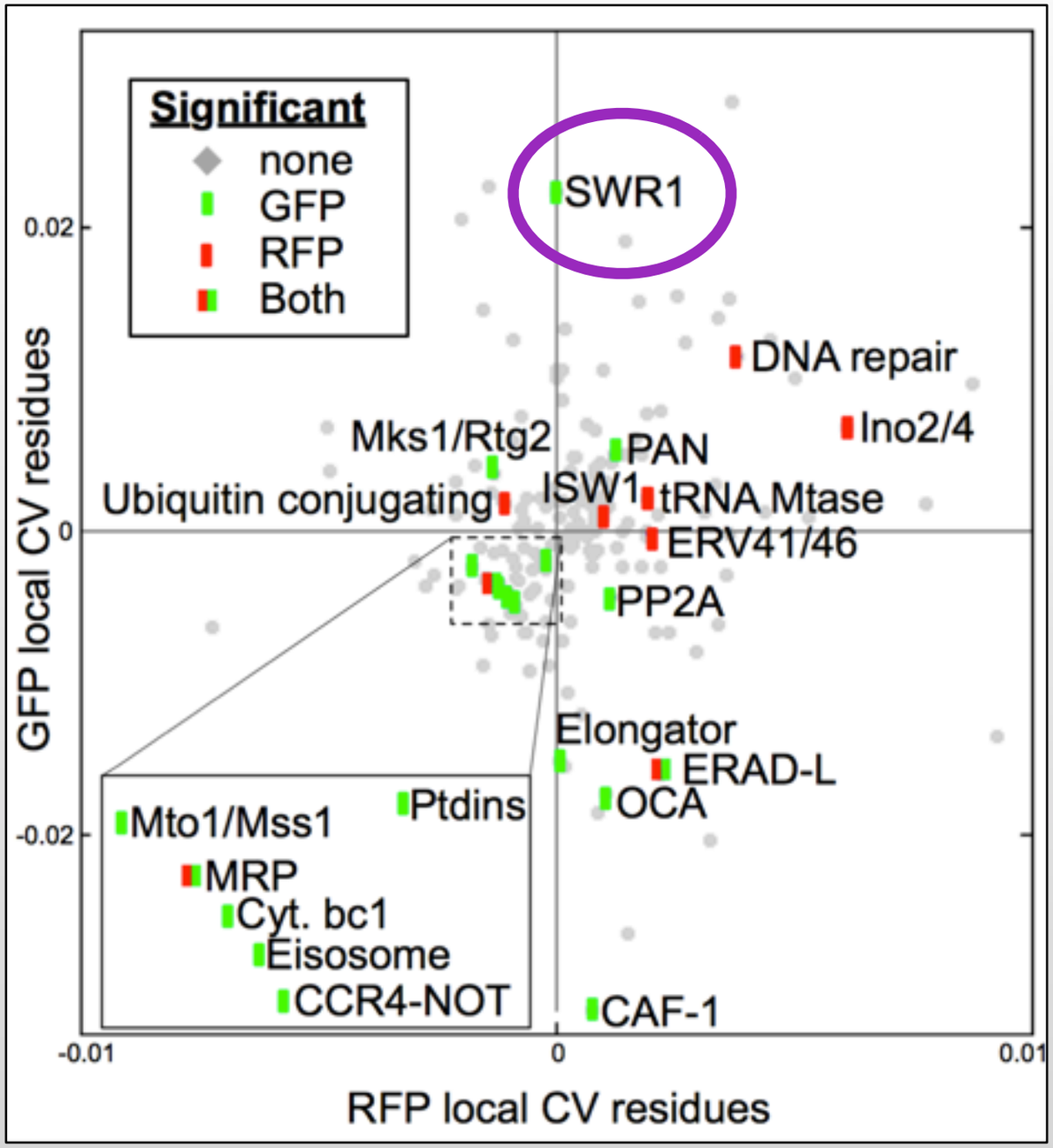

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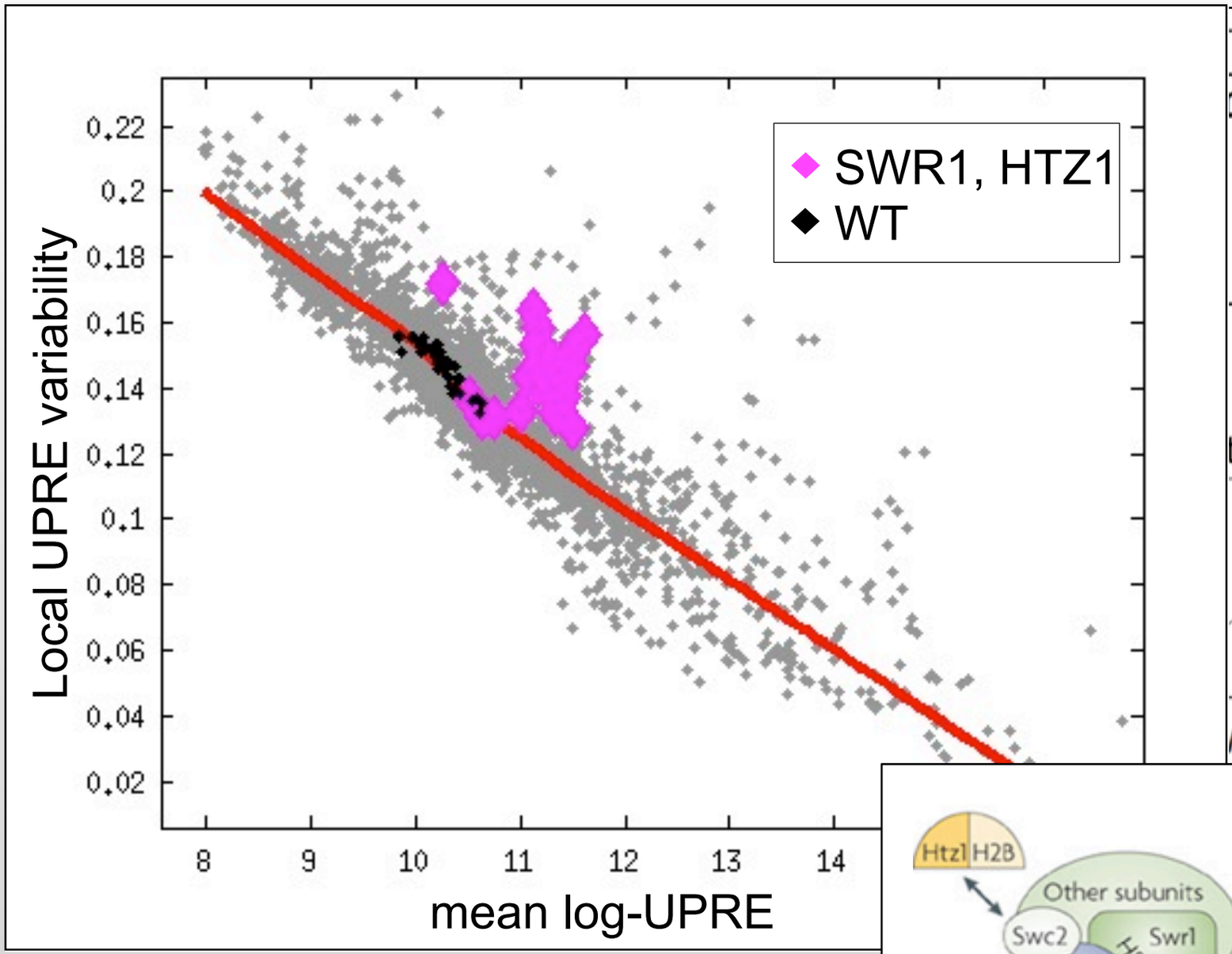
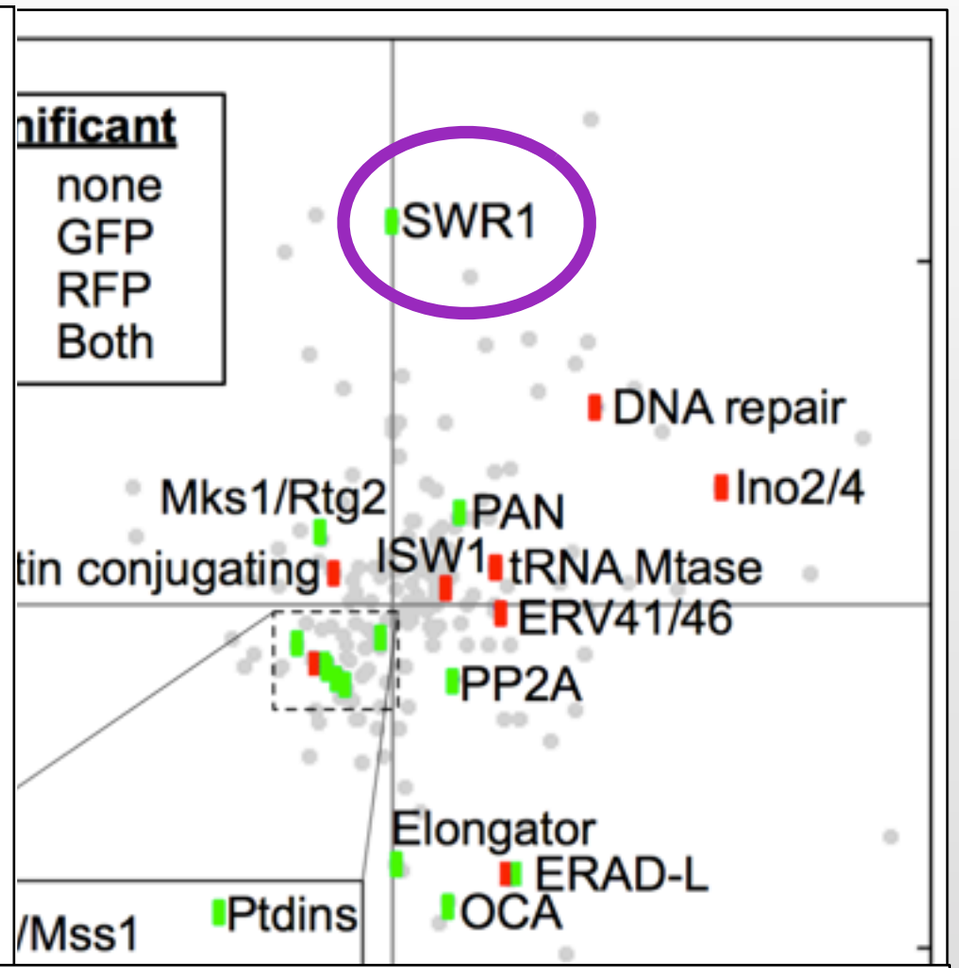
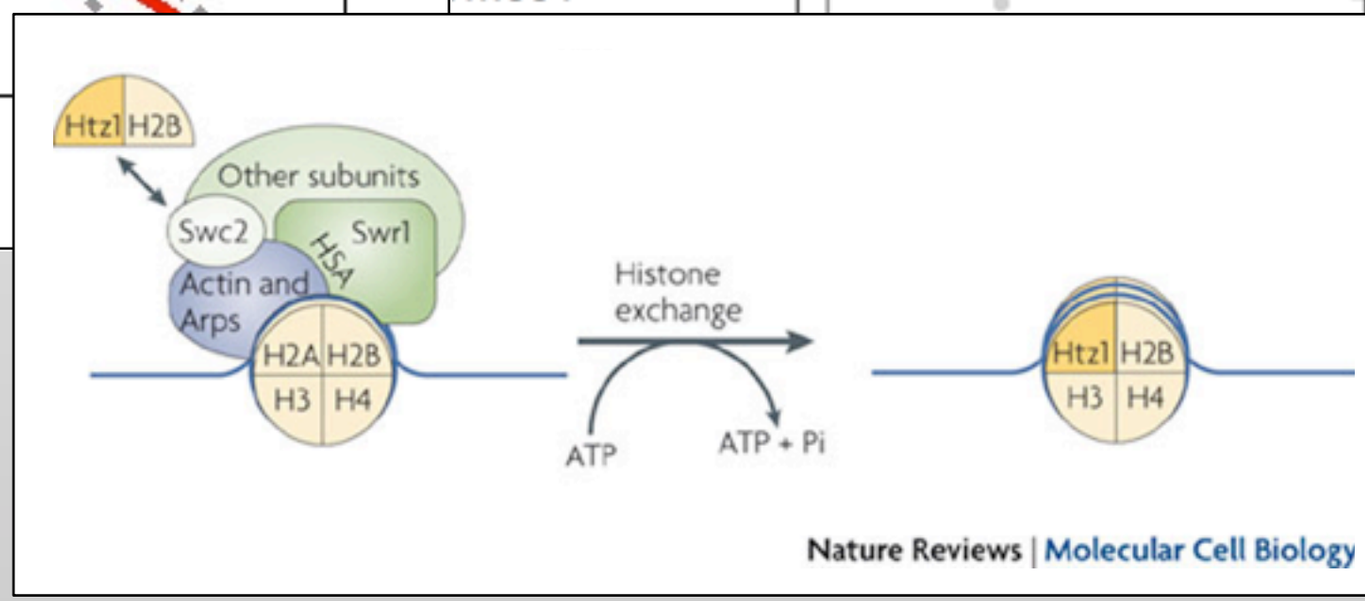
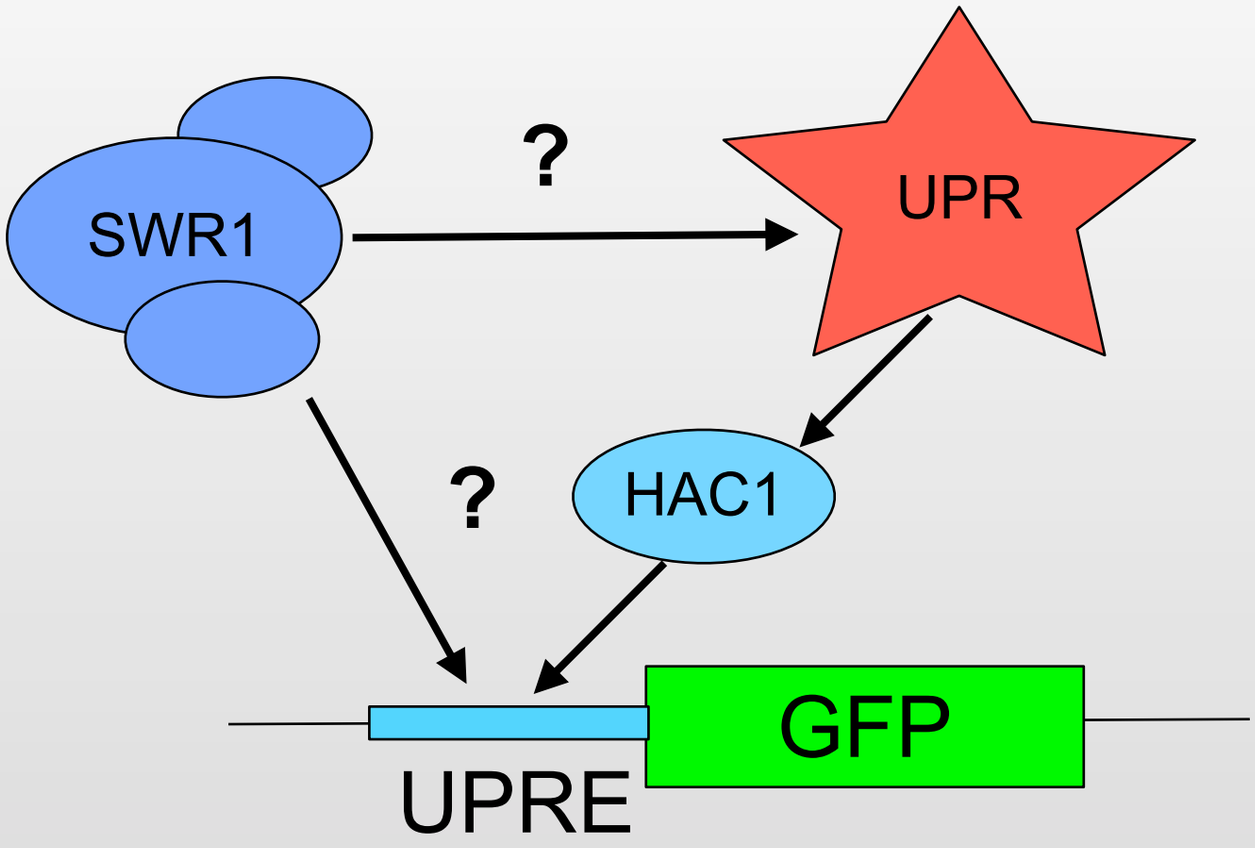


# Changes in Local Variability: Direct or Indirect Effect?



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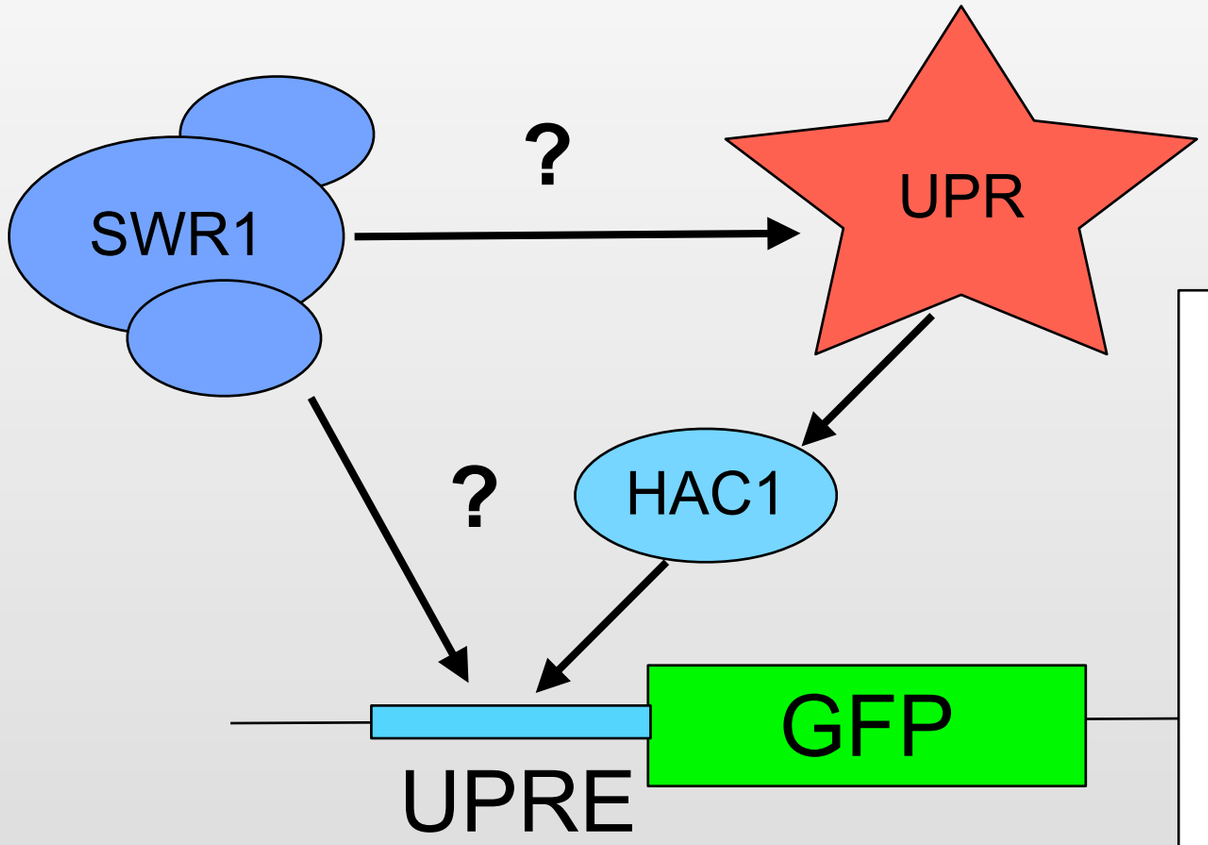


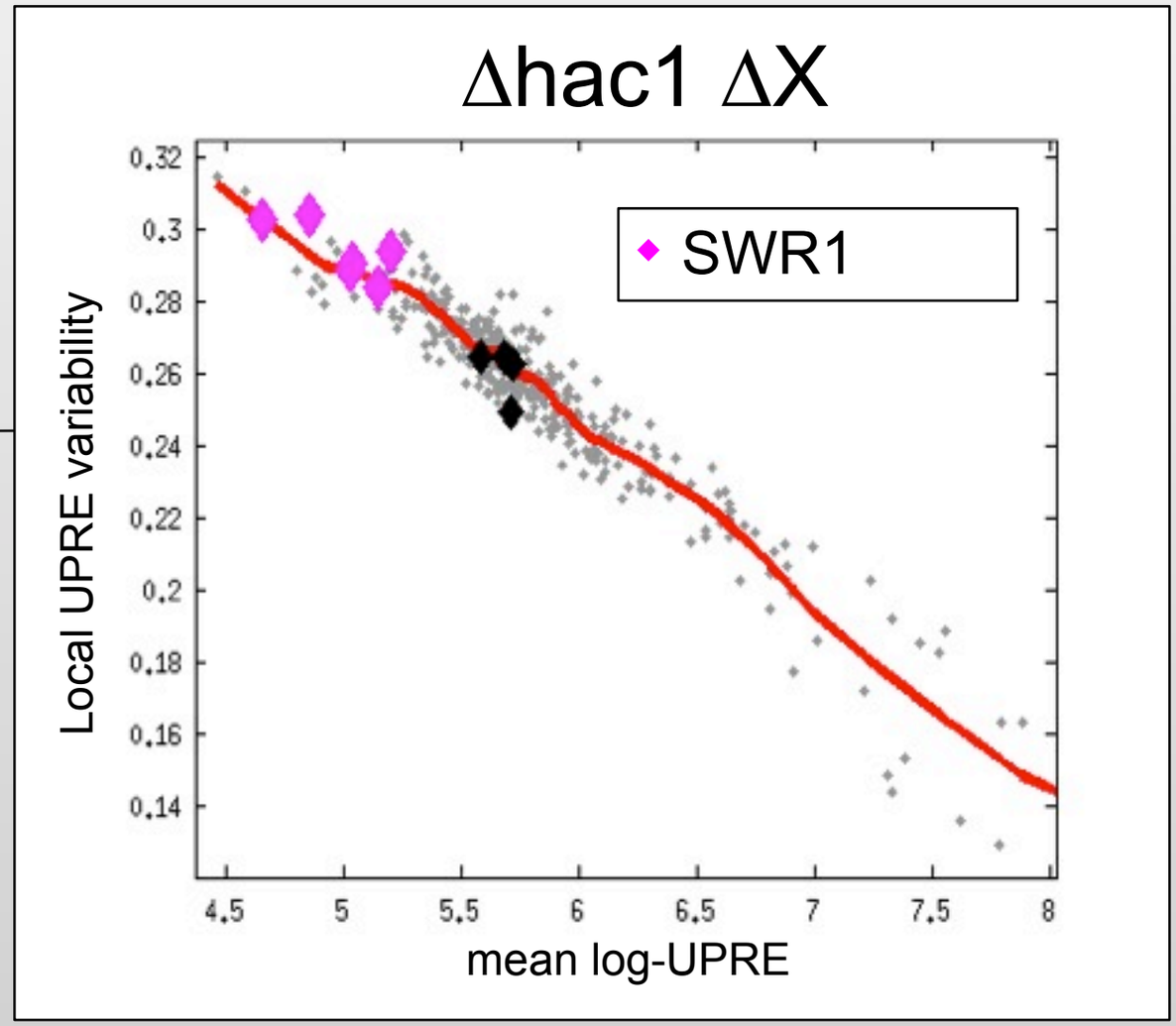

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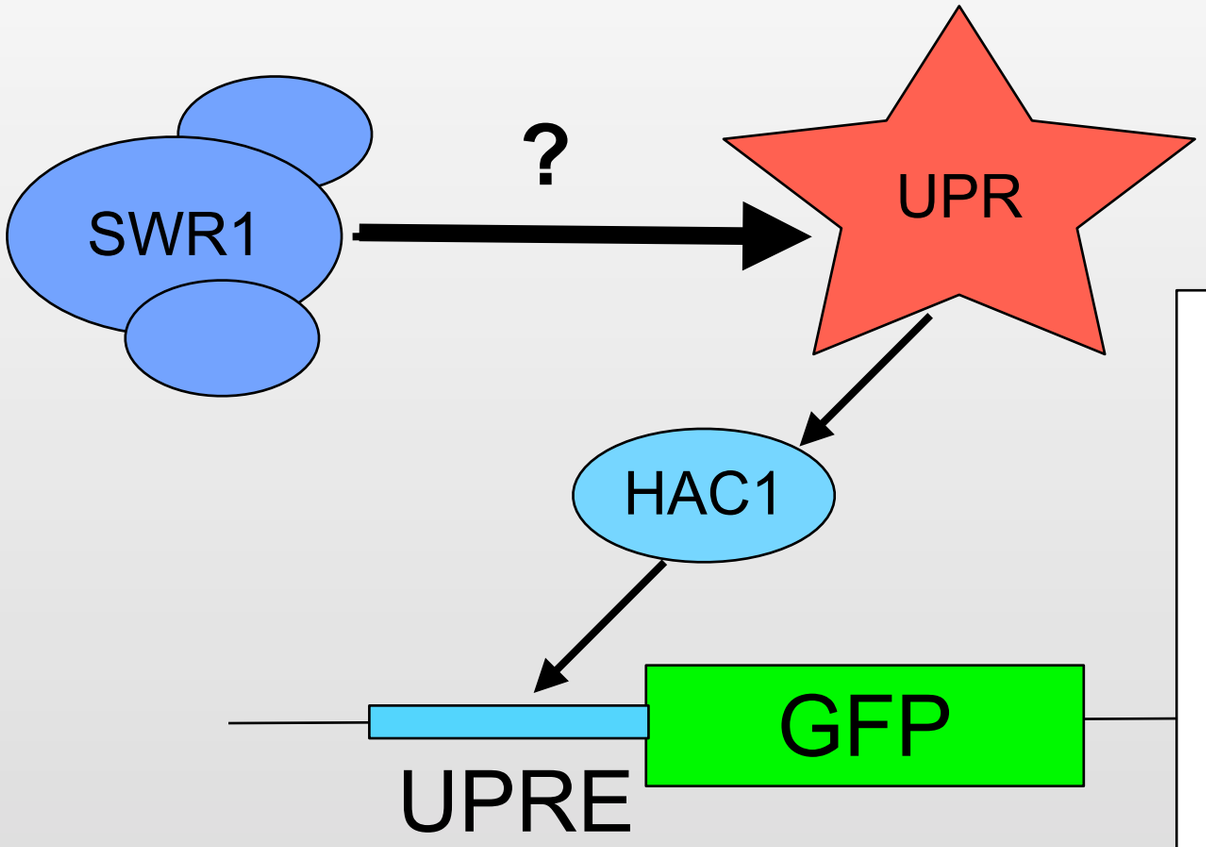


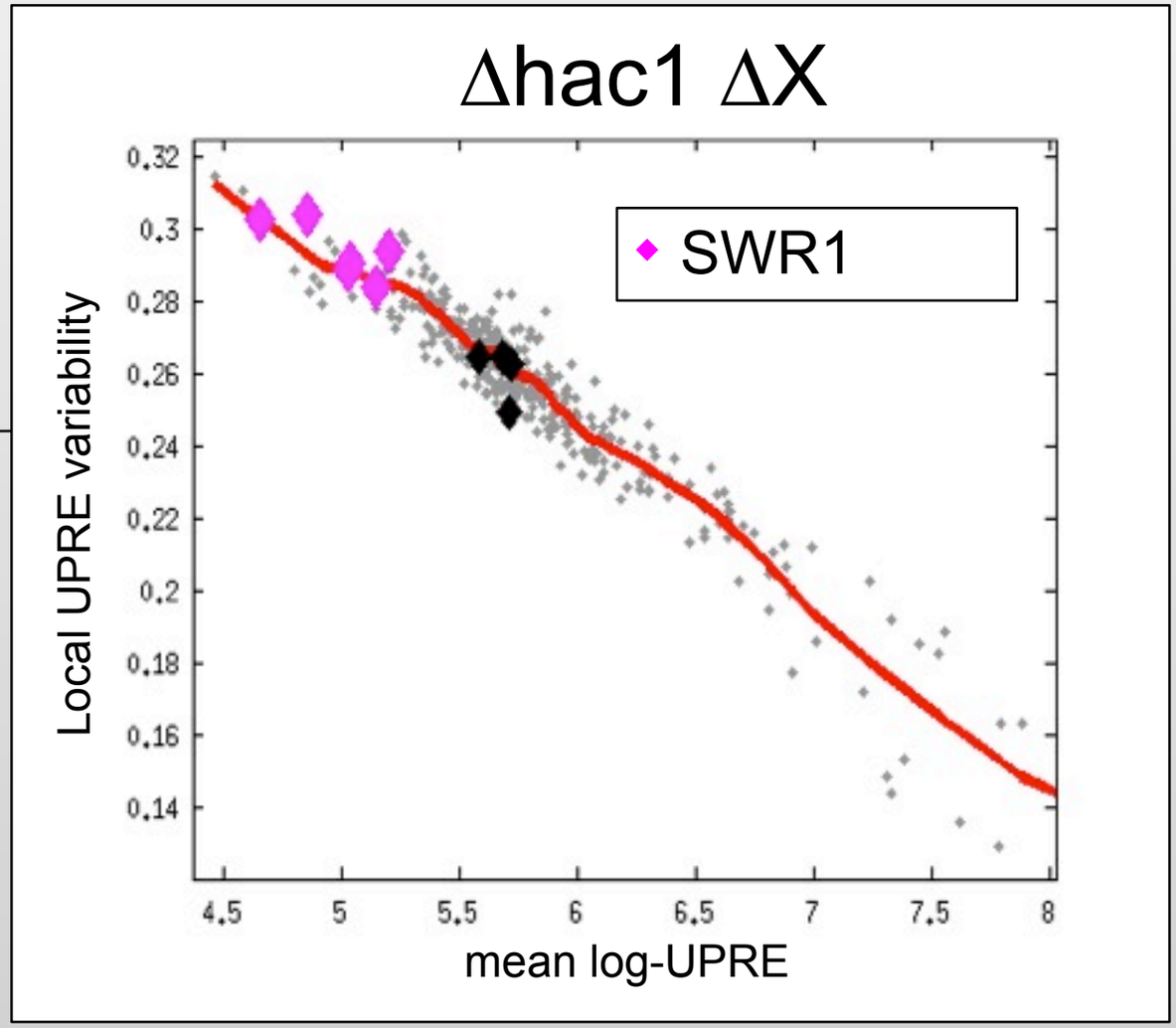

# Indirect Mechanism

- Search for genes X such that:
  - X is down-regulated in ∆swr1 (published data)
  - ∆X increases local UPRE noise

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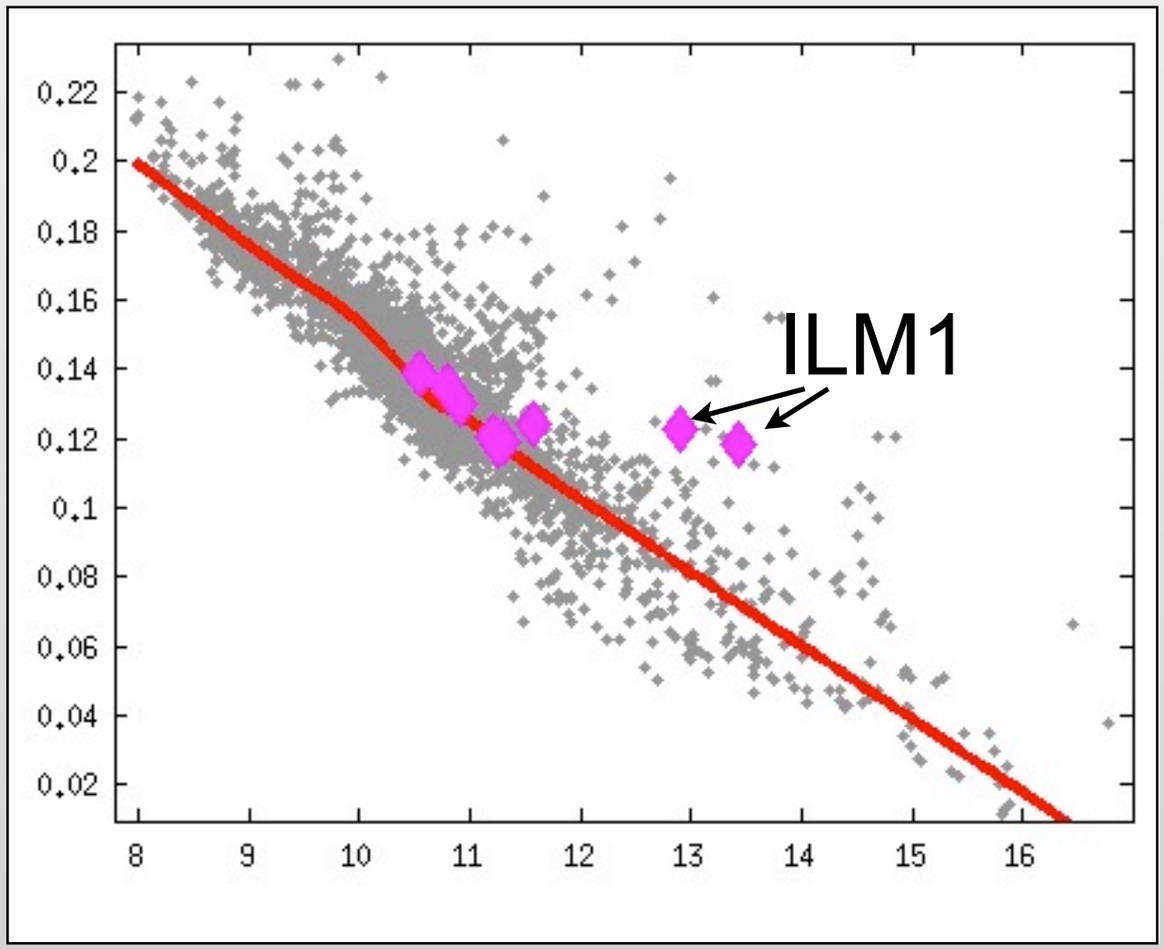

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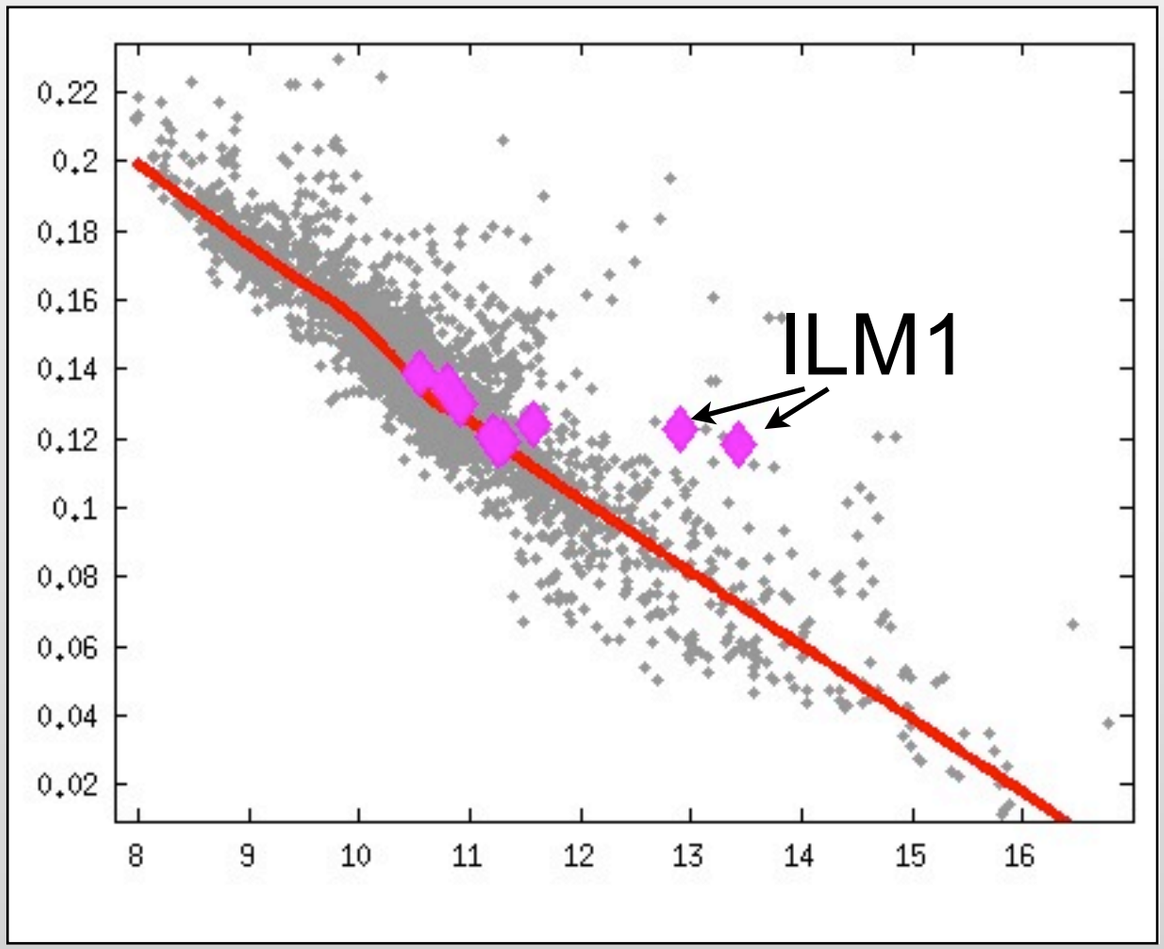


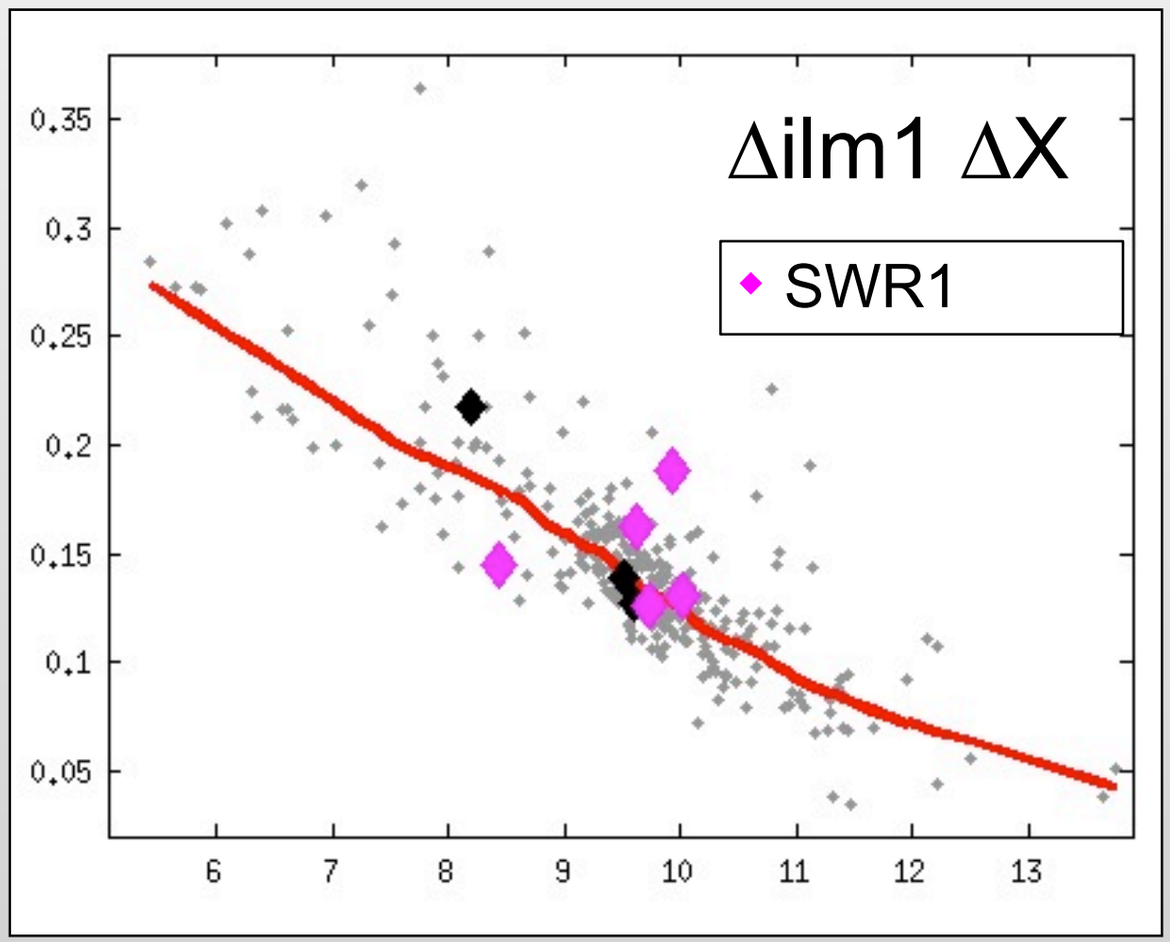

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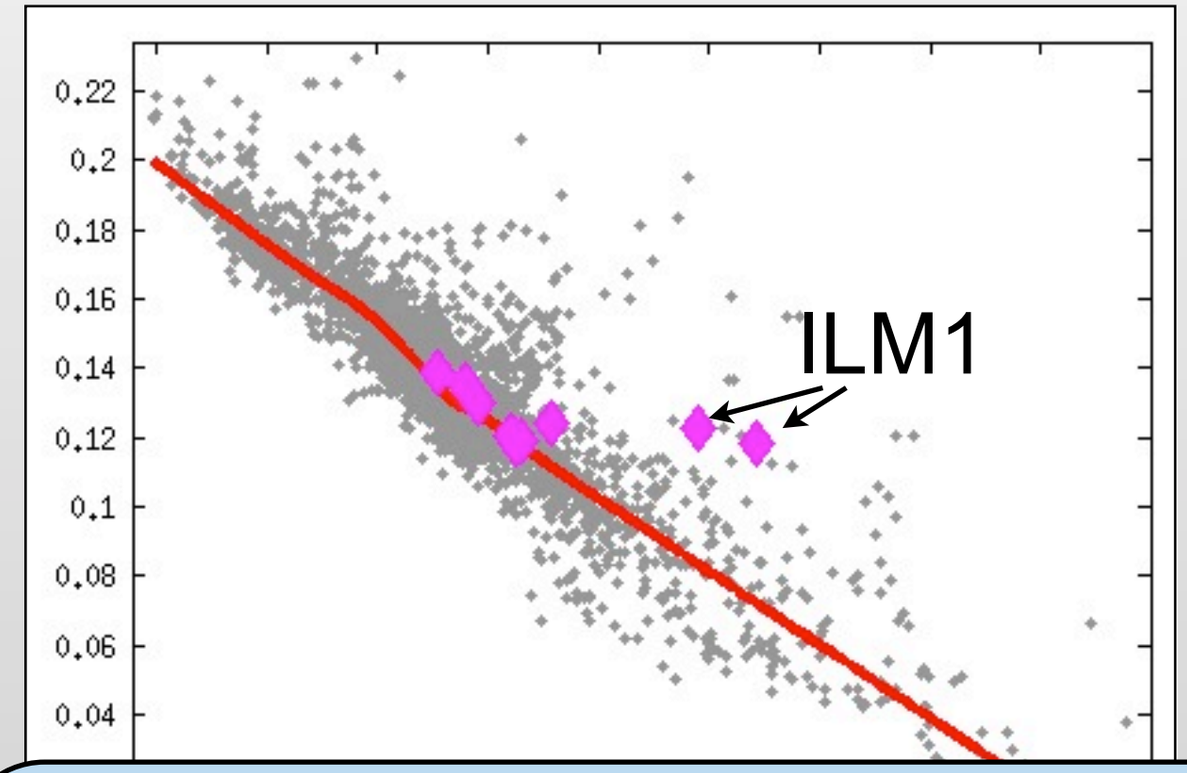
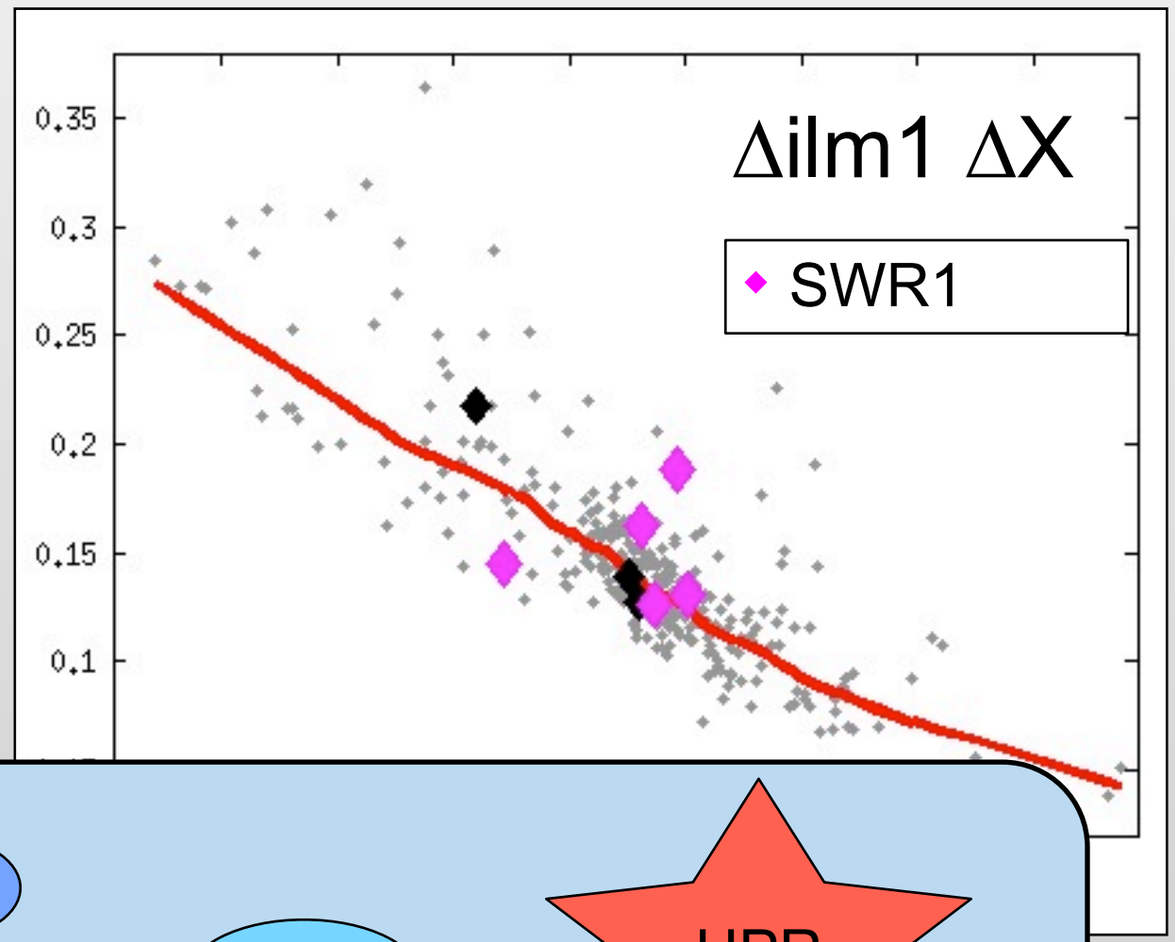
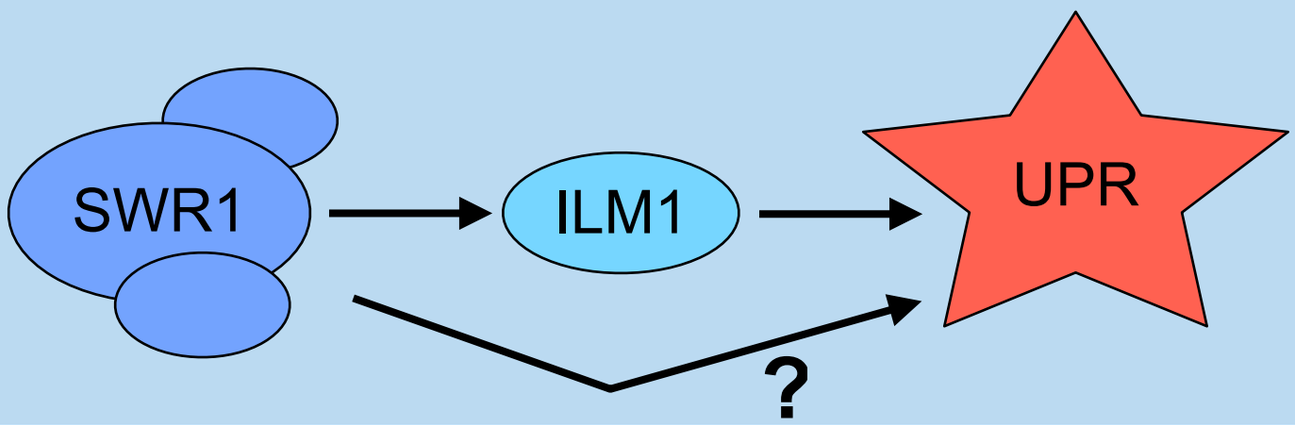


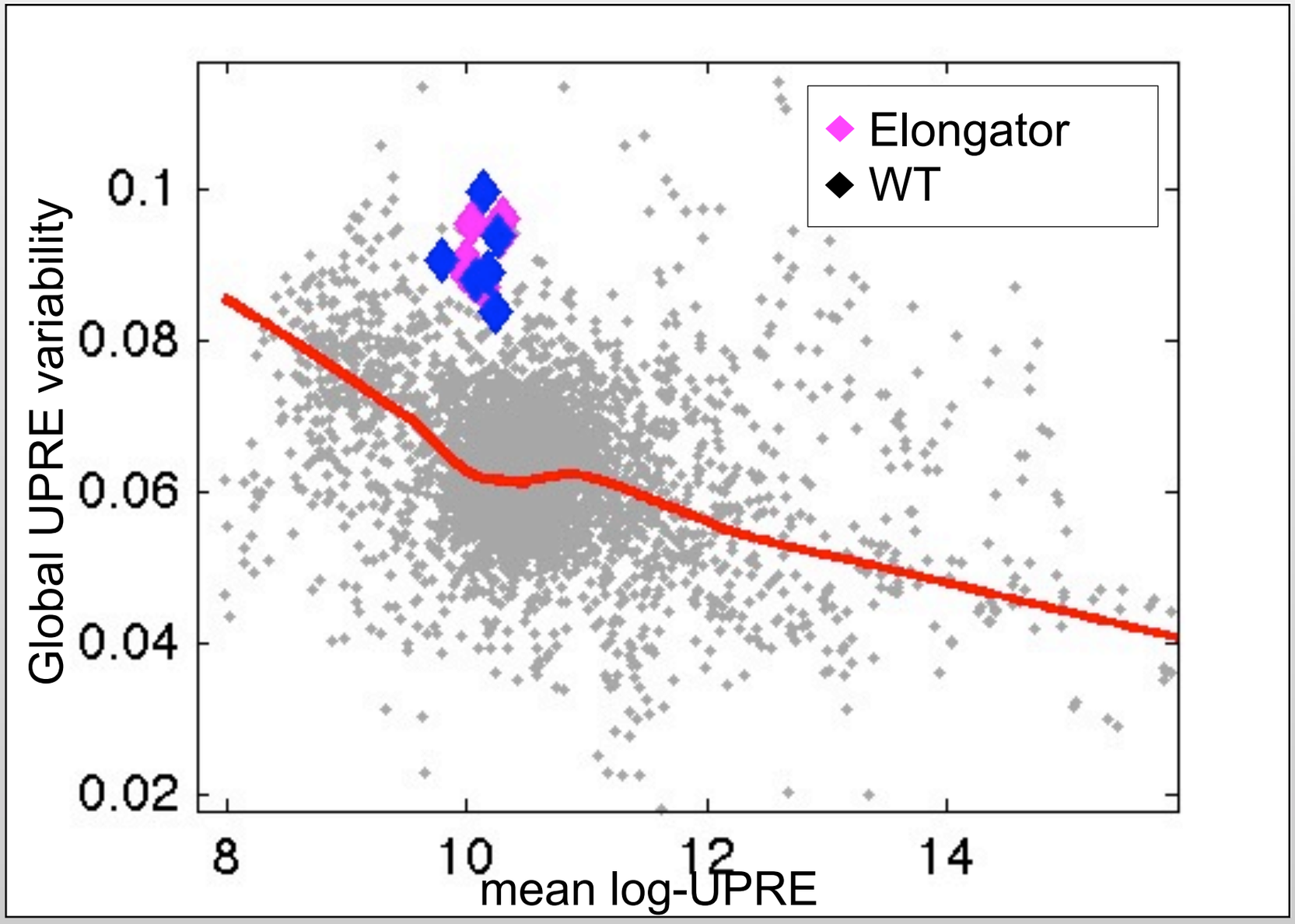




Partial epistasis with ILM1

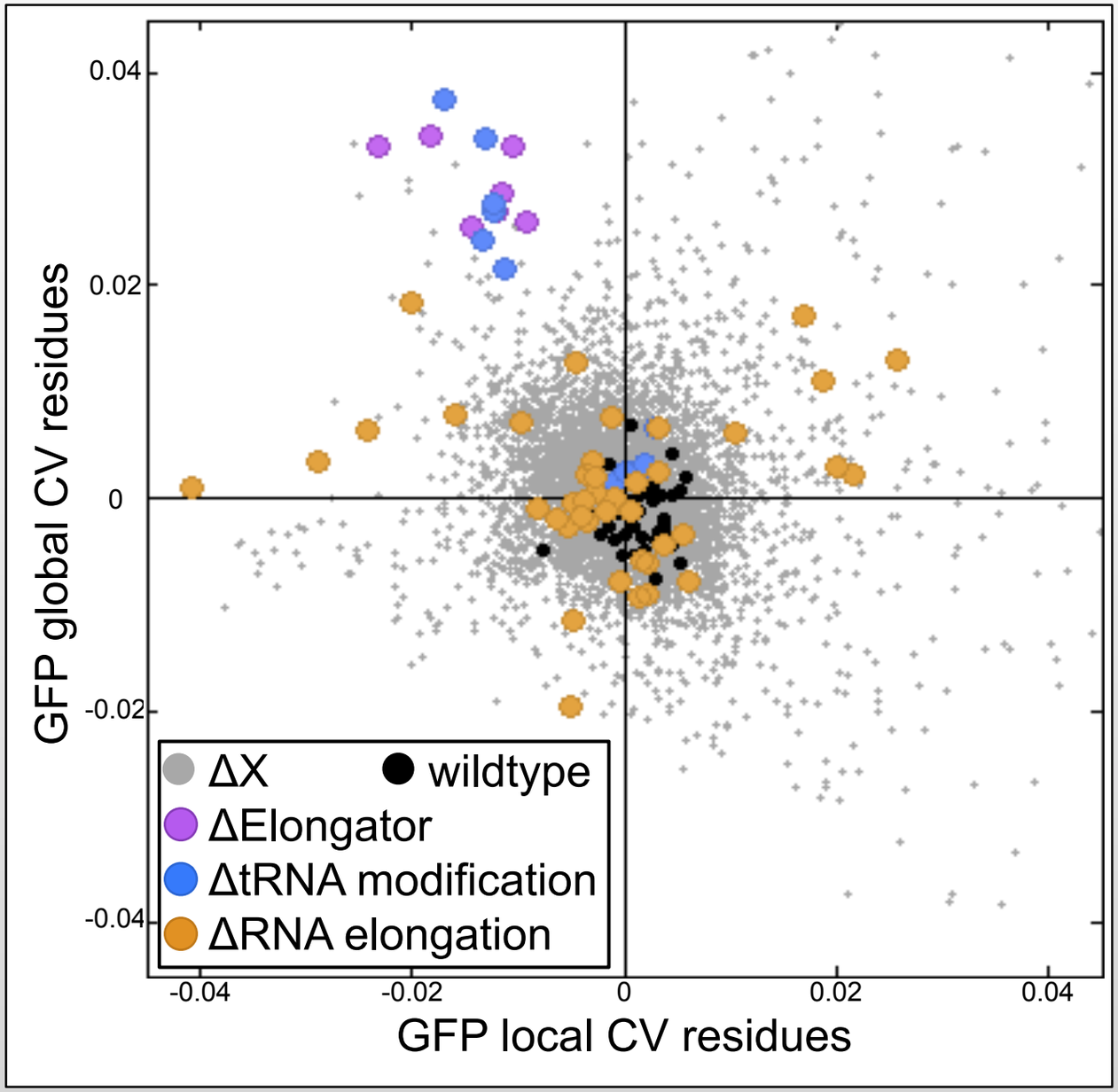


# Increasing Global Variability: Elongator Complex

- Known functions:
  transcription elongation, tRNA modification



# Implicating Specific Function




- Variability “signature” implicates tRNA modification in high global variability of elongator

# Summary

- Variability as a phenotype for genetic screens
  - Provides different perspective than mean
  - Clues to mechanism

- Analysis of specific promoters across KO library
  - Highlights interesting regimes of variability
  - Systematic screen uncovers unexpected candidates

**Prospects**

- Using double KO for epistatic analysis
- Combination of multiple phenotypes

# Team Effort

**Ruty Rinott**

**Ariel Jaimovich**

## My lab

- Ayelet Rahat
- **Ruty Rinott**
- **Ariel Jaimovich**
- Tommy Kaplan
- Assaf Weiner
- Avital Klien
- Adam Nitzan
- Ohad Fried
- Noa Novershtern
- Naomi Habib
- Moran Yassour
- Aharon Novogordoski
- Ofer Meshi
- Tal El-Hay
- Eli Shapira

## Collaborators

- Andrew Capaldi
- Erin O'Shea
- Aviv Regev
- Hanah Margalit
- Oliver Rando
- Raz Kupferman