Gene expression regulation during cellular differentiation

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CRBM
11/10/2022

Outline

1. **Gene expression regulation: why and how?**
2. **Critical roles of transcription co-activators.**
3. **Case study #1: Nutrient-sensing and sexual differentiation in the fission yeast *S. pombe.***
4. **Case study #2: Regulation of interferon-stimulated genes in colorectal cancer cells.**

Defining gene expression regulation and why should you care?

Problem

- **Genes alone can account for the extraordinary complexity of a living organism.**
- **Genes interact with each other and with their environment (medium, cells):**
  - **House-keeping genes: ‘always’ ON**
  - **Regulated genes: ON or OFF**

Gene expression regulation

Difference prokaryotes / eukaryotes

- **Prokaryotes: ON is the default state**

  regulators = repressors

- **Eukaryotes: OFF is the default state**

  regulators = activators

Difference prokaryotes / eukaryotes

- **Prokaryotes: ON is the default state**

  regulators = repressors

  <u>although</u>: archeal histones…

- **Eukaryotes: OFF is the default state**

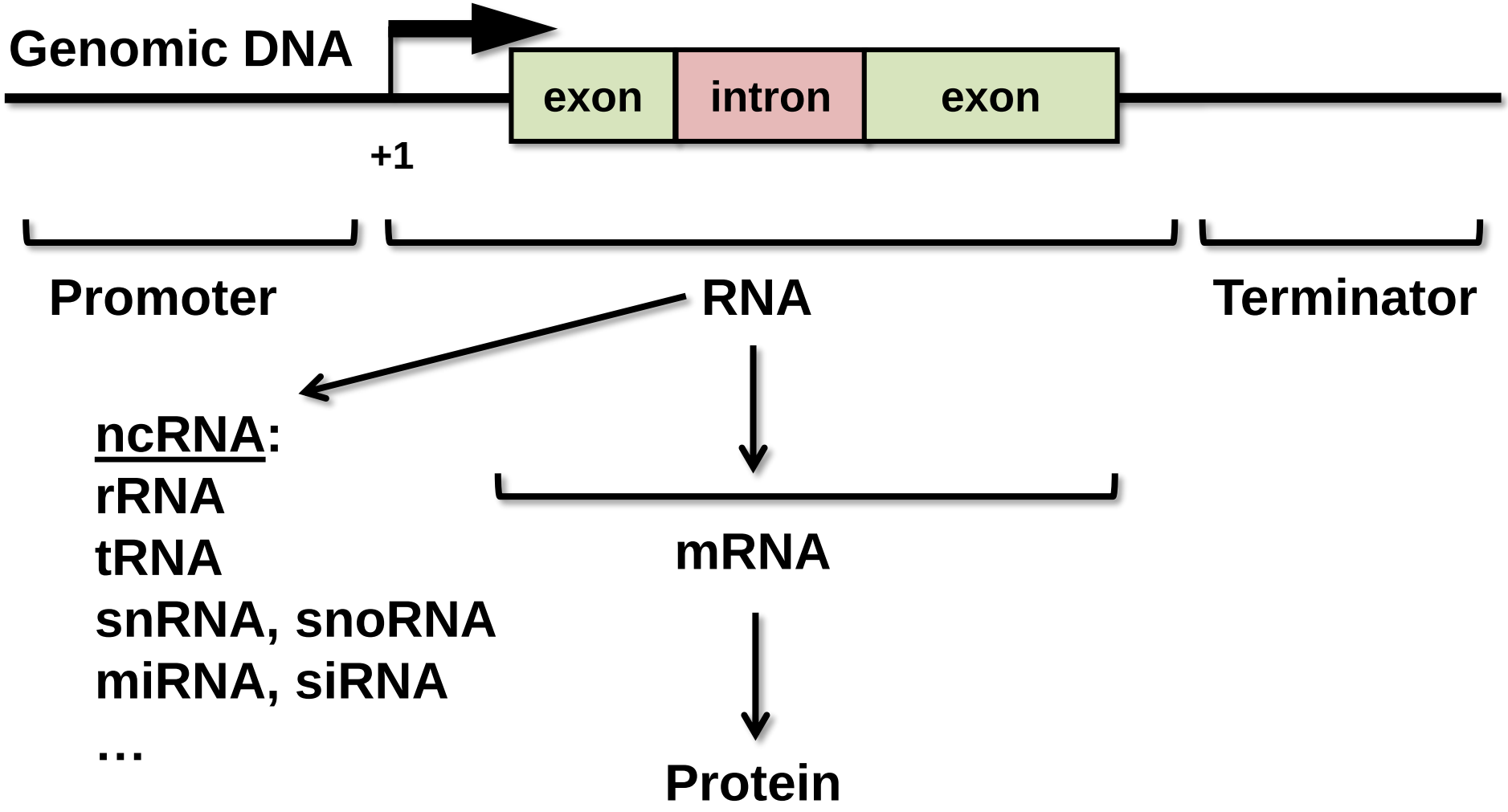
  regulators = activators

  <u>although</u>: pervasive transcription…

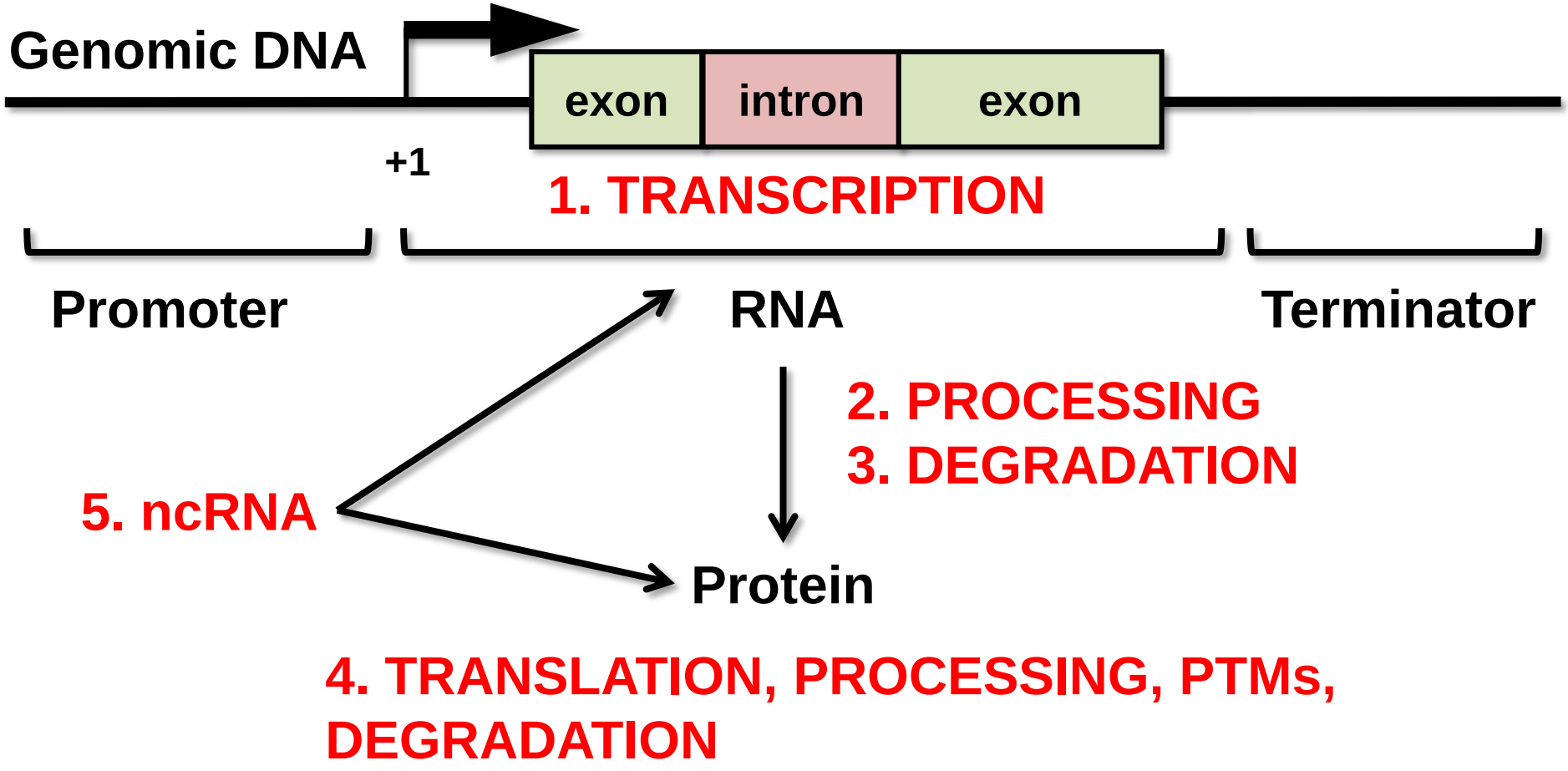
Importance of gene regulation

- **Self-renewal and cell-type specification.**
- **Adaptation to the environment and evolutionary novelty.**
- **Perturbations during oncogenesis.**

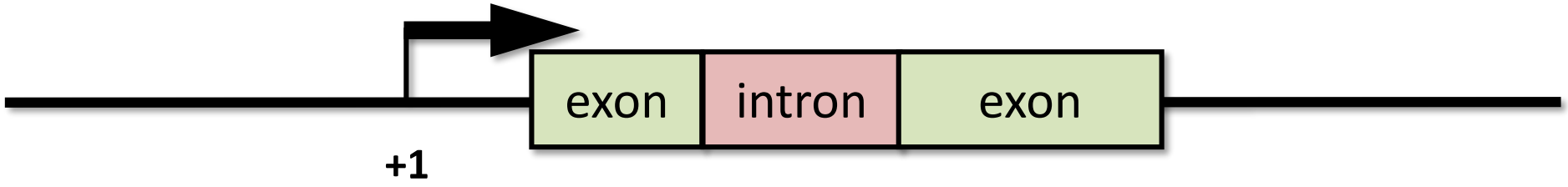
Structure of an eukaryotic gene



Regulating the expression of a gene

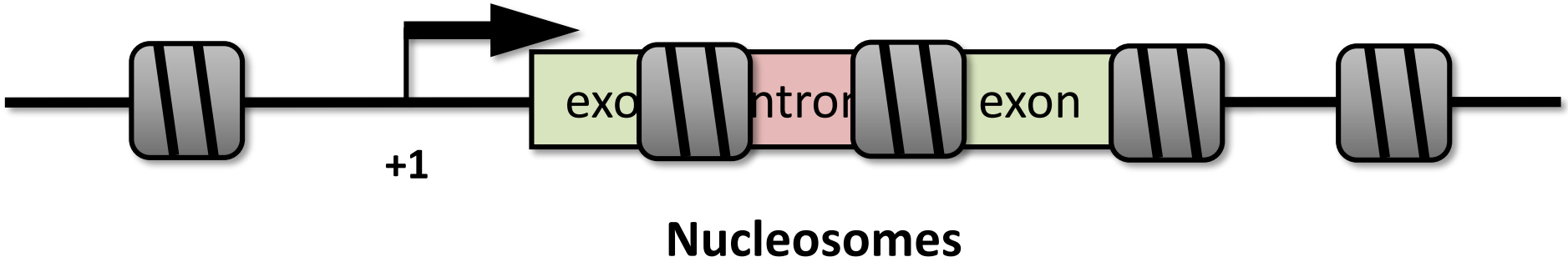


Regulating transcription



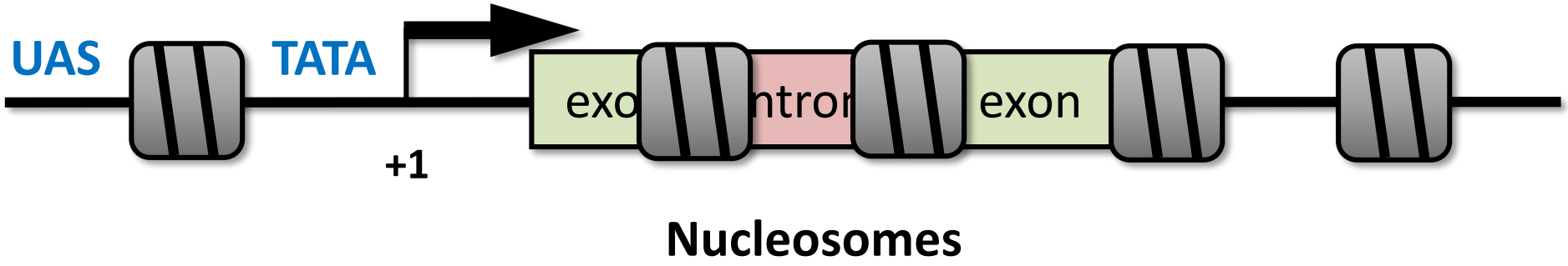
Regulating transcription

1. Chromatin structure



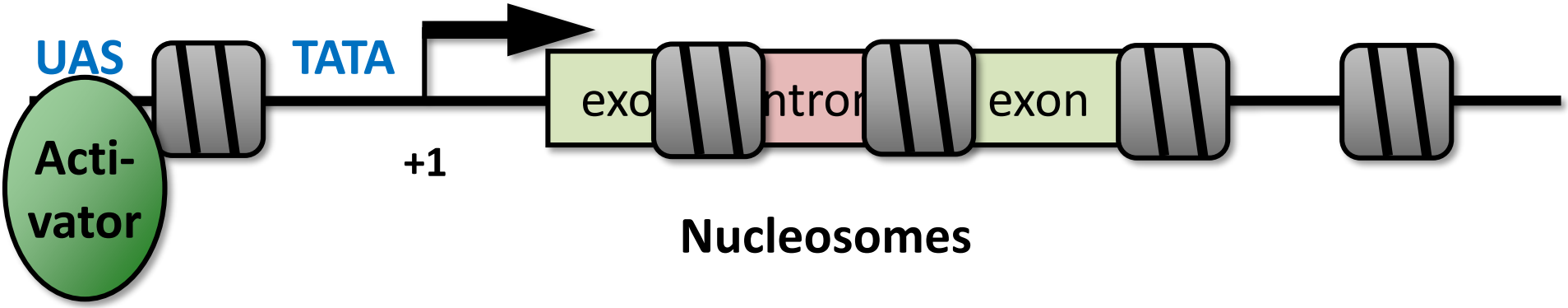
Regulating transcription

1. **Chromatin structure**
2. **Sequences (*cis*-regulatory elements)**



Regulating transcription

1. Chromatin structure
2. Sequences (*cis*-regulatory elements)
3. DNA-binding factors (*trans*-acting factors)



Regulating transcription

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2. Sequences (*cis*-regulatory el
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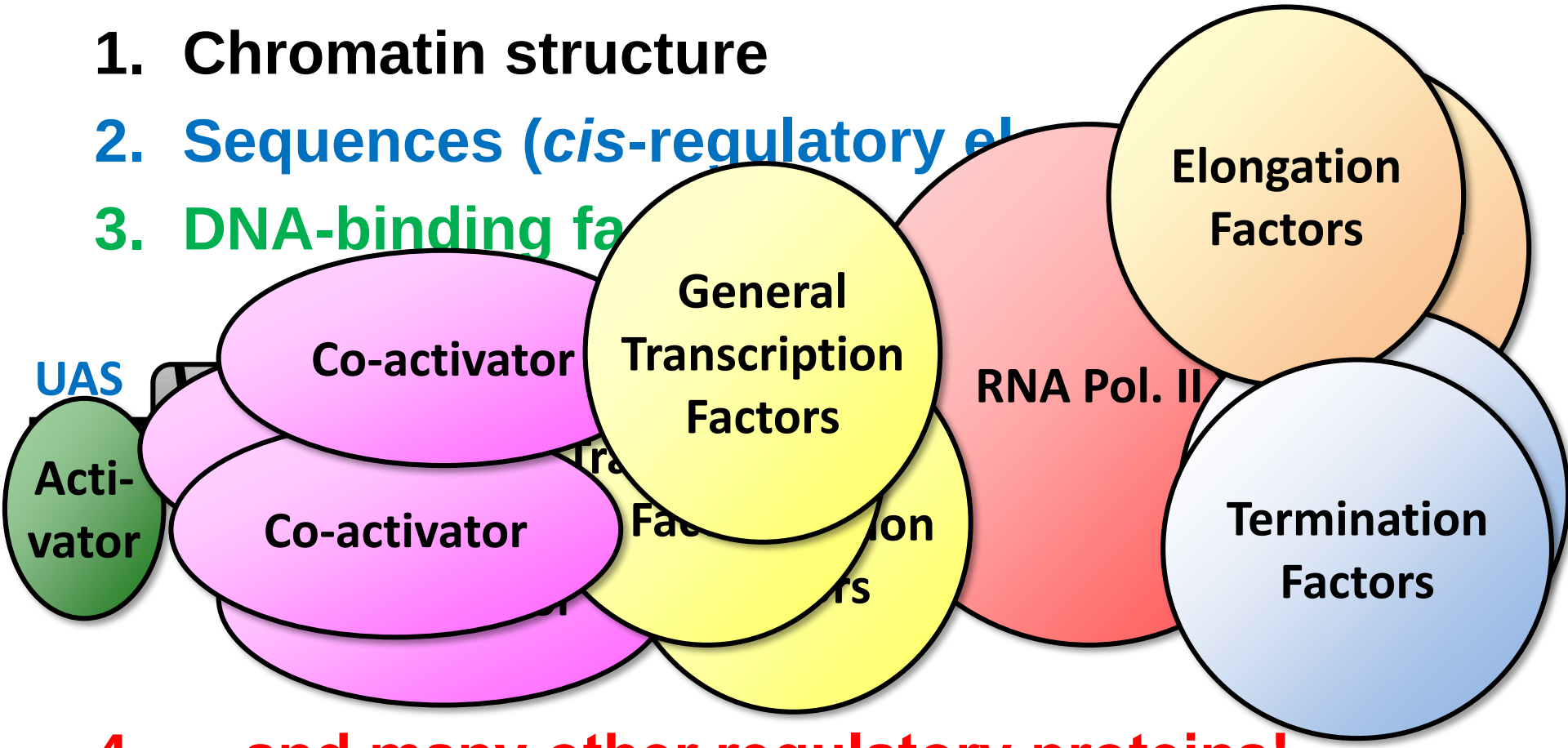


4. …and many other regulatory proteins!

Regulation of transcription initiation

- ***cis*-regulatory elements**
- **Specific transcription factors:**
  - **DNA binding domain: binds to specific motifs, 6-10 bp, within promoters / enhancers**
  - **Trans-activation domain: recruits co-activators and general transcription factors**
- **General transcription factors:**
  - **DNA binding: TATA box-binding protein (TBP)**
  - **Initiations (vs. elongators + terminators)**

Regulation of transcription initiation

- **Nucleosomes positioning: ATP-dependent chromatin remodeling complexes**
- **Histone modifications ('histone code'): histone-modifying complexes**

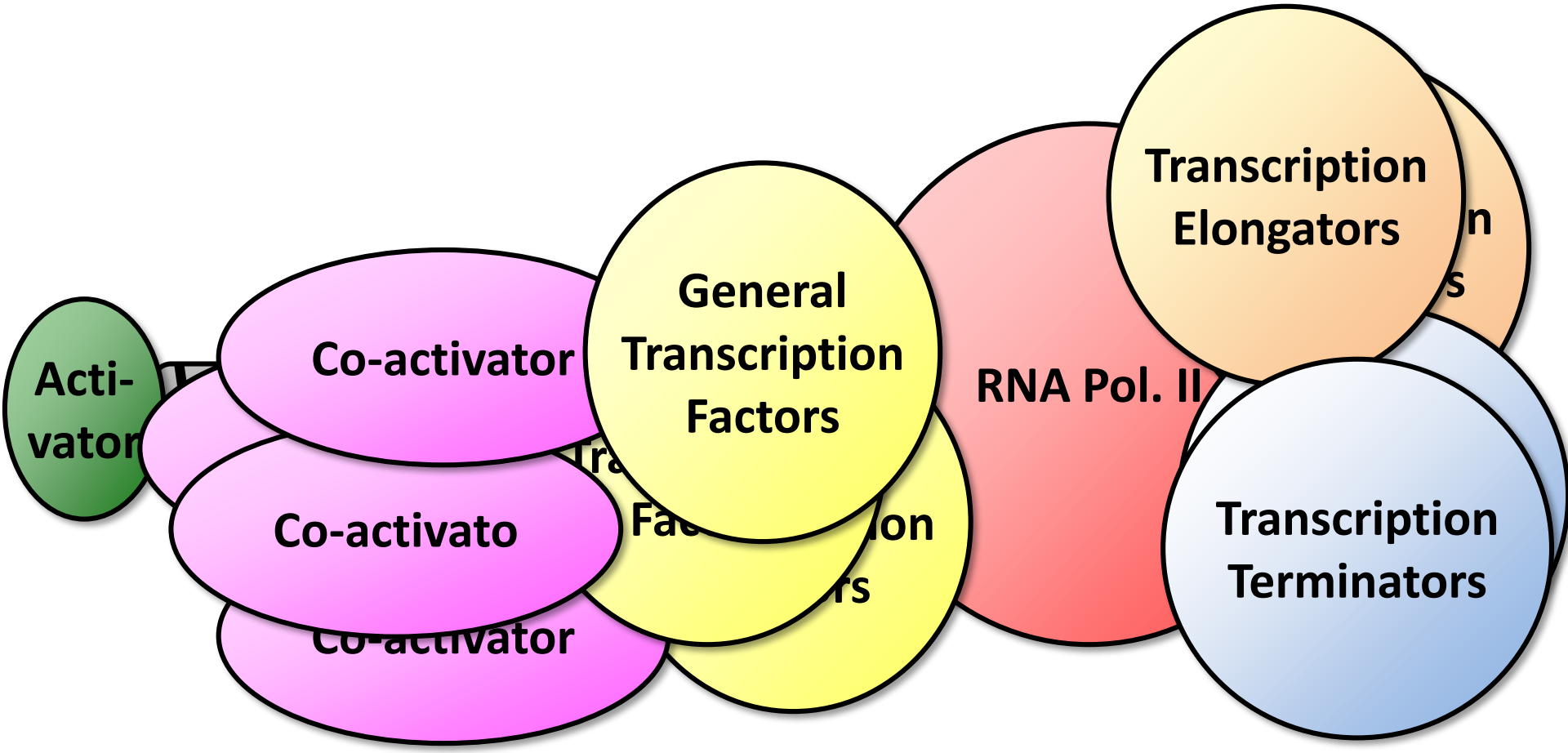
= transcription co-activators

- **Additional layer: non coding RNAs, DNA methylation**

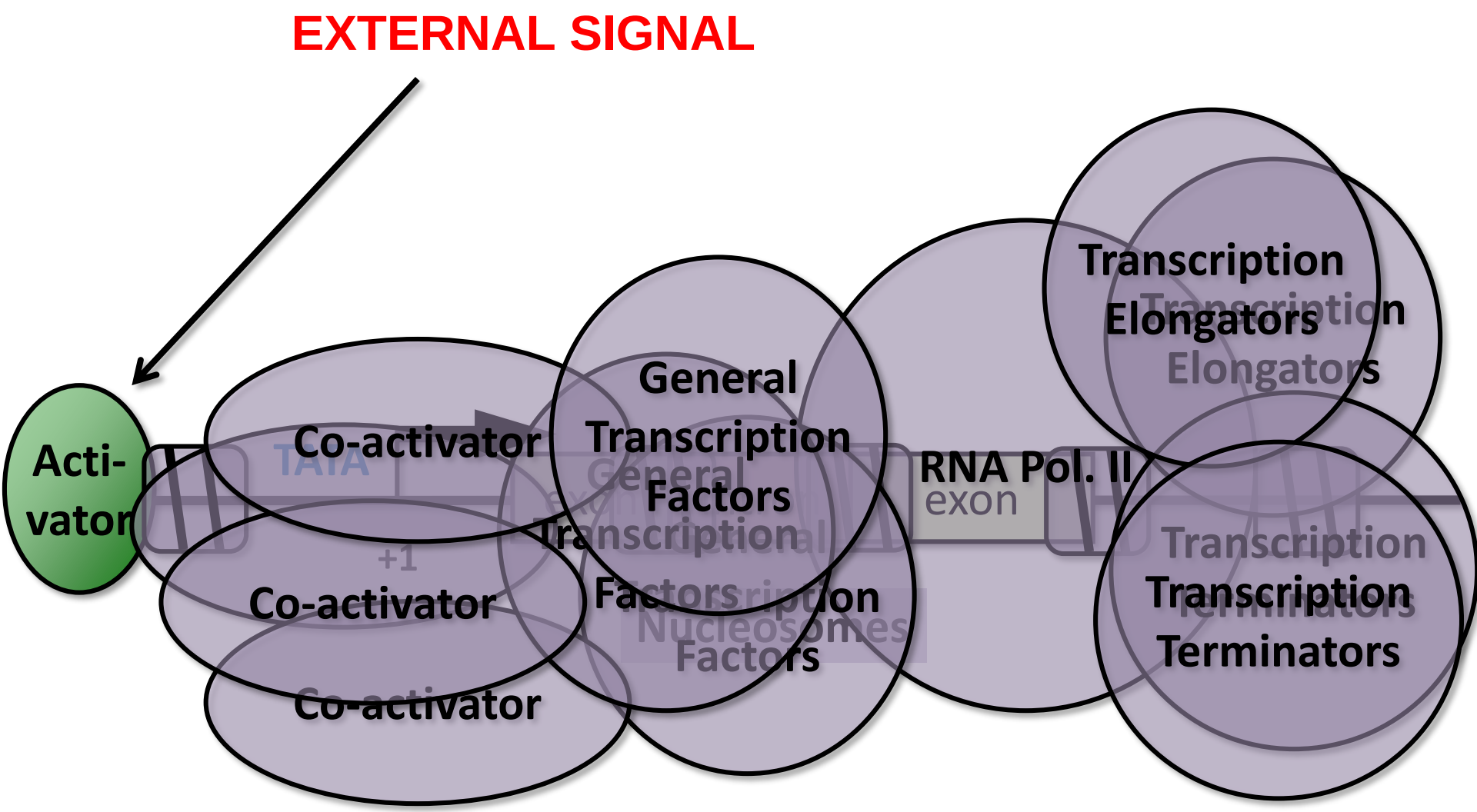
= Epigenetic regulation of transcription

Overall objective:

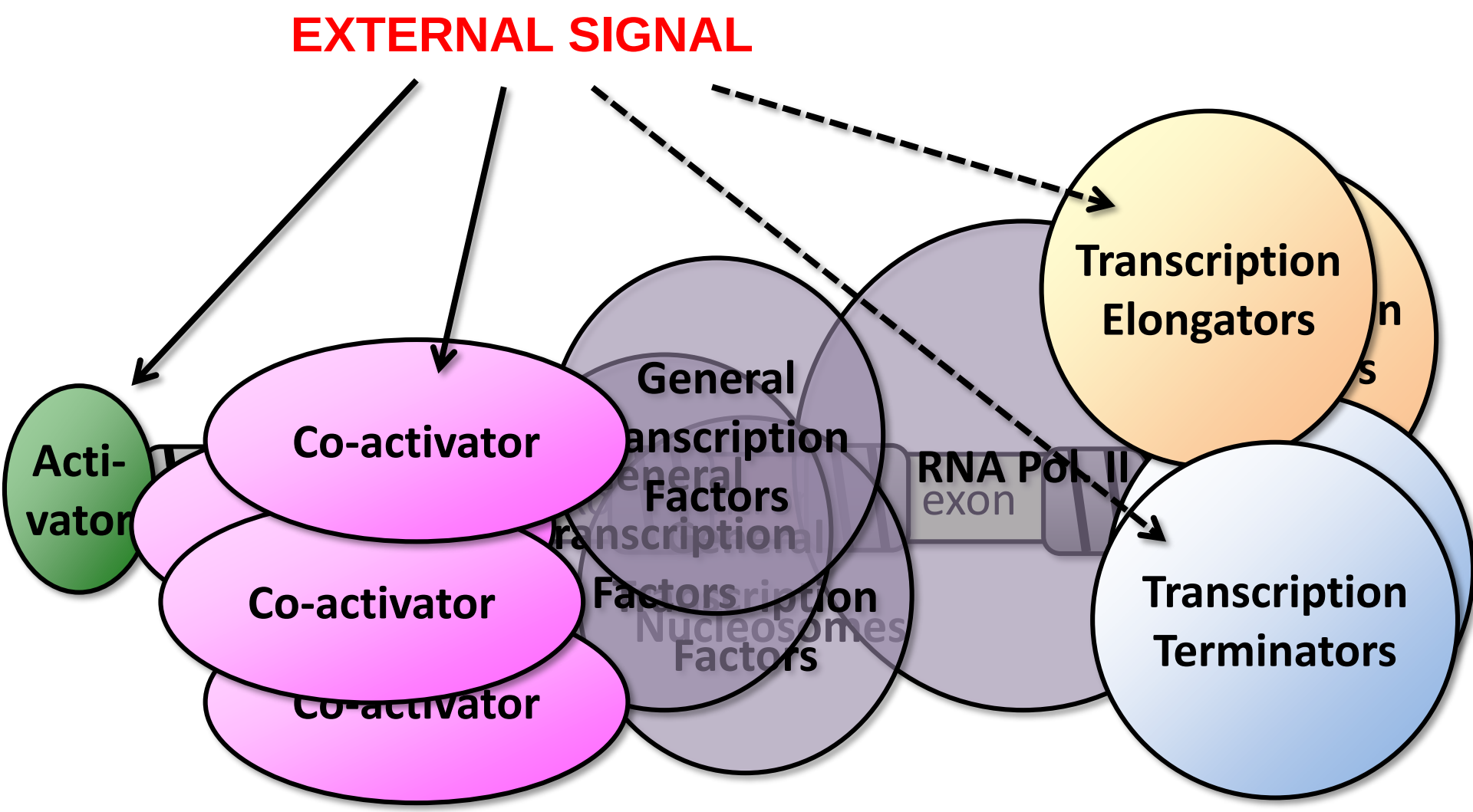
To understand the mechanisms of transcription initiation and its regulation by external factors.



Classical view

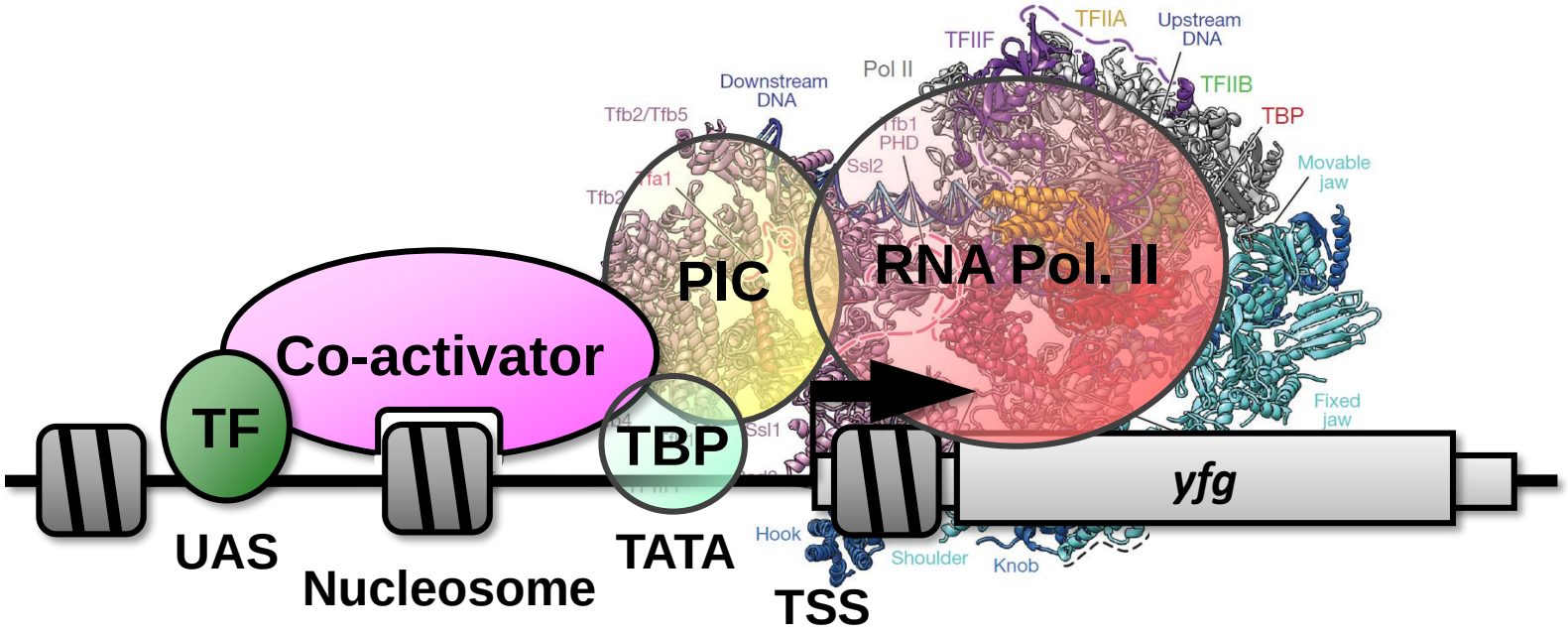


Recent discoveries



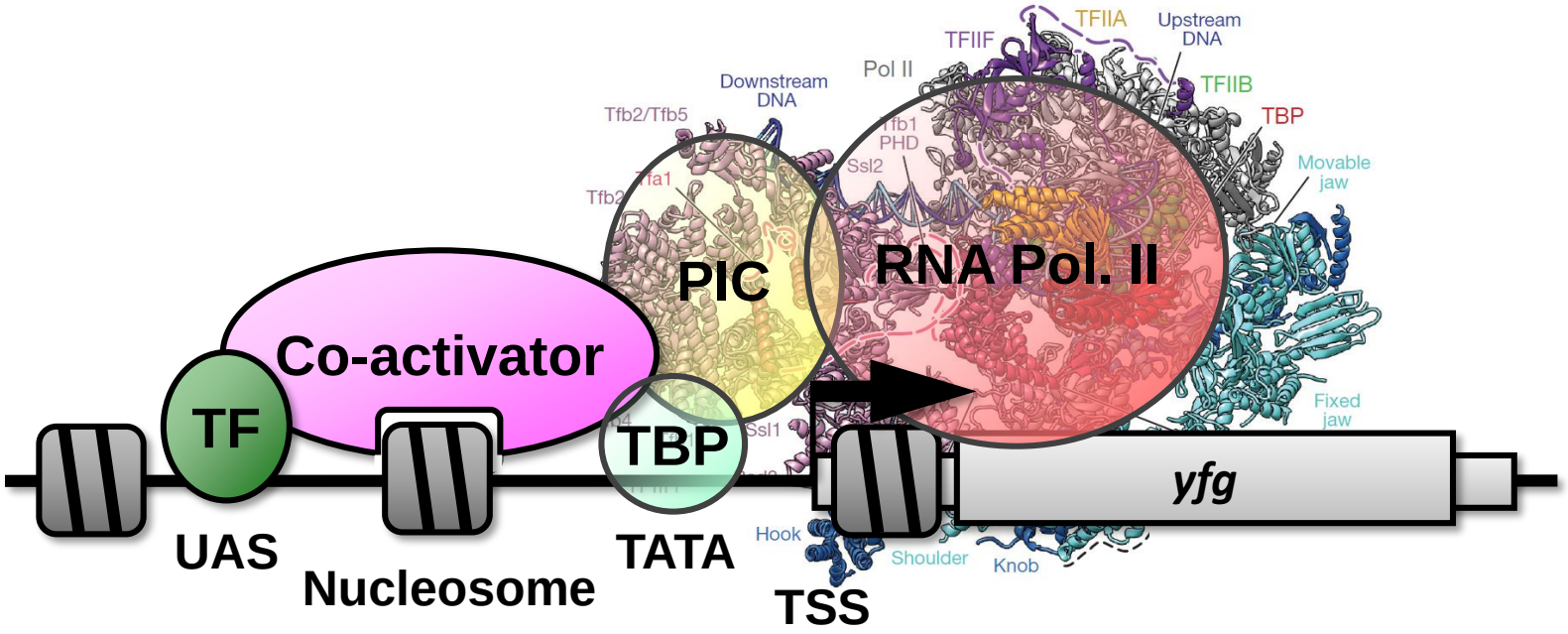
Transcriptional co-activators

- **Bridge promoter-bound activators to general transcription machinery.**



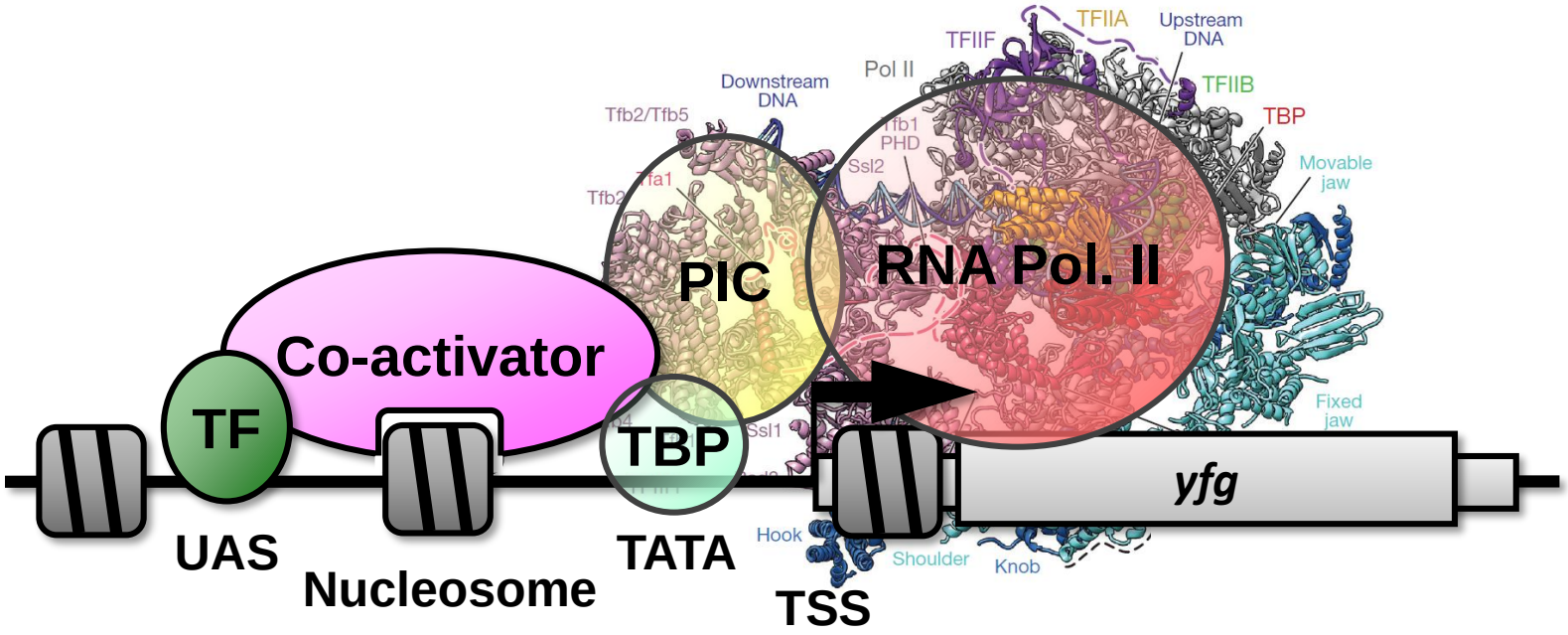
Transcriptional co-activators

- **Bridge promoter-bound activators to general transcription machinery.**
- **Chromatin-modifying and –remodeling activities.**



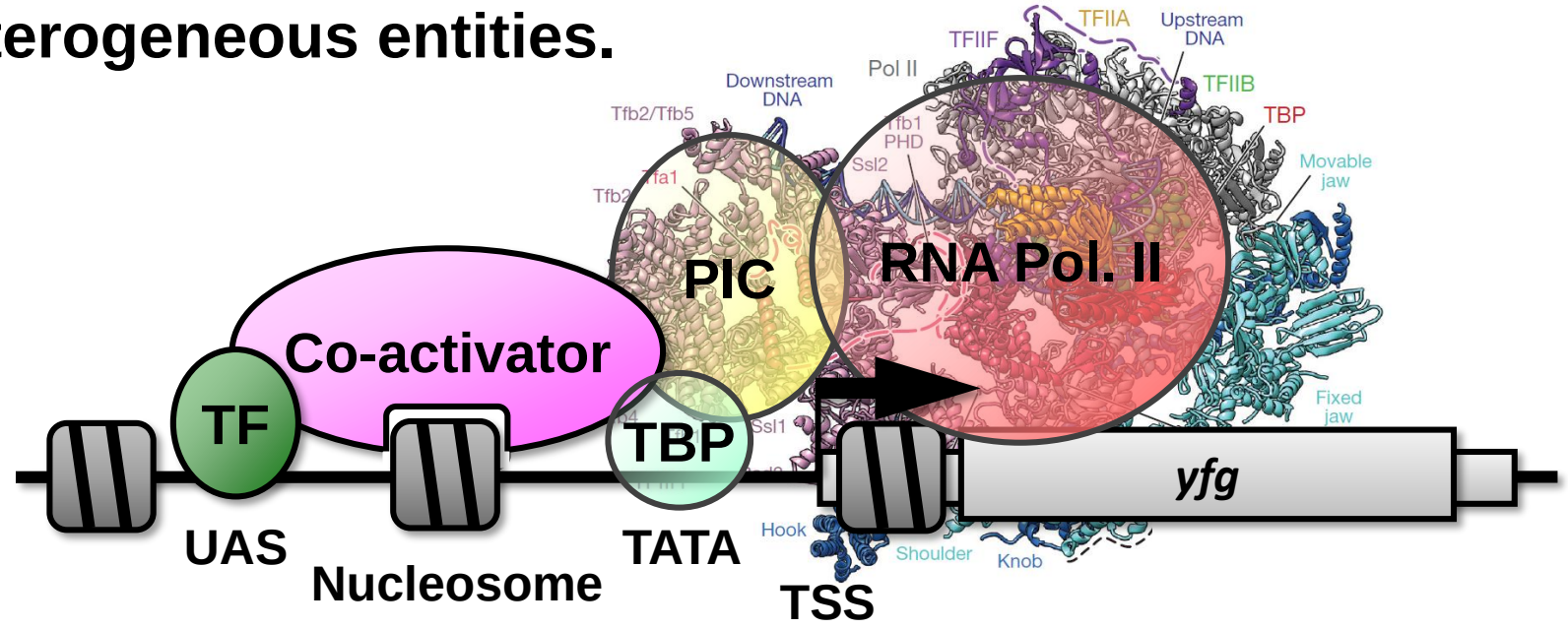
Transcriptional co-activators

- **Bridge promoter-bound activators to general transcription machinery.**
- **Chromatin-modifying and –remodeling activities.**
- **Multifunctional: modular organization.**



Transcriptional co-activators

- **Bridge promoter-bound activators to general transcription machinery.**
- **Chromatin-modifying and –remodeling activities.**
- **Multifunctional: modular organization.**
- **Heterogeneous entities.**



Open questions

1. Regulatory input from signaling cues?

➔ Conditions / Factors modulating their activities.

2. Direct target genes?

➔ Mechanisms of transcription initiation.

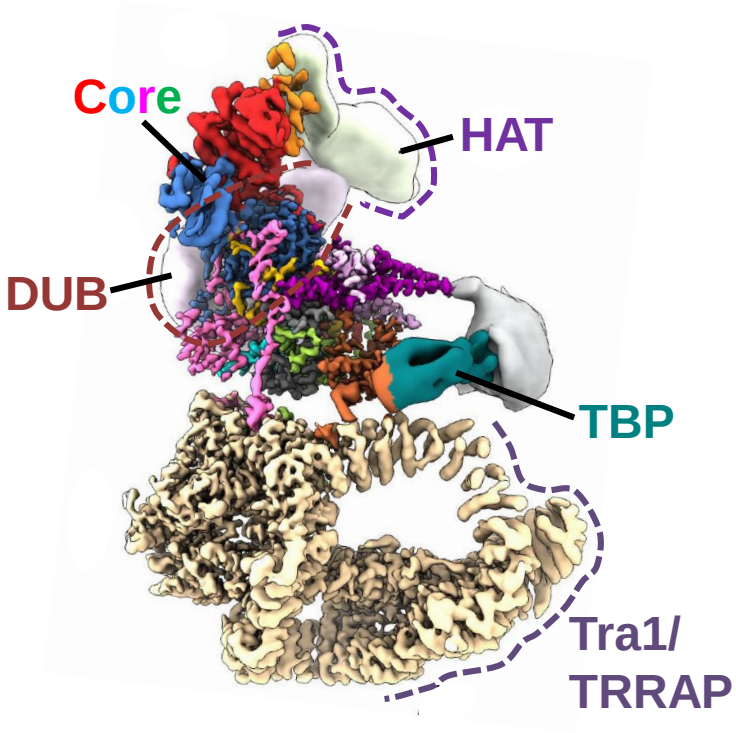
3. Principles of assembly?

➔ Functional relevance of their modularity and heterogeneity.

Which experimental systems are available to address these questions?

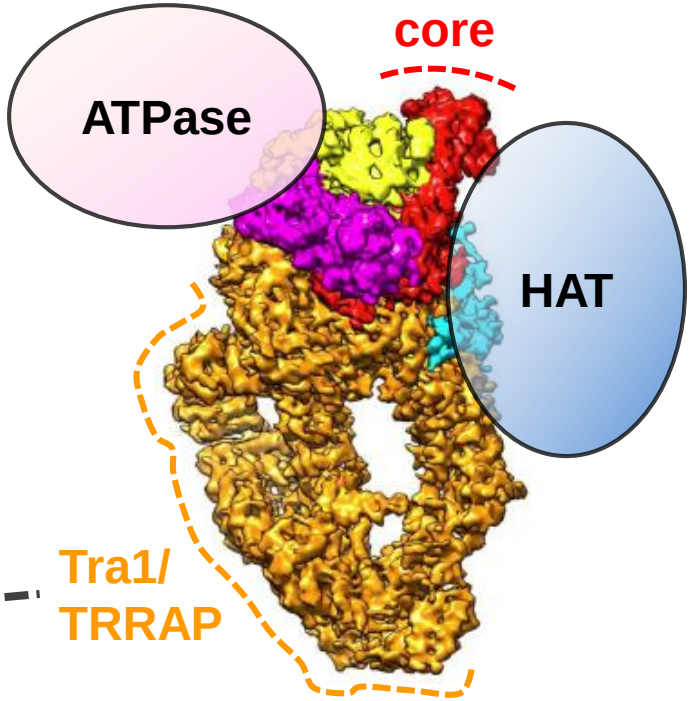
Our model co-activator complexes

SAGA



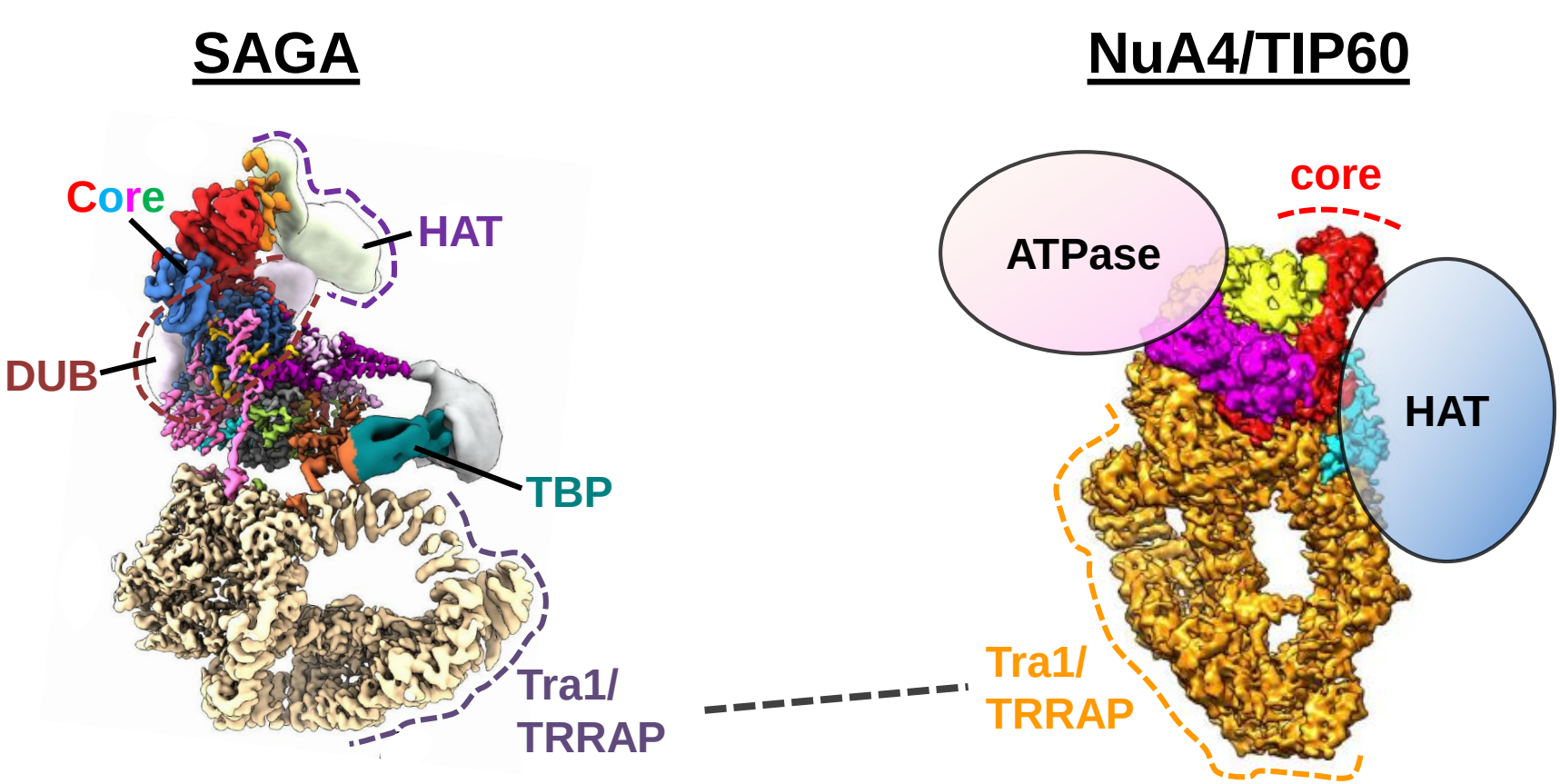
Papai et al., 2020

NuA4/TIP60



Wang et al., 2018

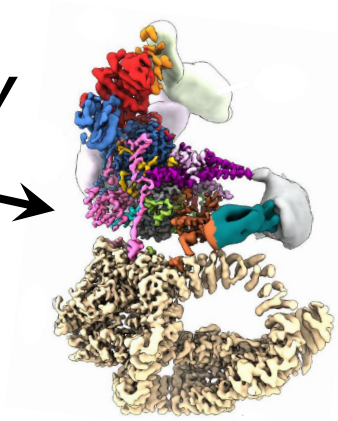
Our model co-activator complexes



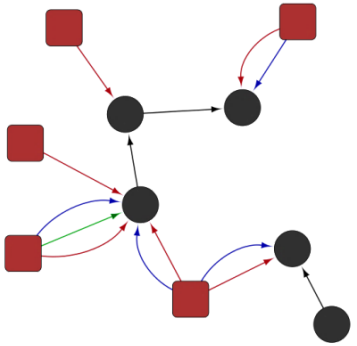
Structural organization = Functional organization

Overall goals of our research

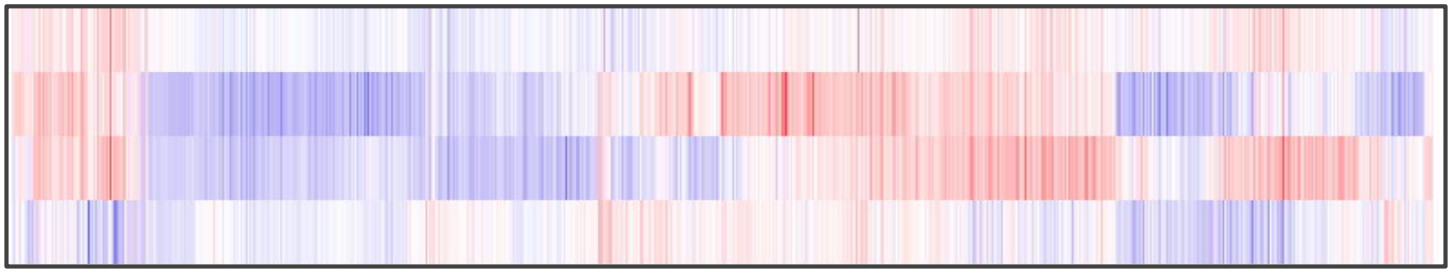
AIM 1: ***assembly***



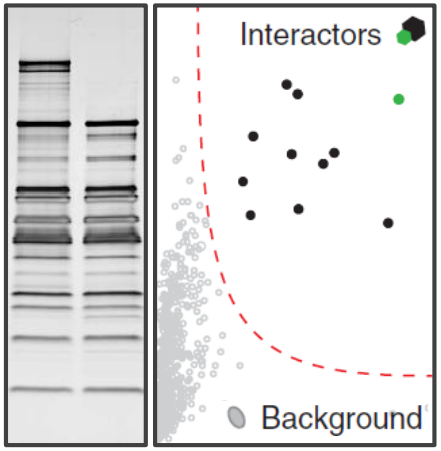
AIM 2: ***regulation***



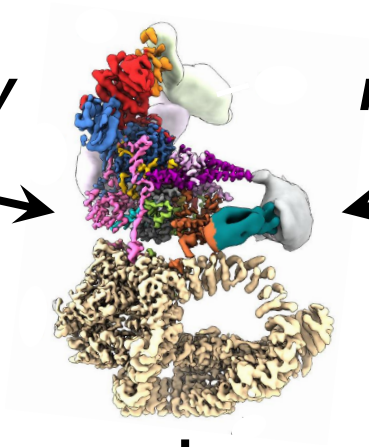
AIM 3: ***functions***



Experimental approaches

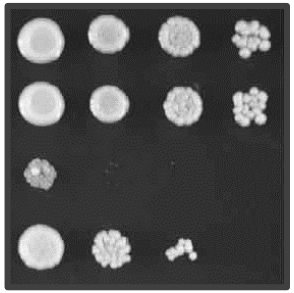


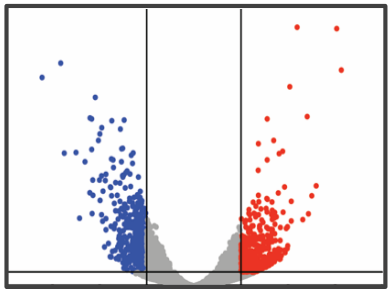
AIM 1: ***assembly***

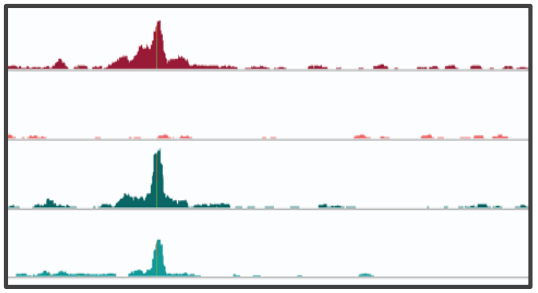


AIM 2: ***regulation***

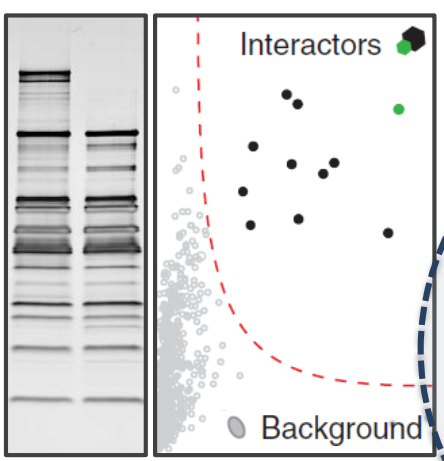
AIM 3: ***functions***



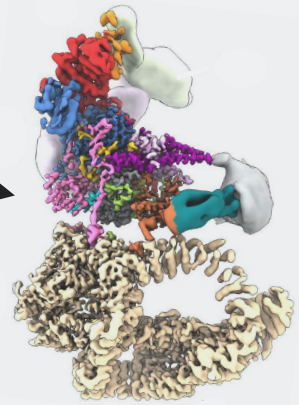




Model systems

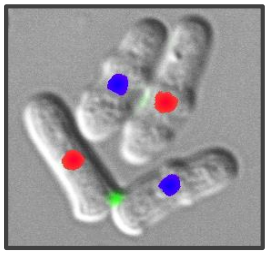


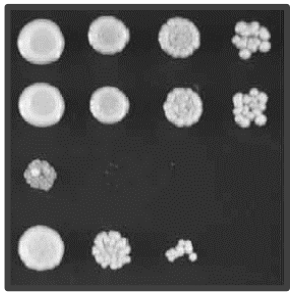
AIM 1: ***assembly***



AIM 2: ***regulation***

Yeasts





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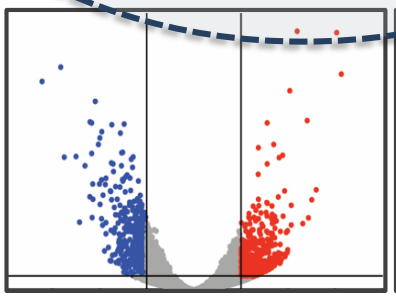
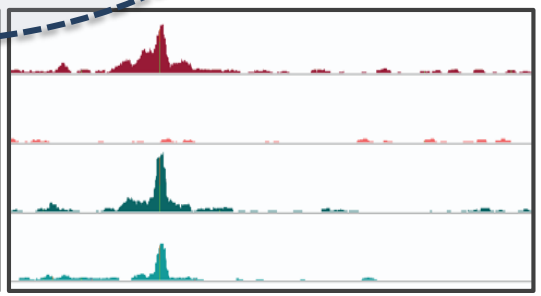
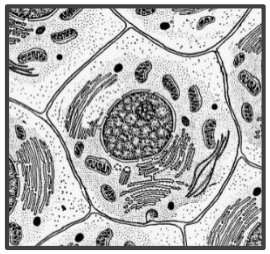
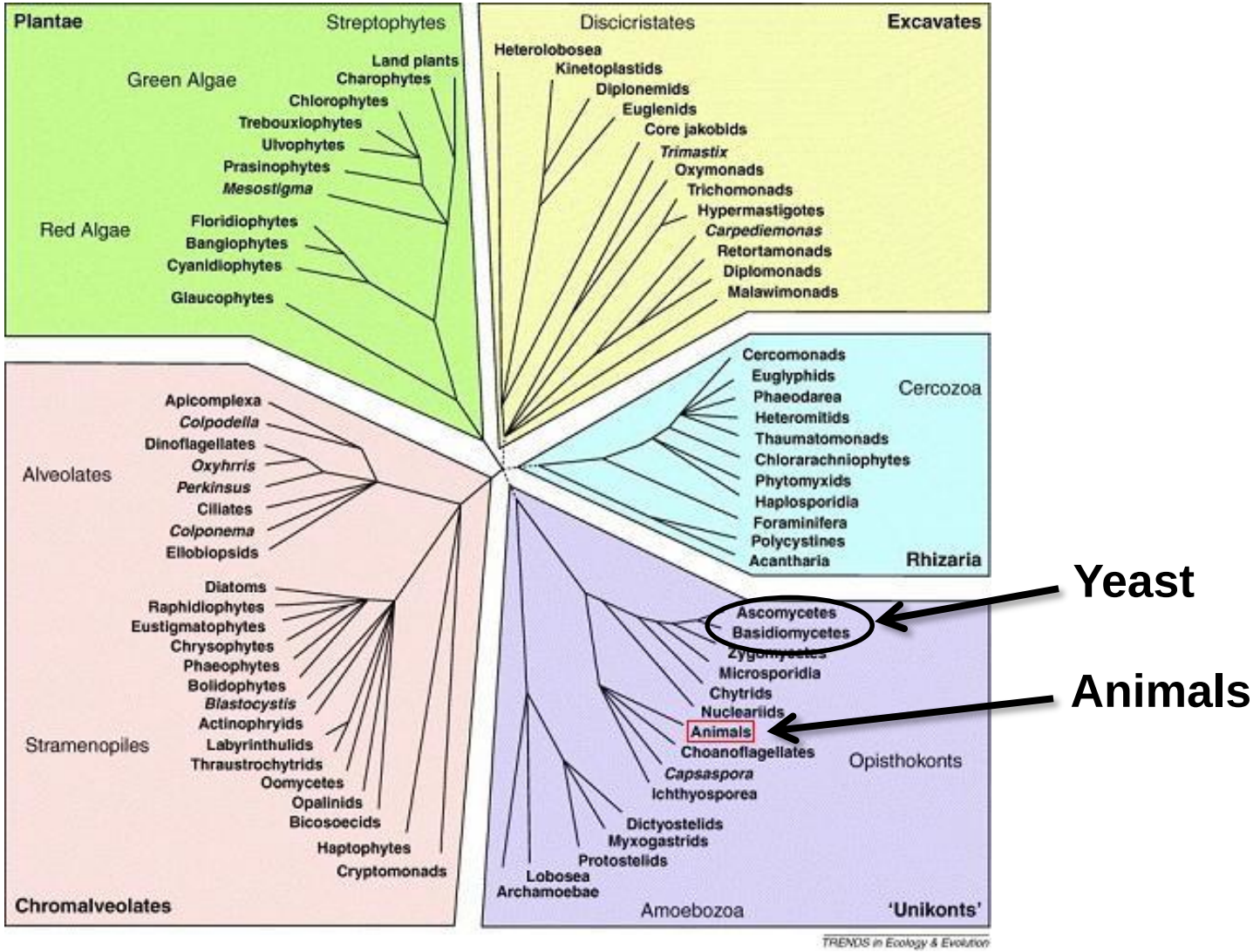




Cancer cells



The tree of all Eukaryotes

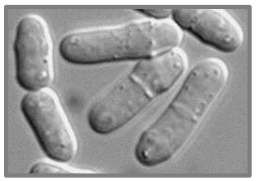


Transcription regulation in yeast

Transcription regulation in yeast

***Saccharomyces cerevisiae* = budding yeast**

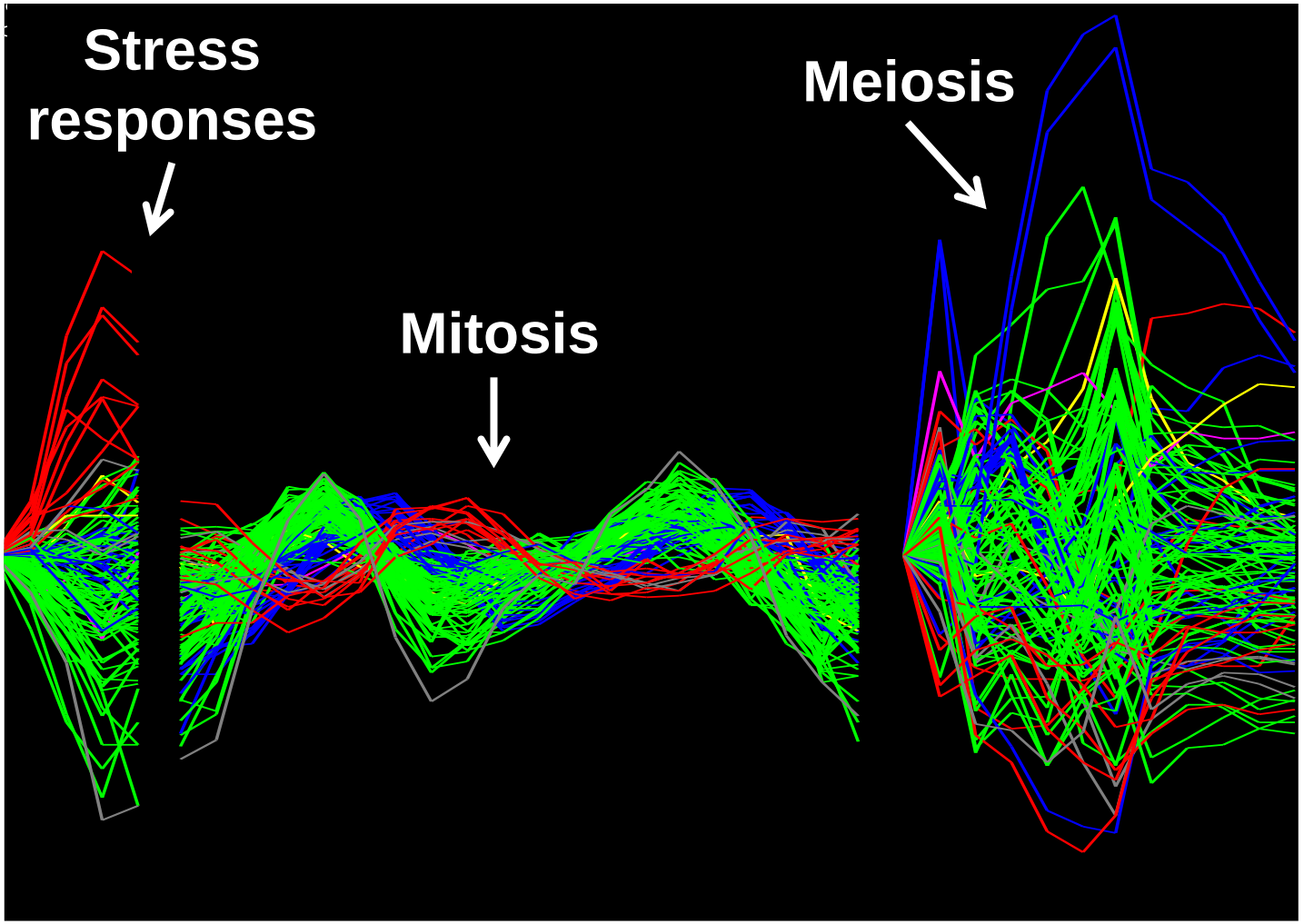
***Schizosaccharomyces pombe* = fission yeast**



Experimental advantages

- **Easy to manipulate, store, maintain.**
- **Unlimited biochemistry, genetic approaches.**
- **Genomes very well annotated.**
- **Fast and easy classical and system wide genetics.**
- **Knock-out, fluorescent tag, purification tag collections.**
- **Best biological characterization of an eukaryotic organism (epigenome, transcriptome, proteome, interactome, metabolome, phenome).**
- **Novel techniques first developed in yeast**

Gene regulation in yeast



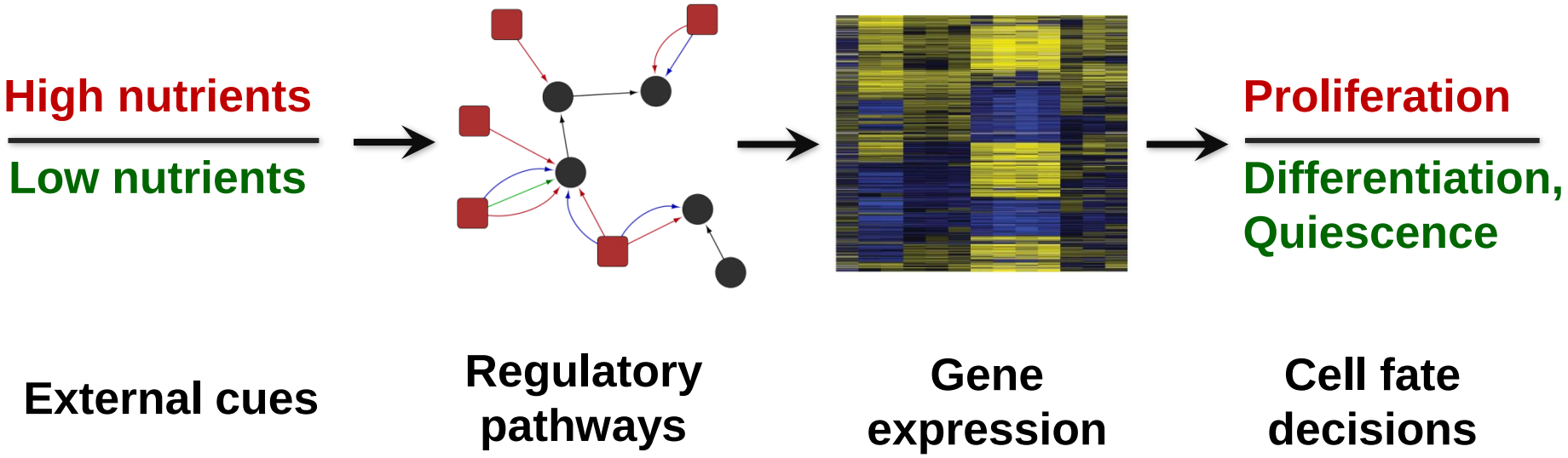
Cell fate determination in yeast

- **Mating-type switching**
- **Proliferation *vs* quiescence *vs* sexual differentiation = conjugation ➔ meiosis ➔ sporulation**
- **Dimorphic switch = yeast-to-hyphae, controls virulence in many pathogens, eg. *C. albicans***

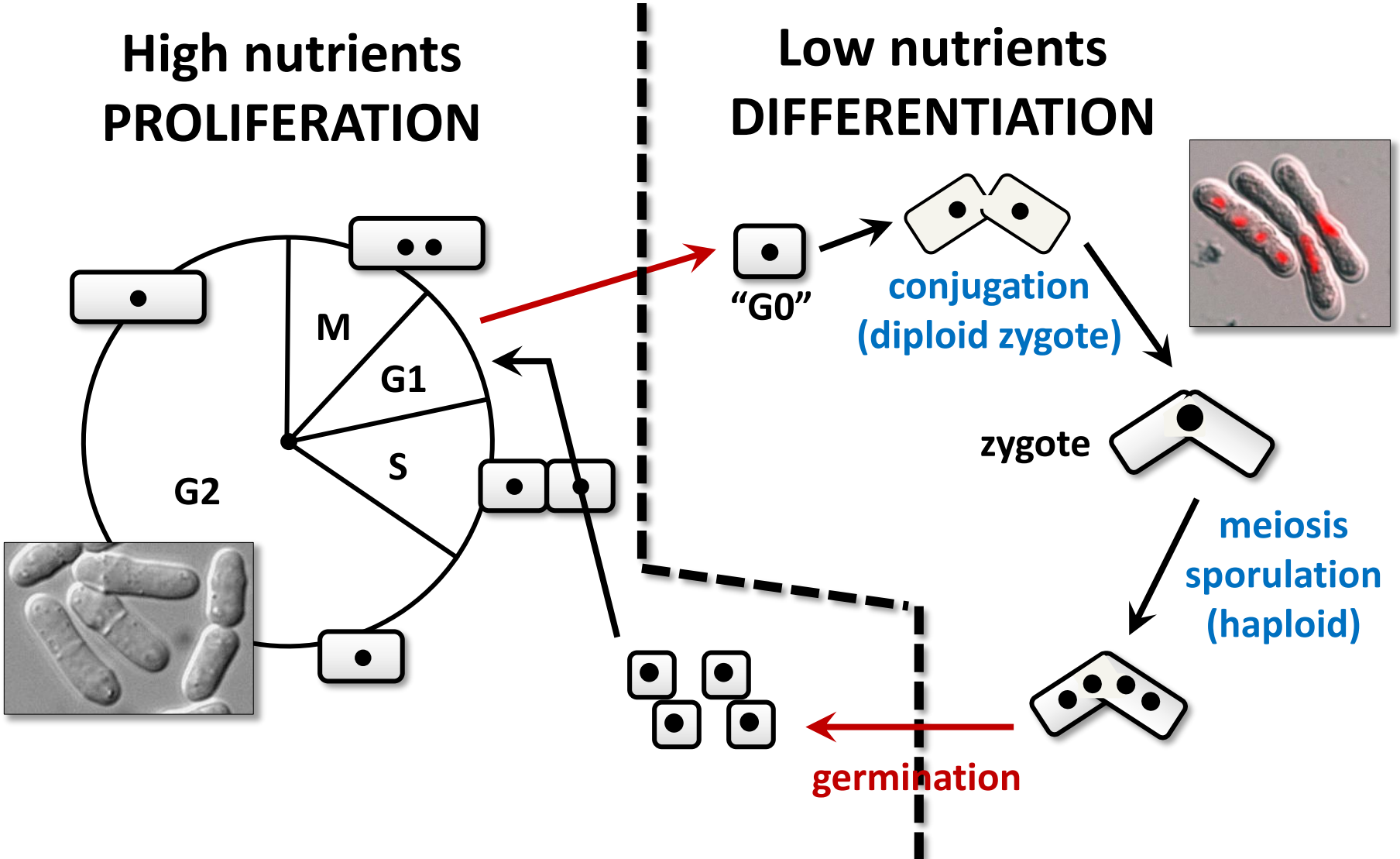
These events are controlled by external cues, from the yeast's environment, typically nutrient quality.

One project in my lab

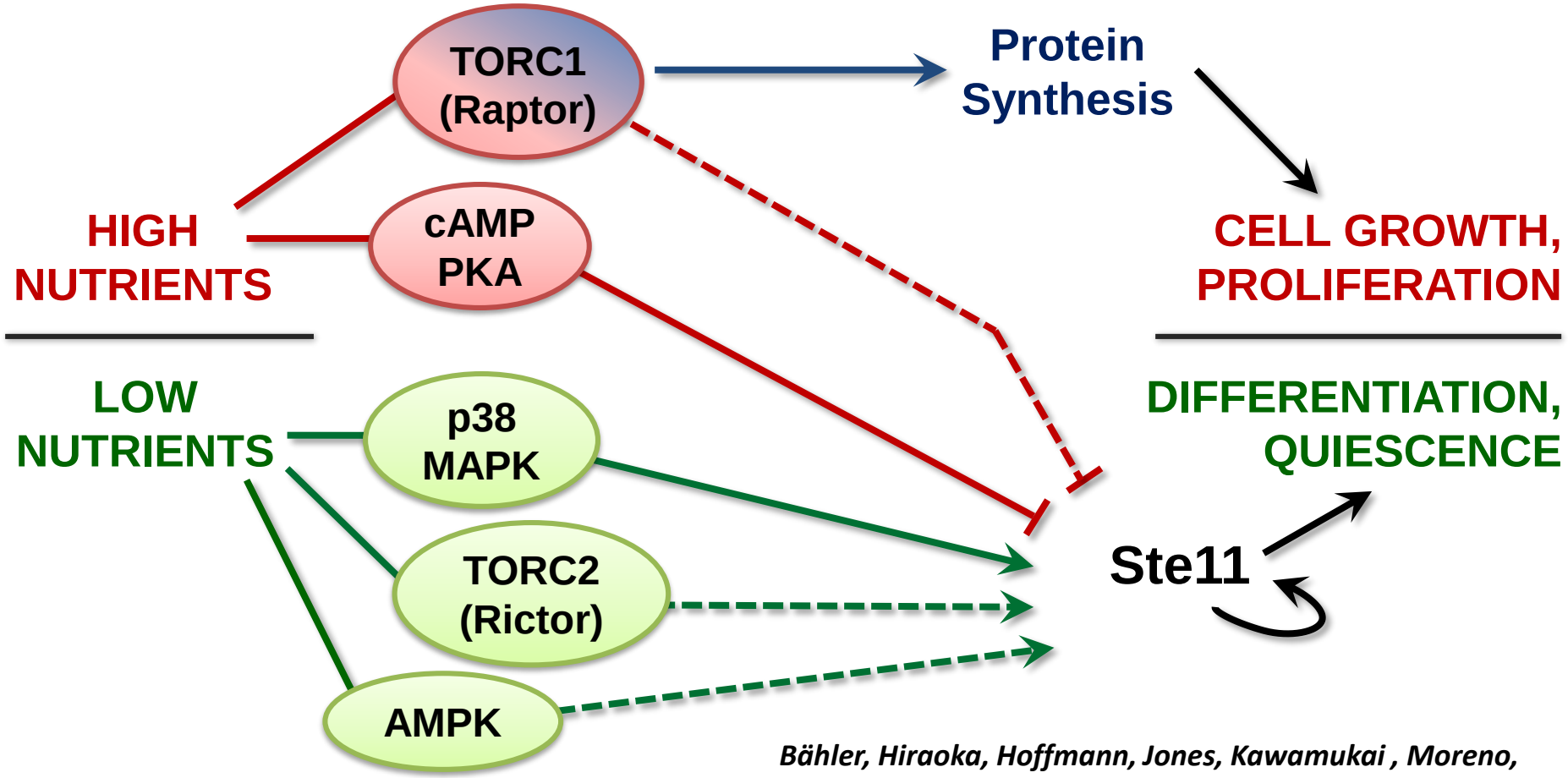
To understand how cells sense nutrient availability to coordinately regulate gene expression and control cell fate.



The life cycle of *S. pombe*



Regulators of the switch proliferation *vs*. differentiation



Bähler, Hiraoka, Hoffmann, Jones, Kawamukai , Moreno, Murakami, Nielsen, Okayama, Petersen, Russell, Toda, Uritani, Weisman, Yamamoto, Yanagida labs

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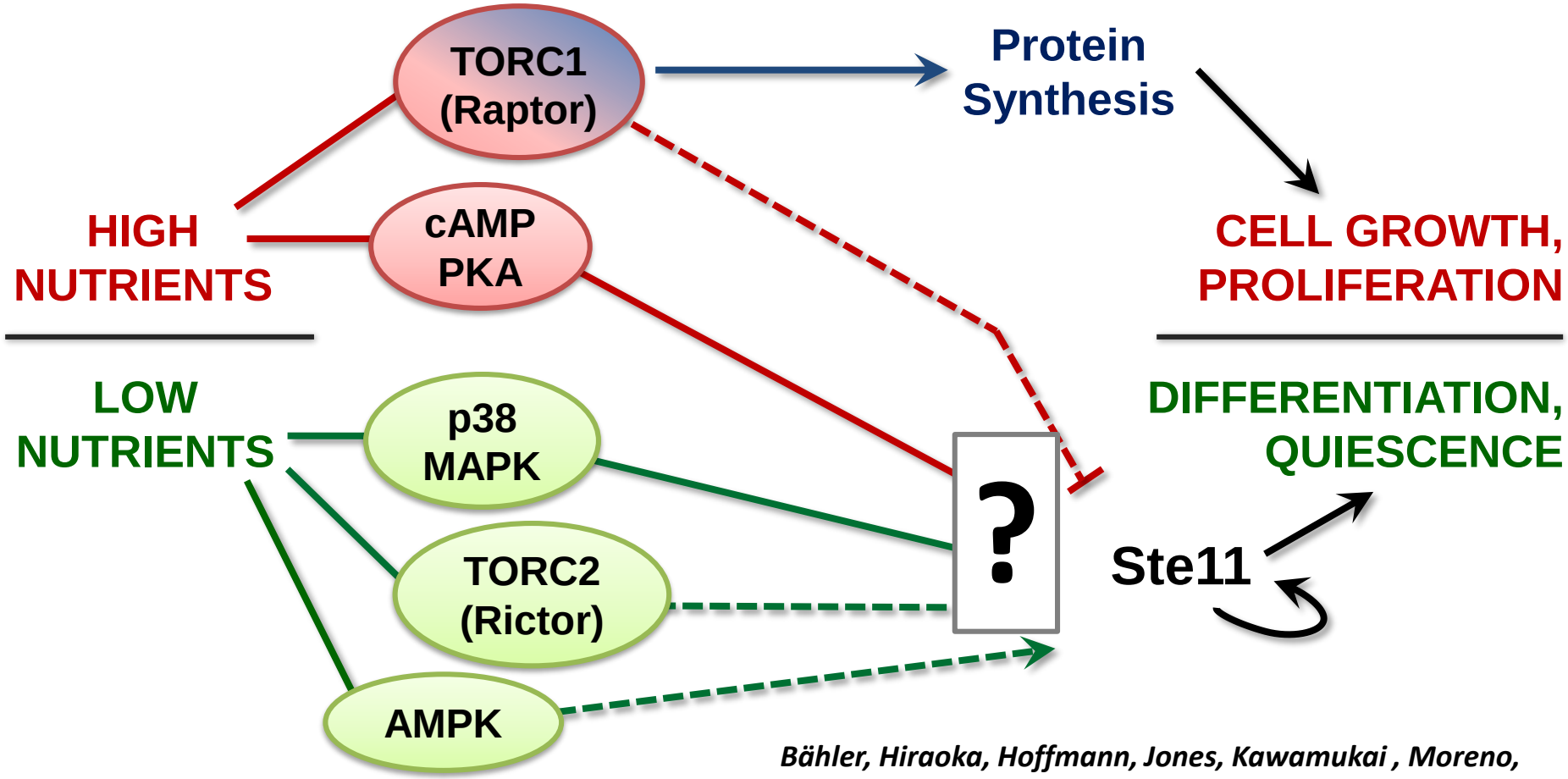

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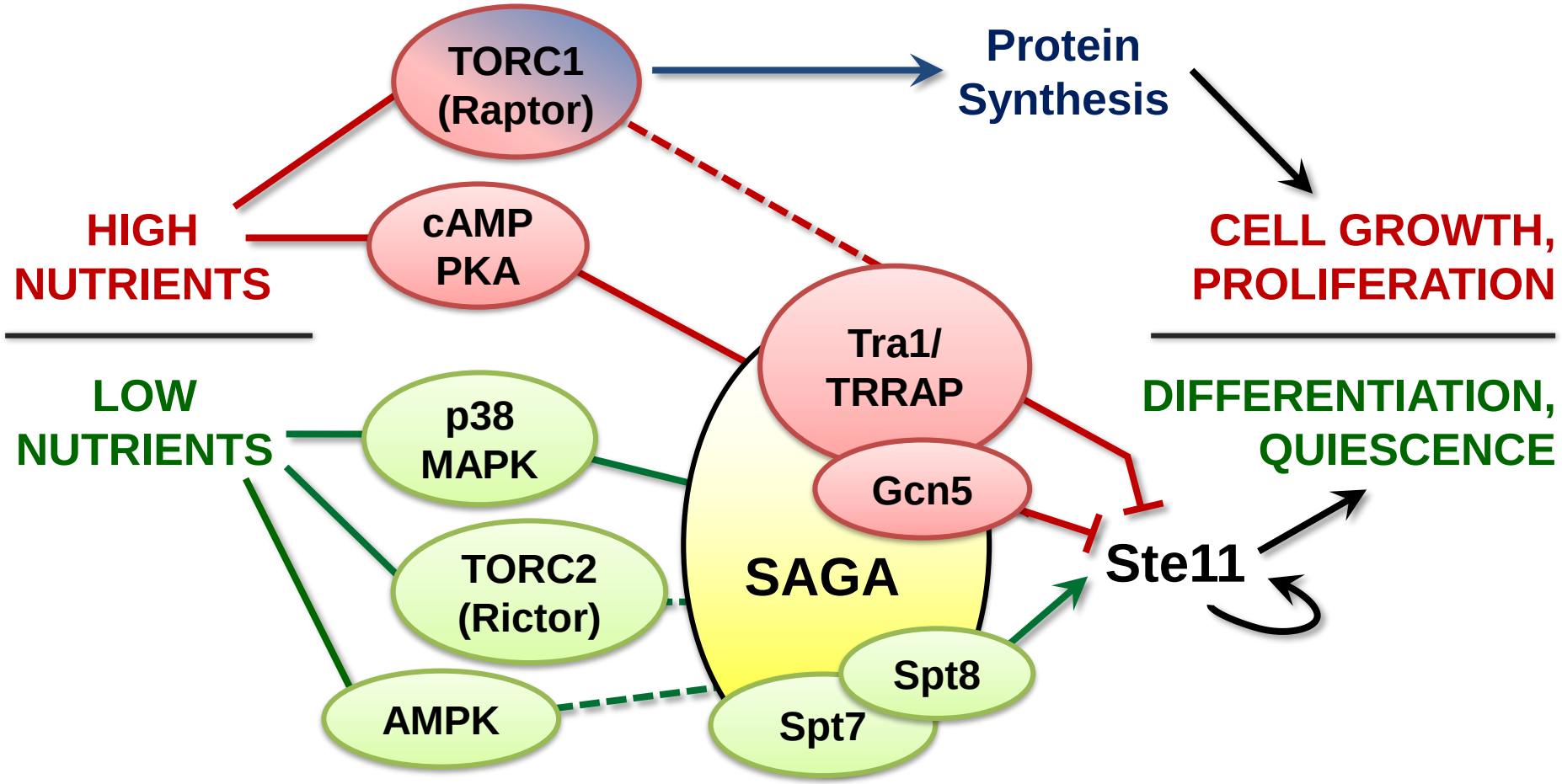
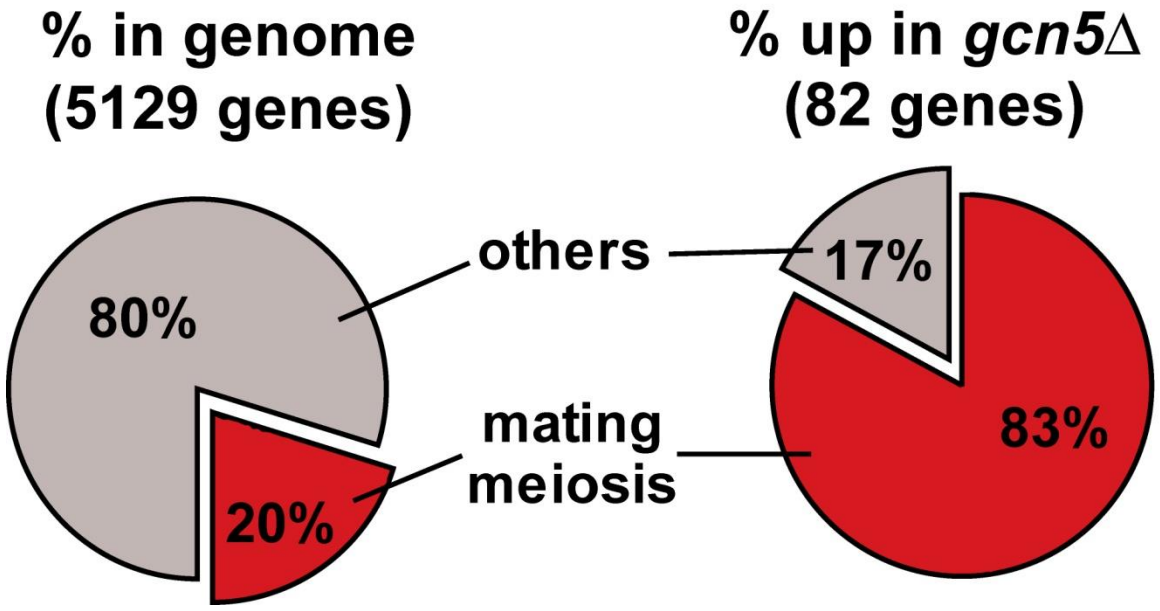


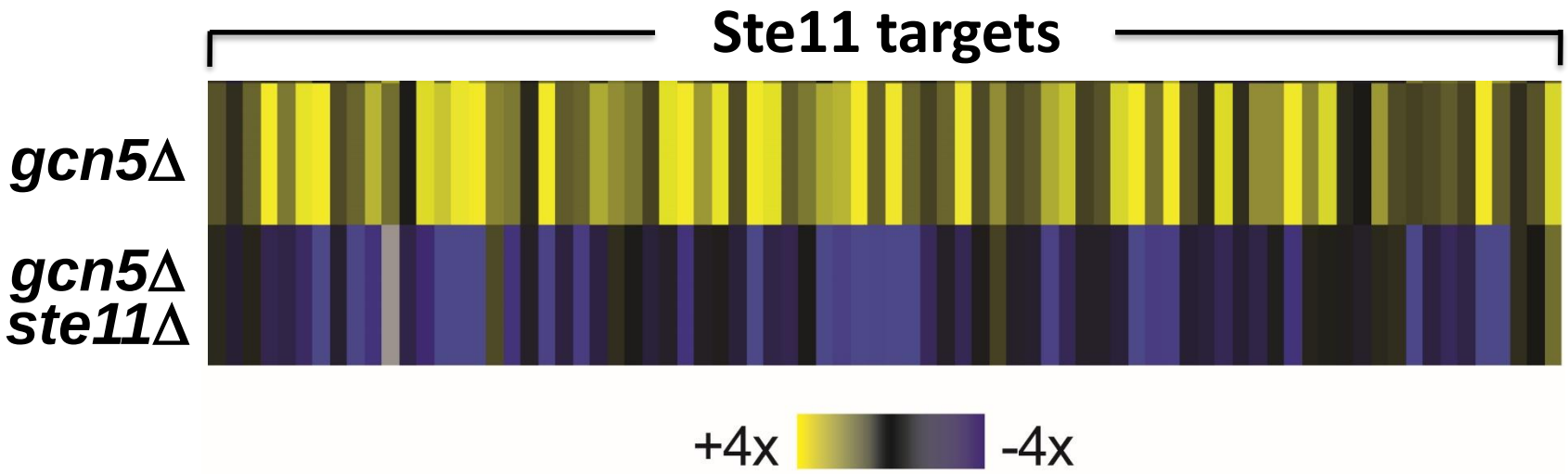
Transcriptome profile of *gcn5*Δ

The transcriptome of *gcn5*Δ mutants = that of cells undergoing differentiation (nutrient starved).

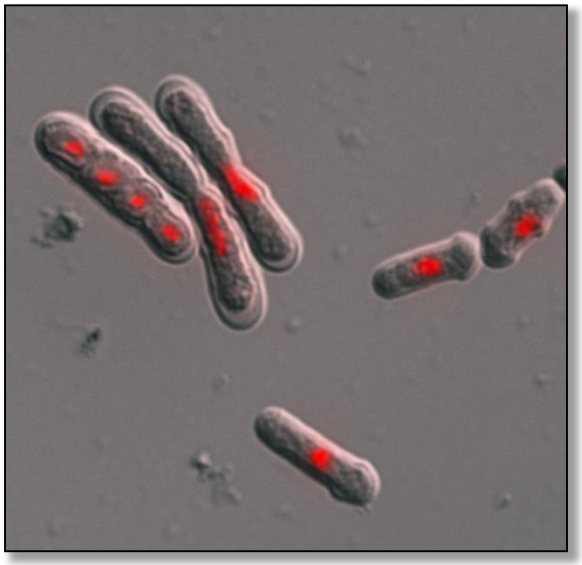


$p = 3.6 \times 10^{-37}$

De-repression of Ste11 target genes



‘Constitutive’ differentiation phenotype

	h^{90} *gcn5*$^{+}$	h^{90} *gcn5*Δ
DAPI + DIC		
% mating	0%	24%

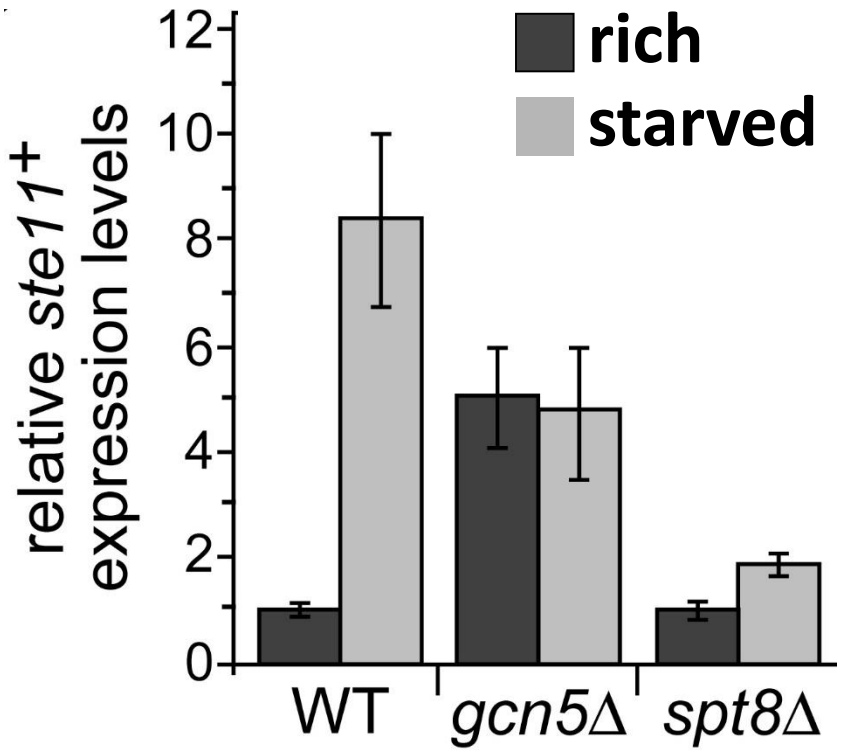
Gcn5 HAT activity is required of the repression of differentiation in high nutrient conditions

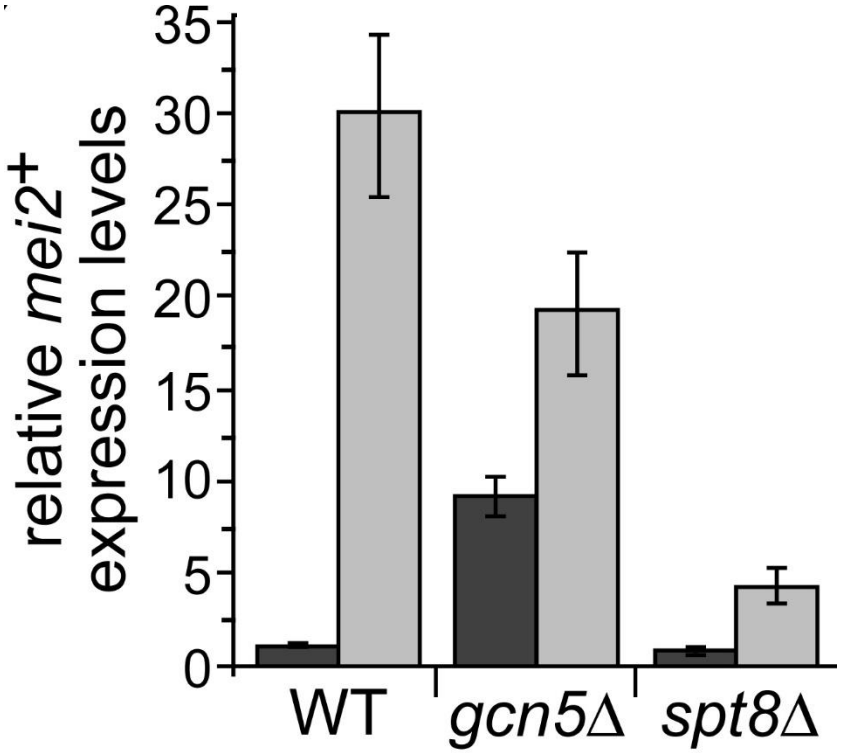
Differentiation phenotype of other SAGA mutants

	Time upon starvation (hrs)			
genotype	0	4	8	24
wild-type	0	4.6	18	37
gcn5Δ	16	23	28	59
spt8Δ	0	0	0	0
ste11Δ	0	0	0	0

Opposing regulatory roles of SAGA subunits in differentiation

Expression of differentiation genes in *spt8Δ* mutants



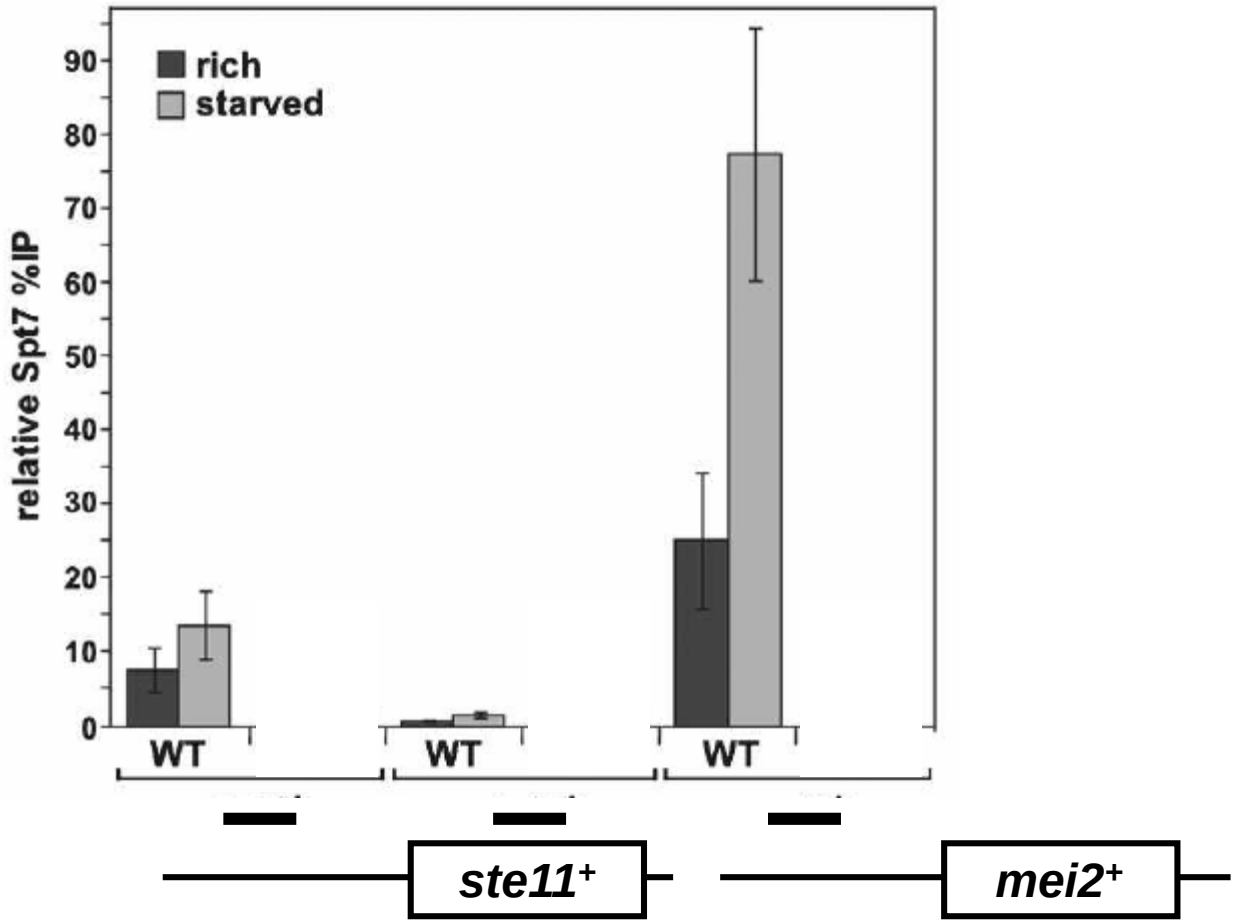


Spt8 is required for the induction of expression of differentiation genes upon nutrient starvation

Are these roles direct?

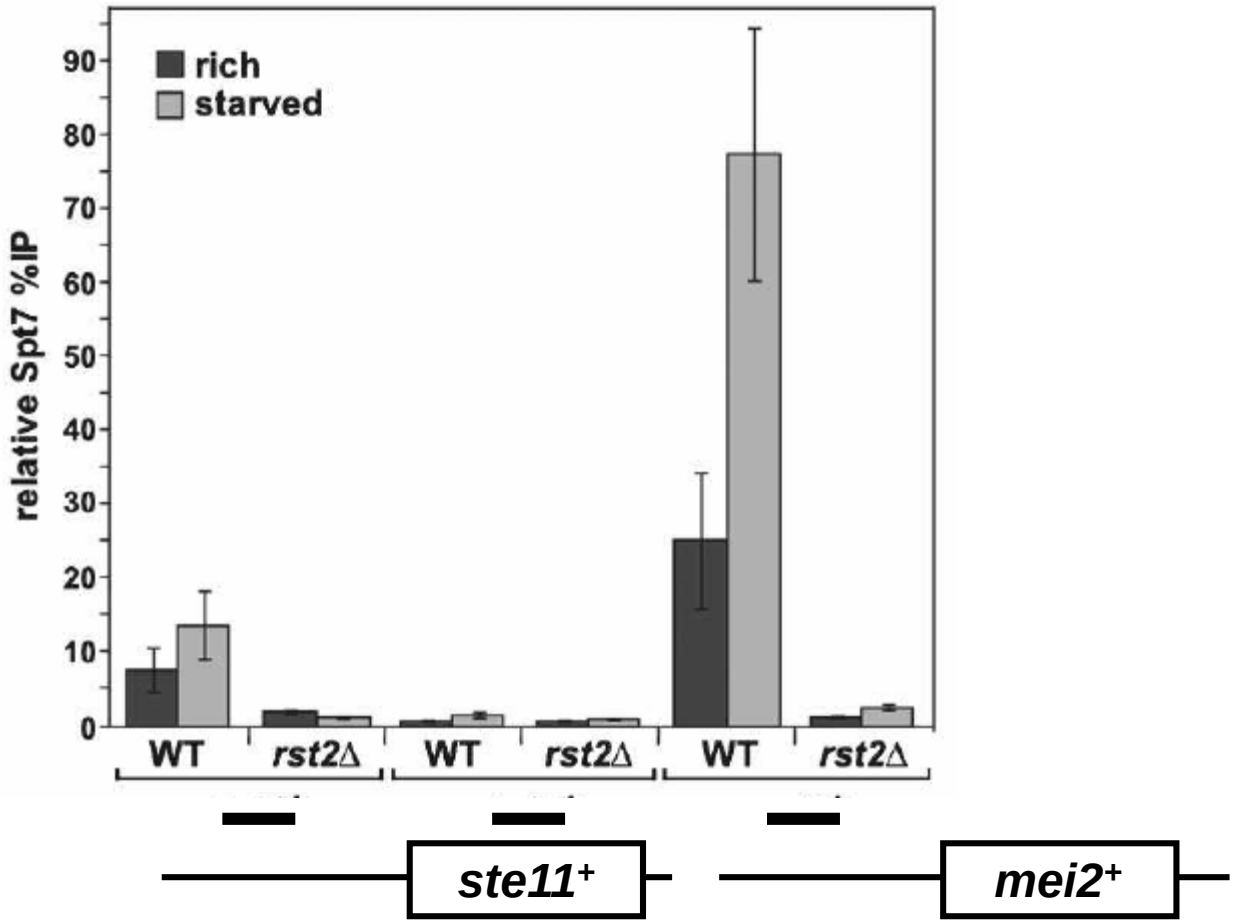
Chromatin Immunoprecipitation (ChIP): is SAGA bound to promoters of differentiation genes?

Are these roles direct?



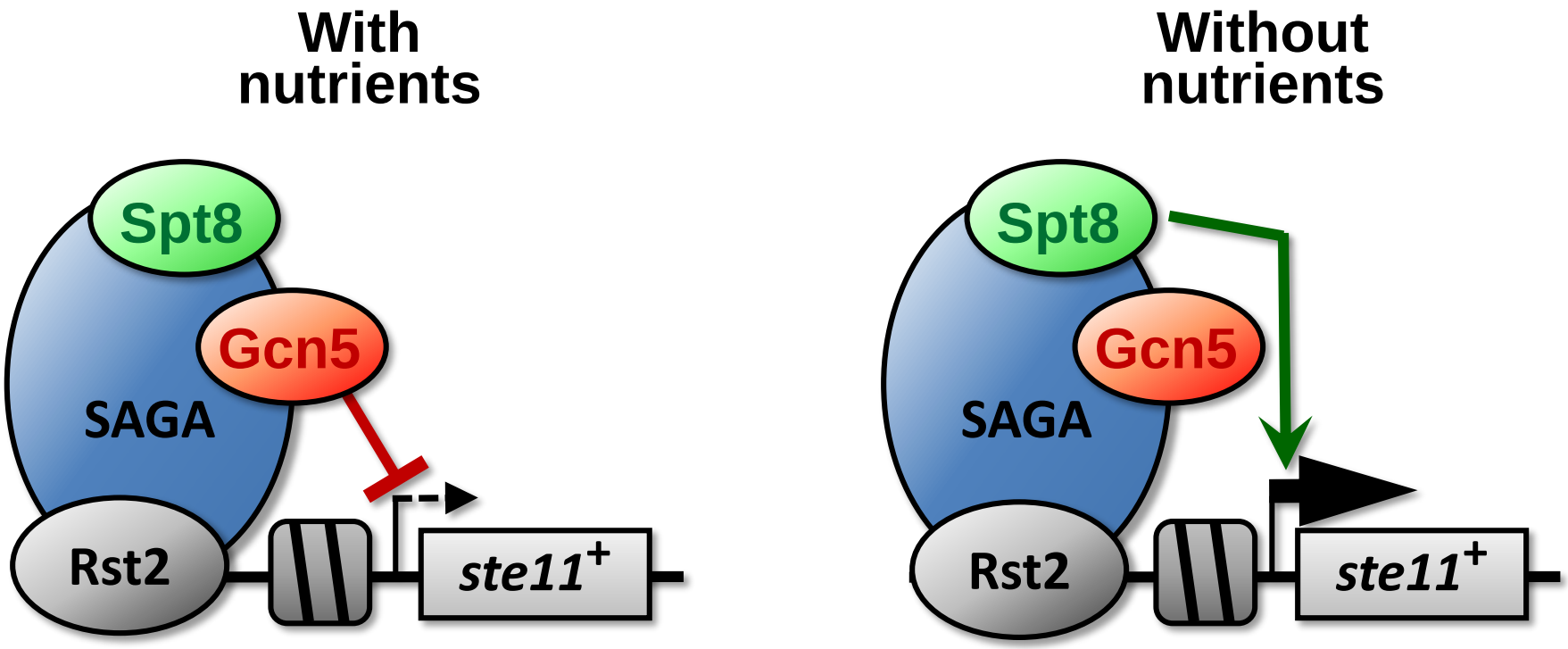
SAGA binds to promoters of differentiation genes, irrespective of nutrient levels

SAGA recruitment



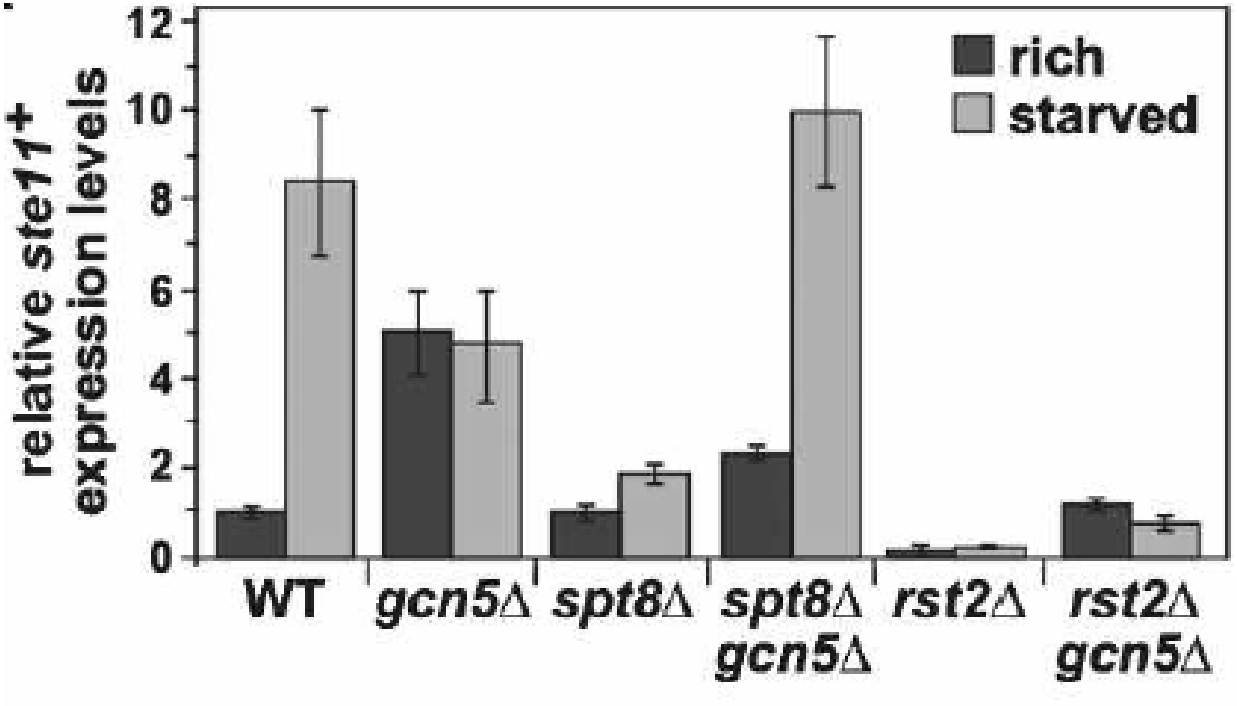
SAGA is recruited by a TF to promoters of differentiation genes, irrespective of nutrients

Thus, so far:



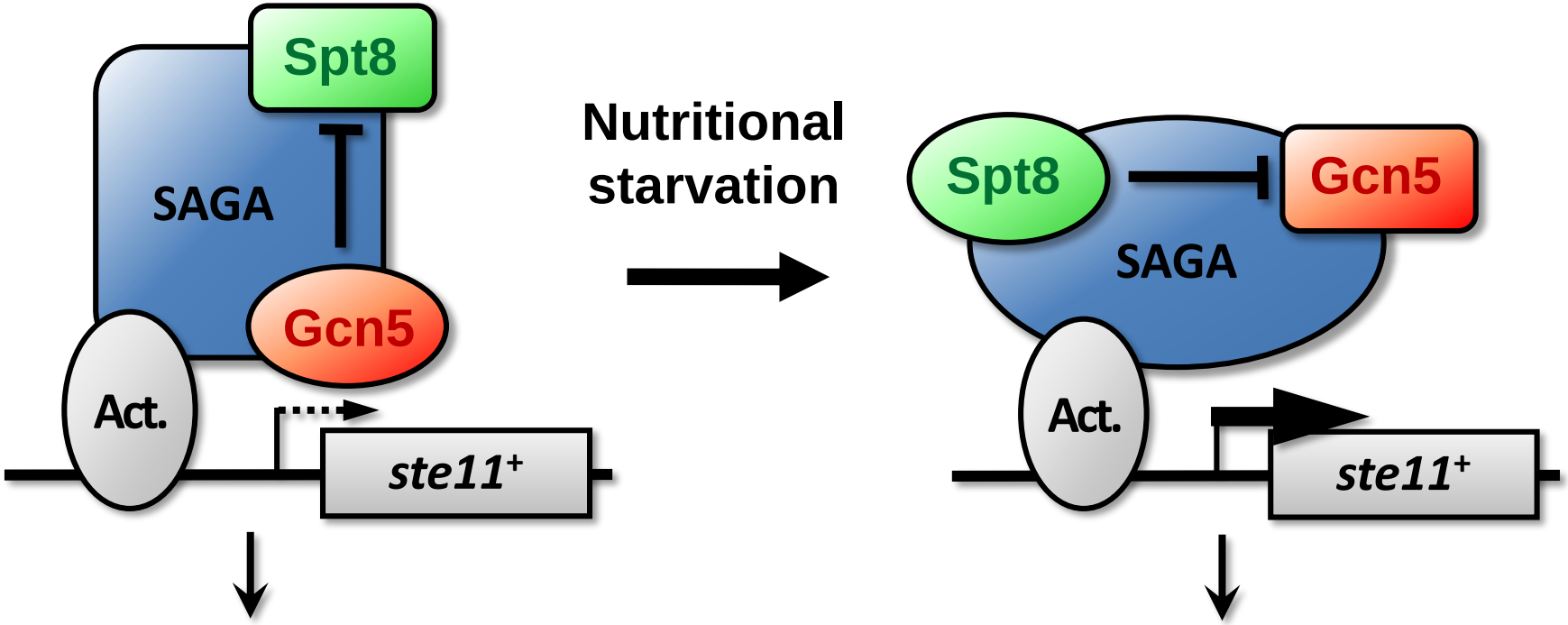
WHAT'S GOING ON?

Which factors act directly? Epistasis analysis (double mutants)



+ nutriments: *spt8Δ* suppress *gcn5Δ*
- nutriments: *gcn5Δ* suppress *spt8Δ*

Working model



Conclusions so far

- **SAGA directly regulates differentiation genes and the switch between proliferation and differentiation.**
- **SAGA switches from a repressor to an activator, depending on nutrients.**

Conclusions so far

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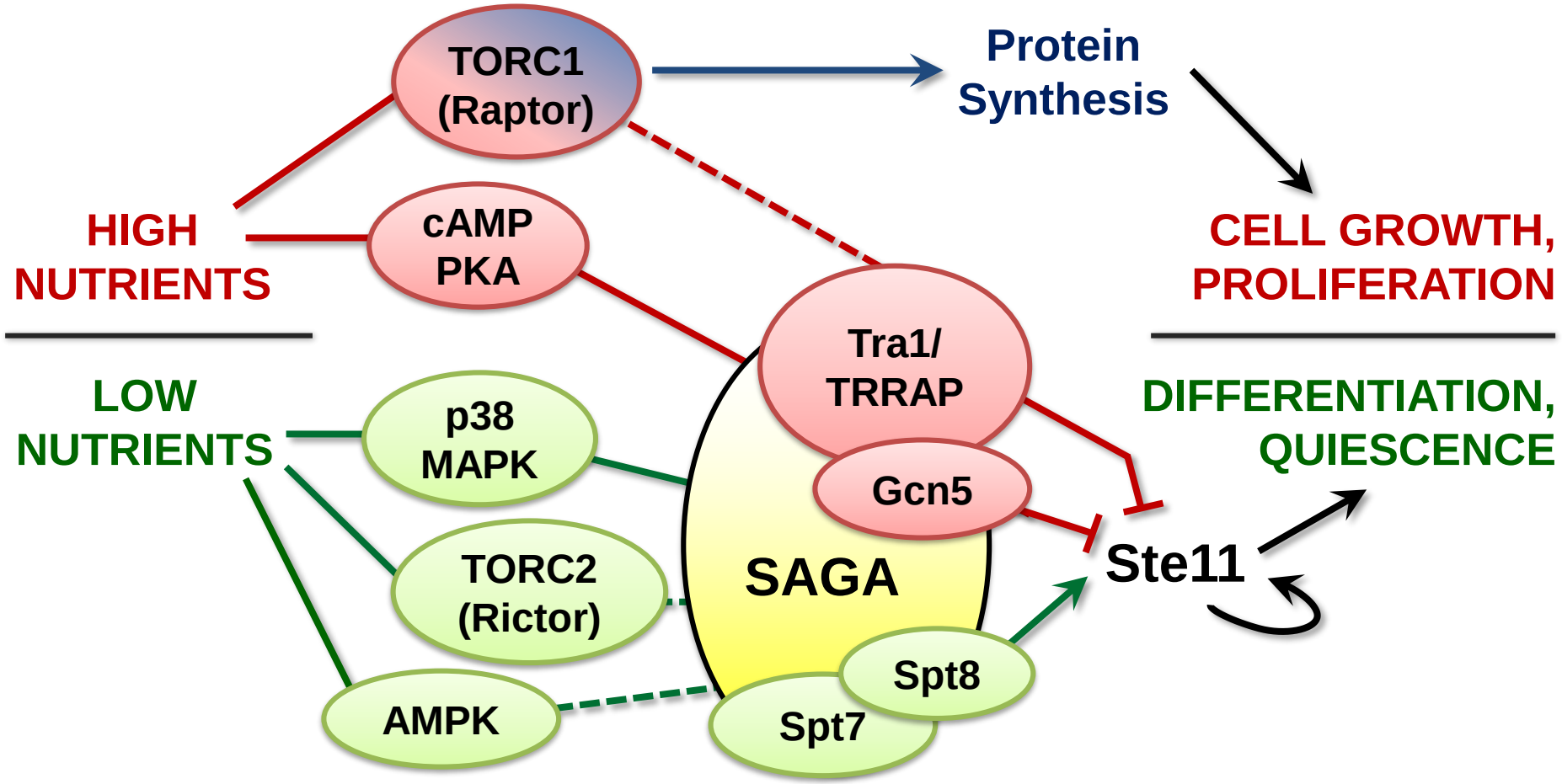
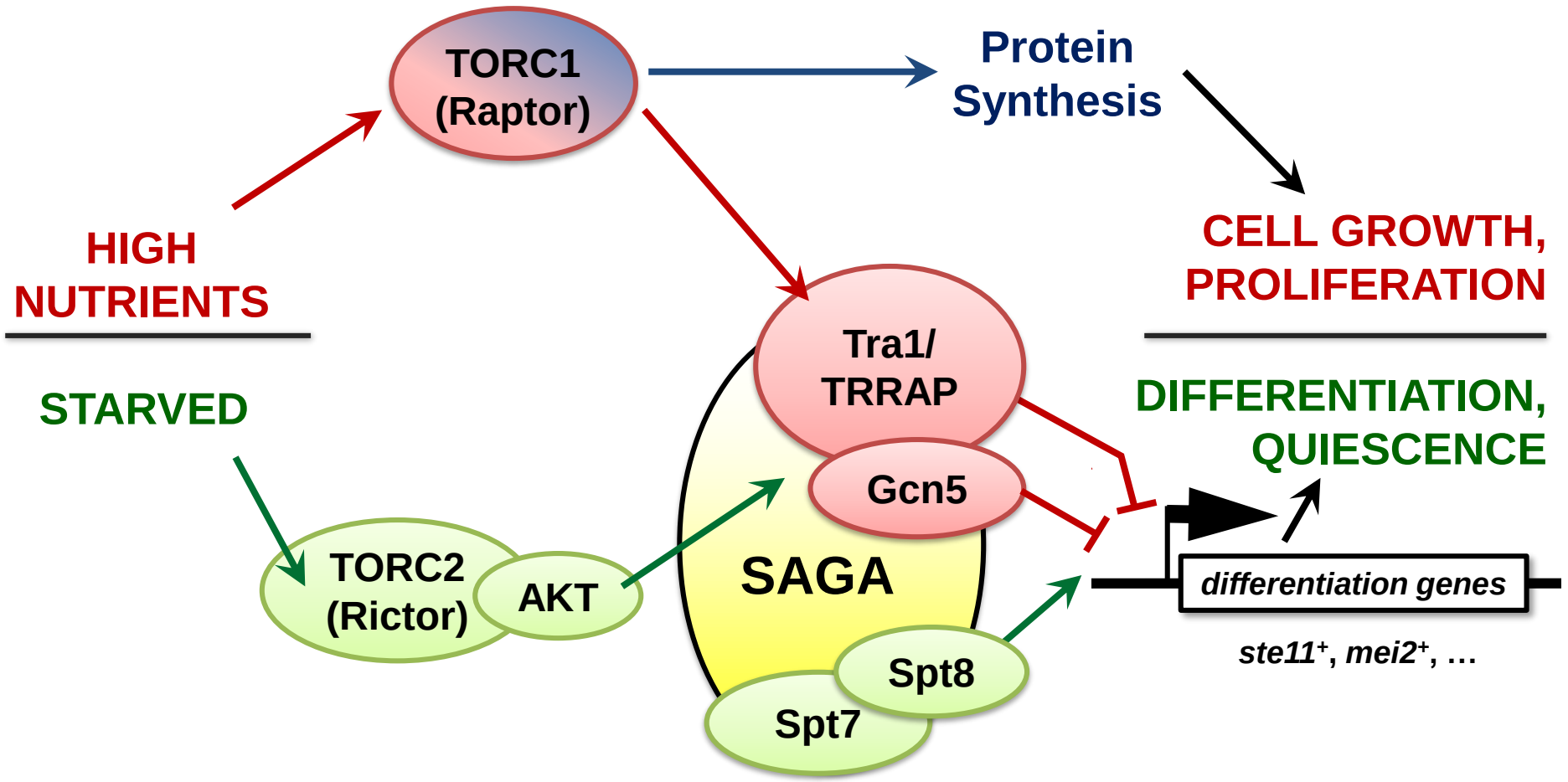
- **SAGA switches from a repressor to an activator, depending on nutrients.**

How does SAGA sense nutrient availability to switch from a repressor to an activator of transcription?

Which regulatory pathway(s) regulate(s) SAGA?

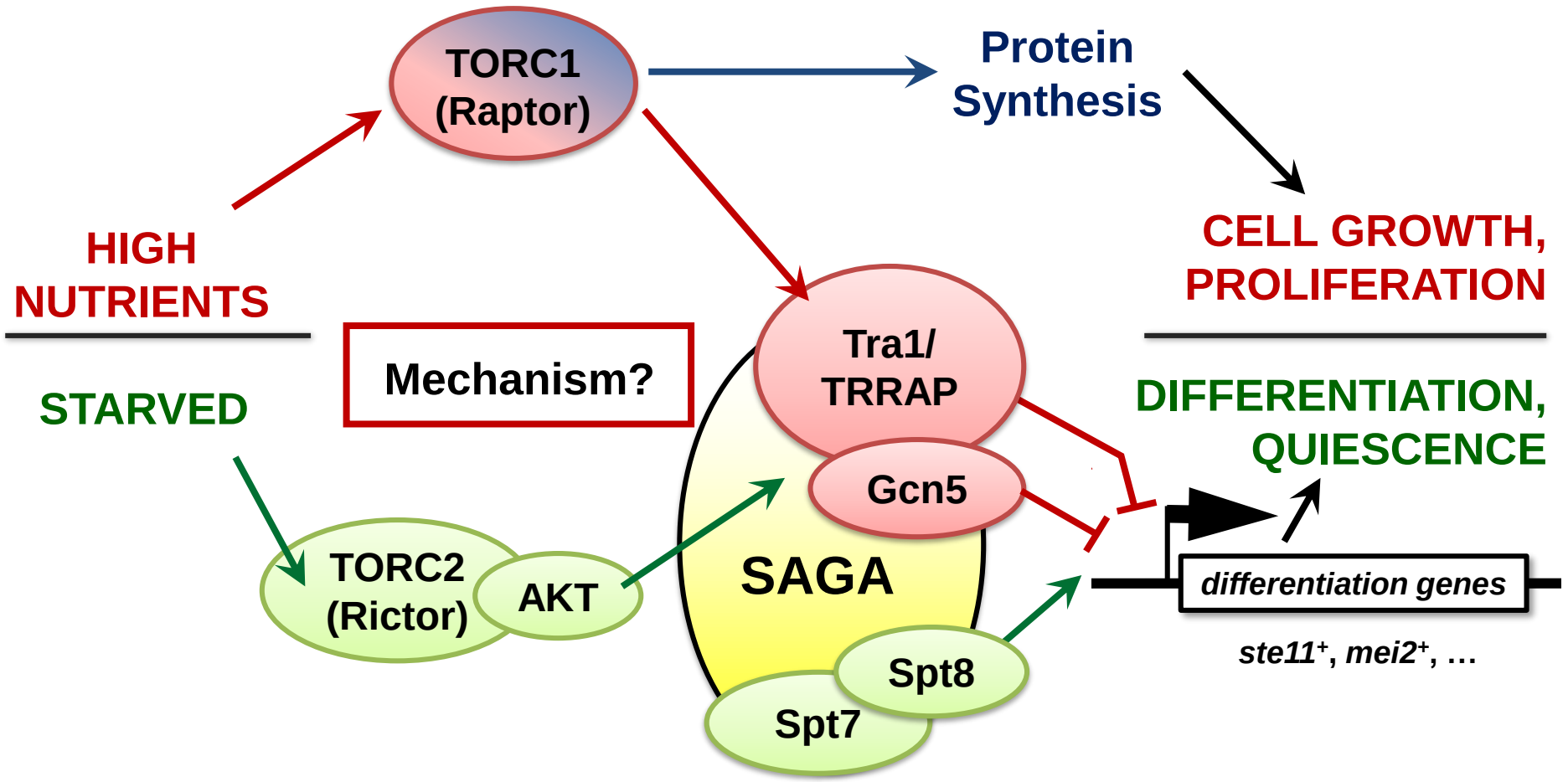


Genetic interaction analyses



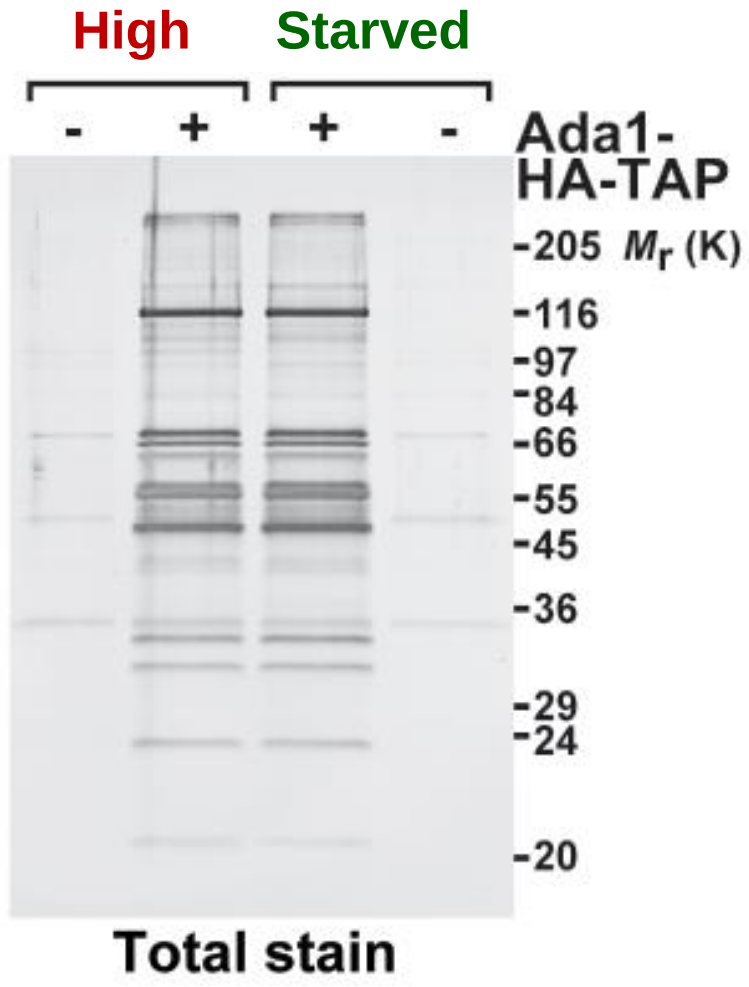
The TORC1 & TORC2 pathways function upstream of SAGA.

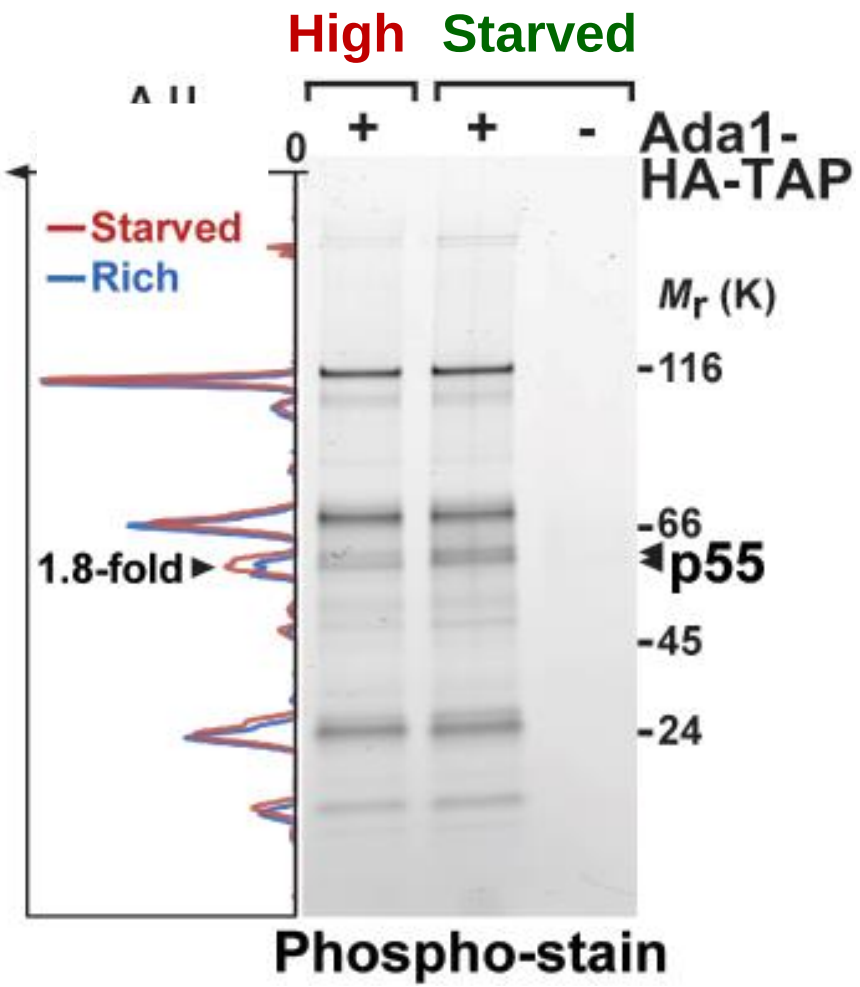
Genetic interaction analyses



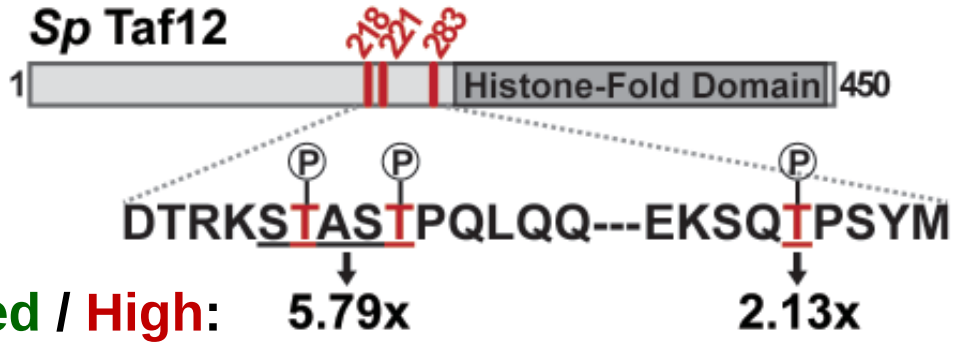
The TORC1 & TORC2 pathways function upstream of SAGA.

SAGA phosphorylation *vs* nutrient levels





Identification of Taf12 by SILAC-MS



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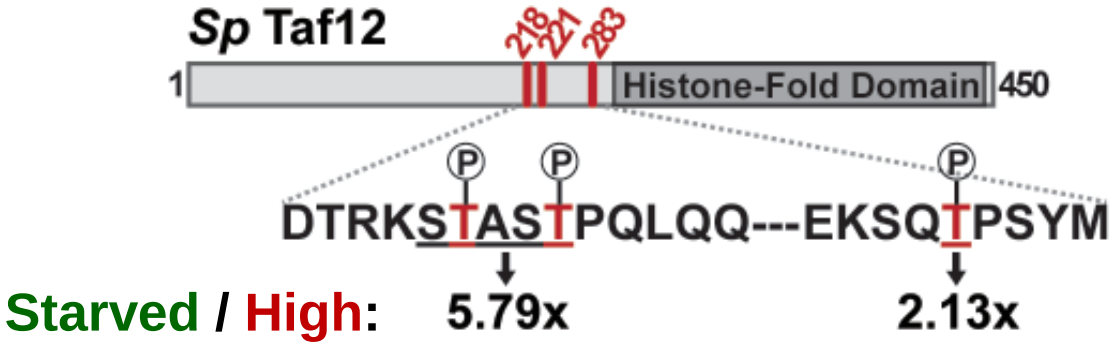

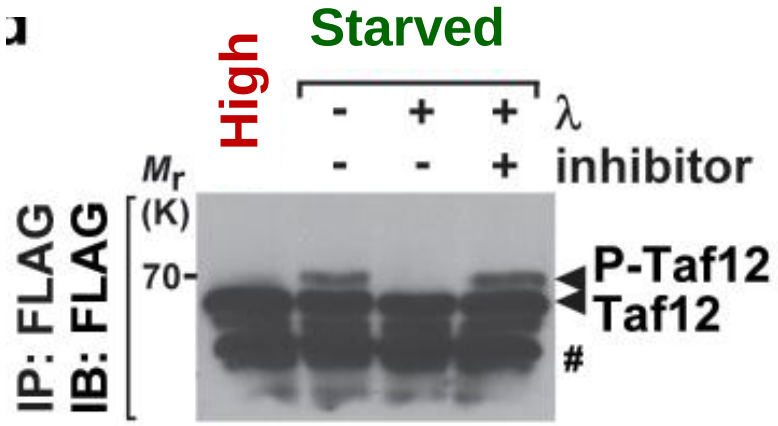

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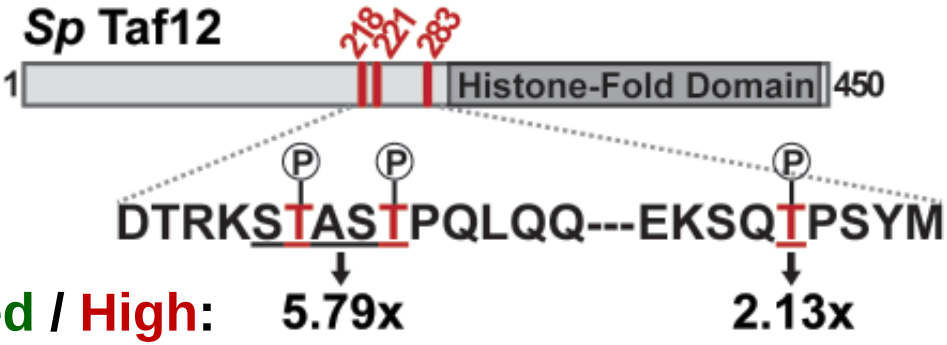

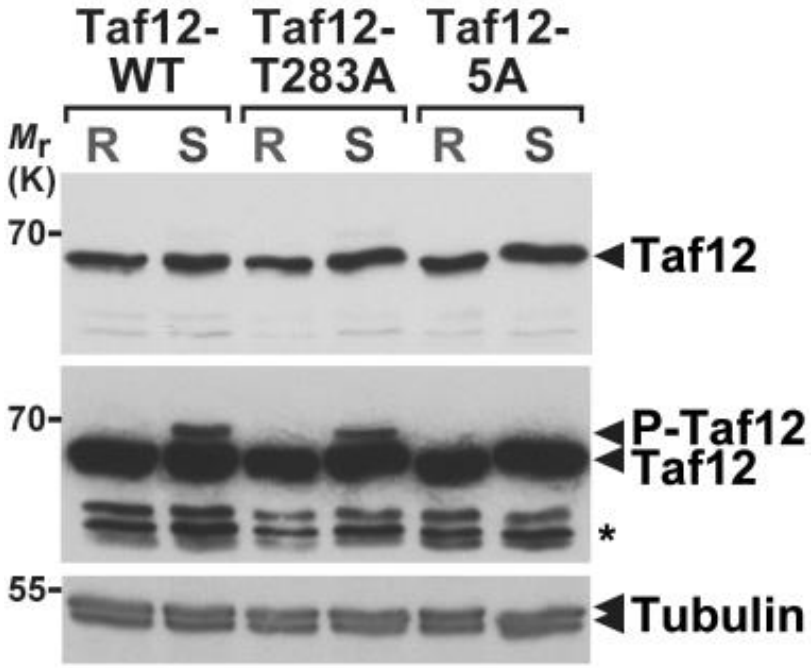

Discrepancy between genetics and biochemistry:

Taf12 is not phosphorylated when TORC1 is active (high nutrients)

Nutrients → TORC1 → ?????? → SAGA ⊣ Differentiation

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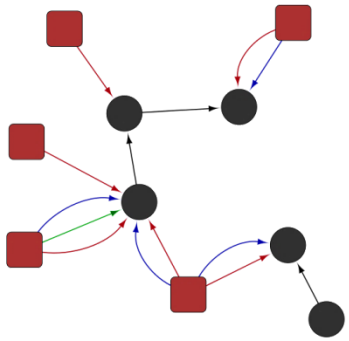
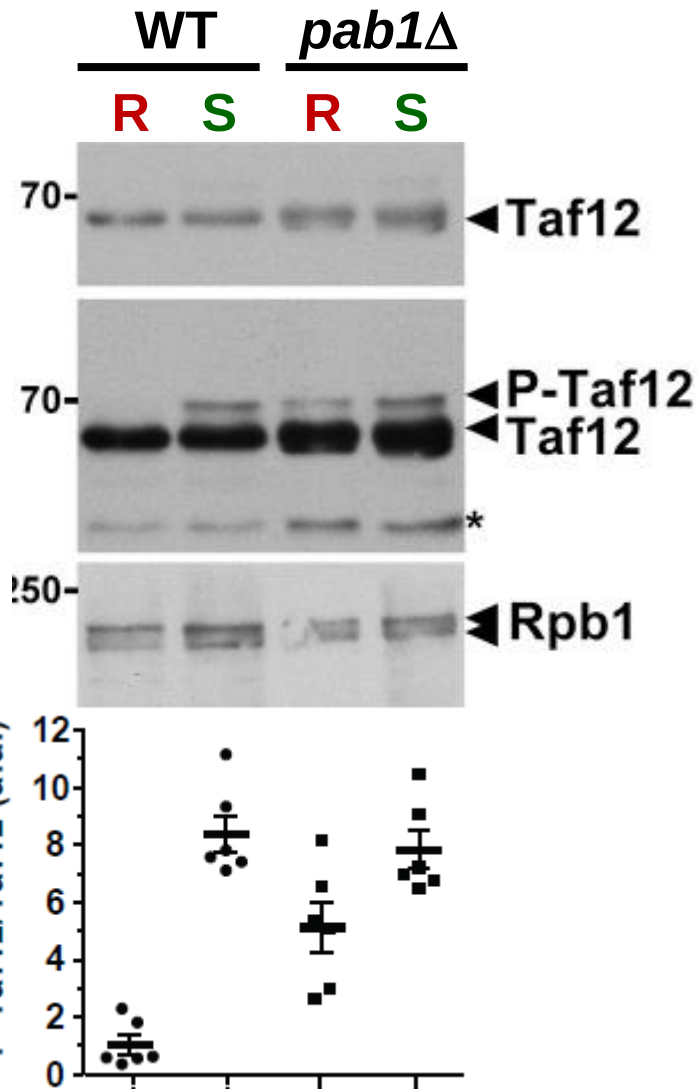
Genetic analyses

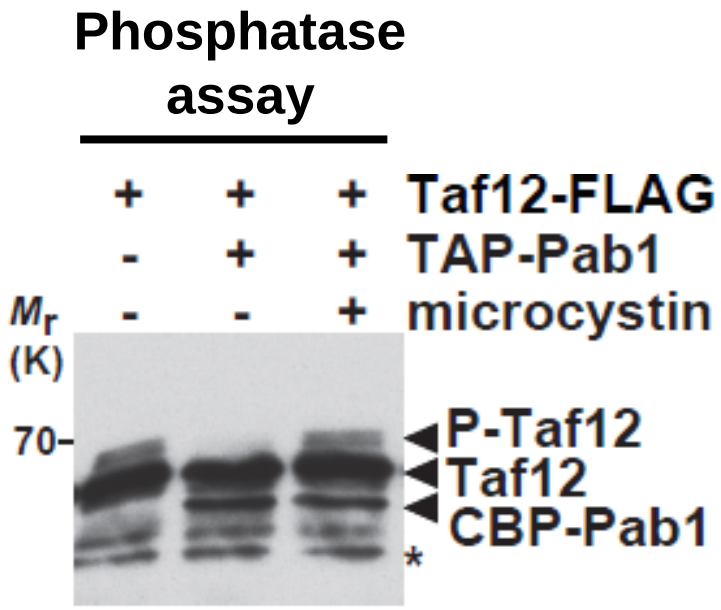


Similar to Gcn5, the PP2A-B55 phosphatase represses differentiation in rich conditions, downstream of TORC1.

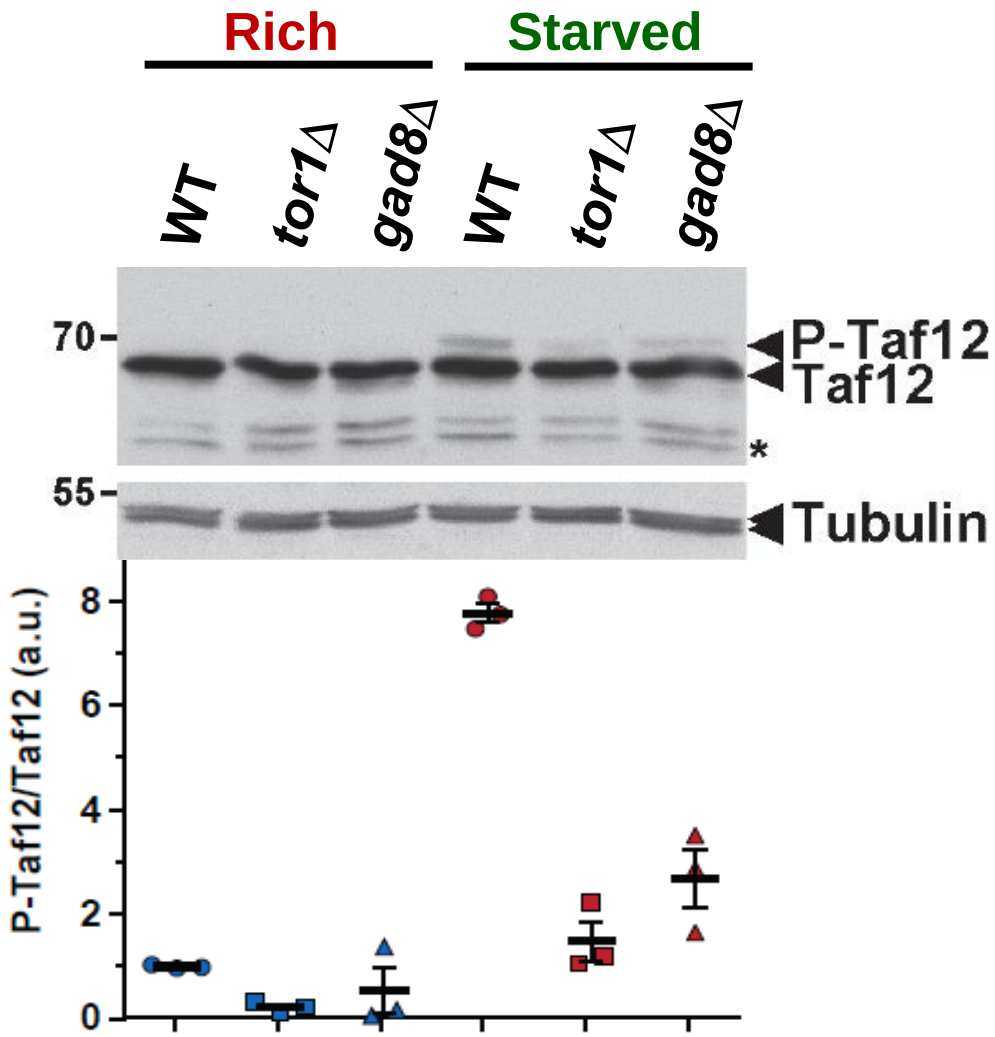
Nutrients → TORC1 → PP2A-Pab1 → SAGA ⊣ Differentiation

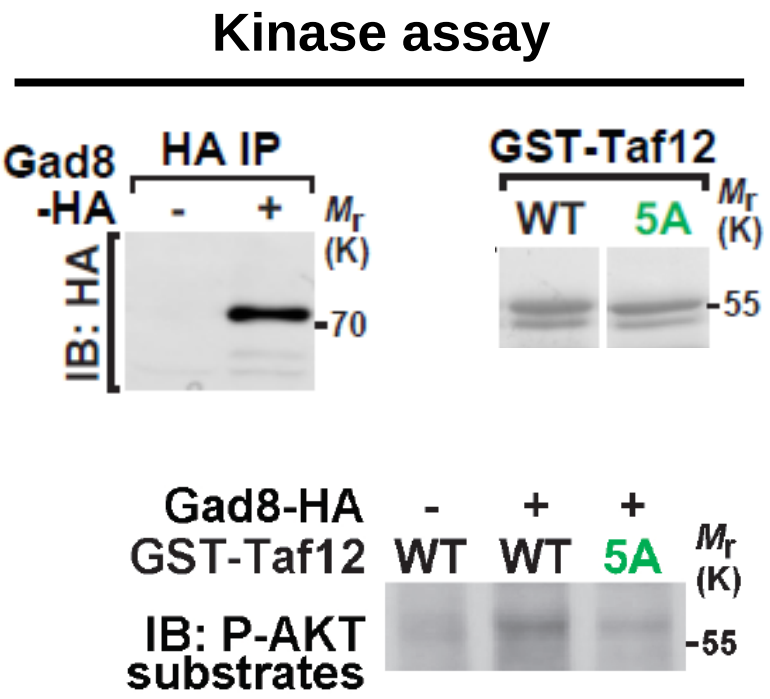
Effect of PP2A on Taf12 phosphorylation



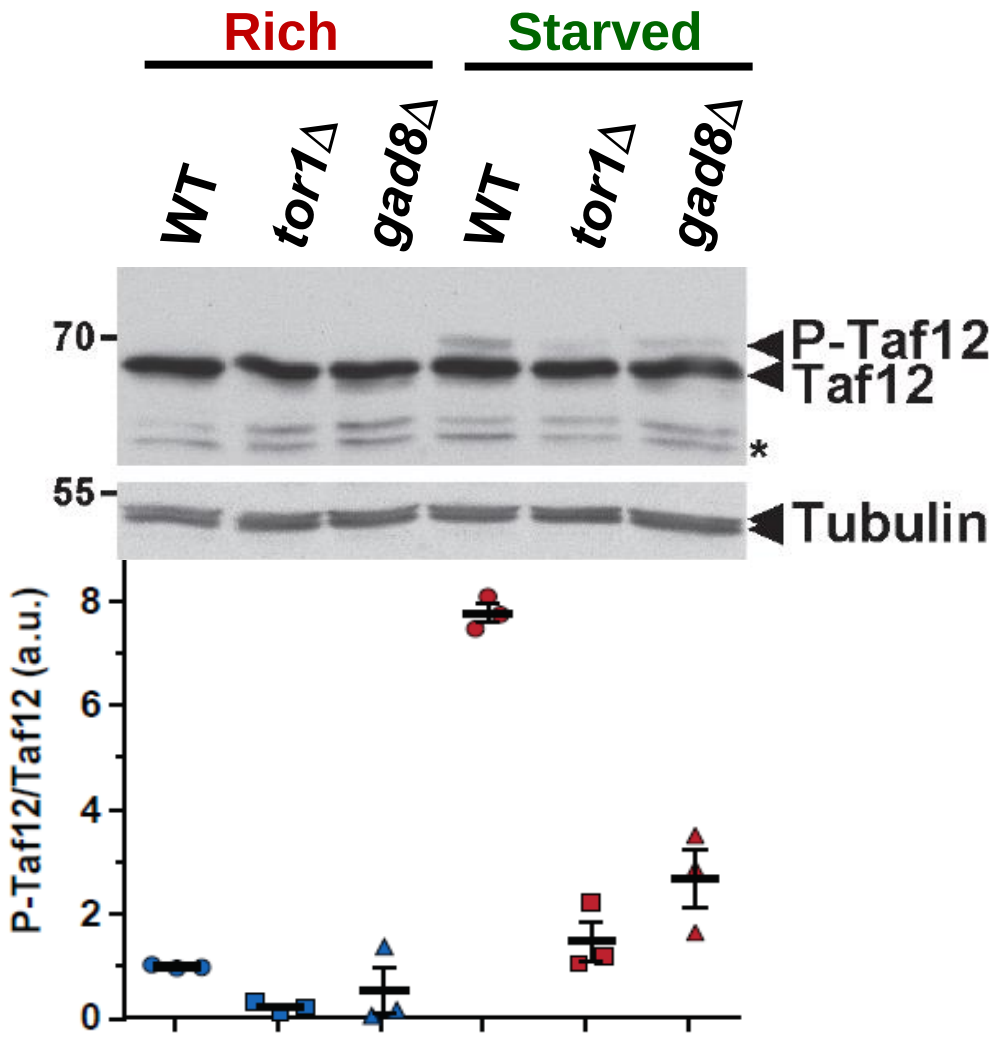


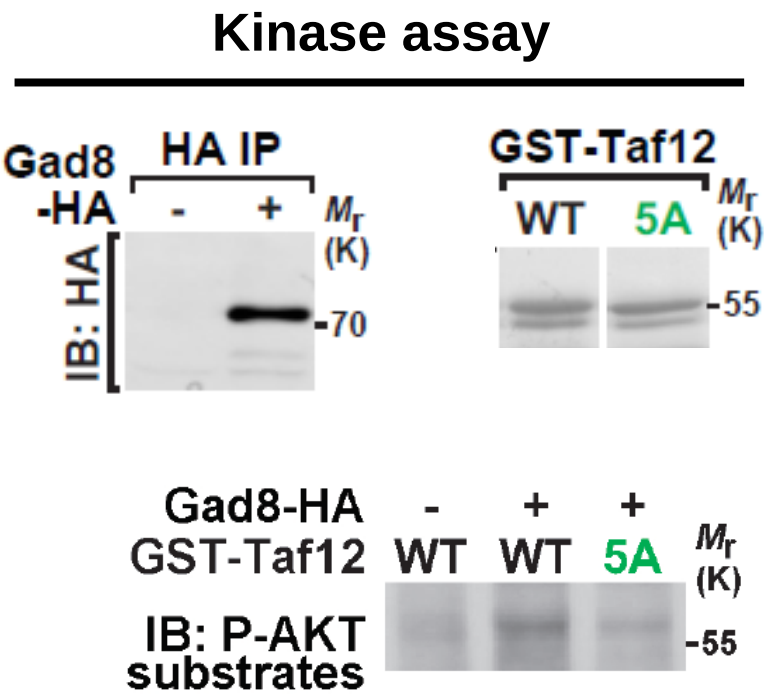
Effect of TORC2/AKT



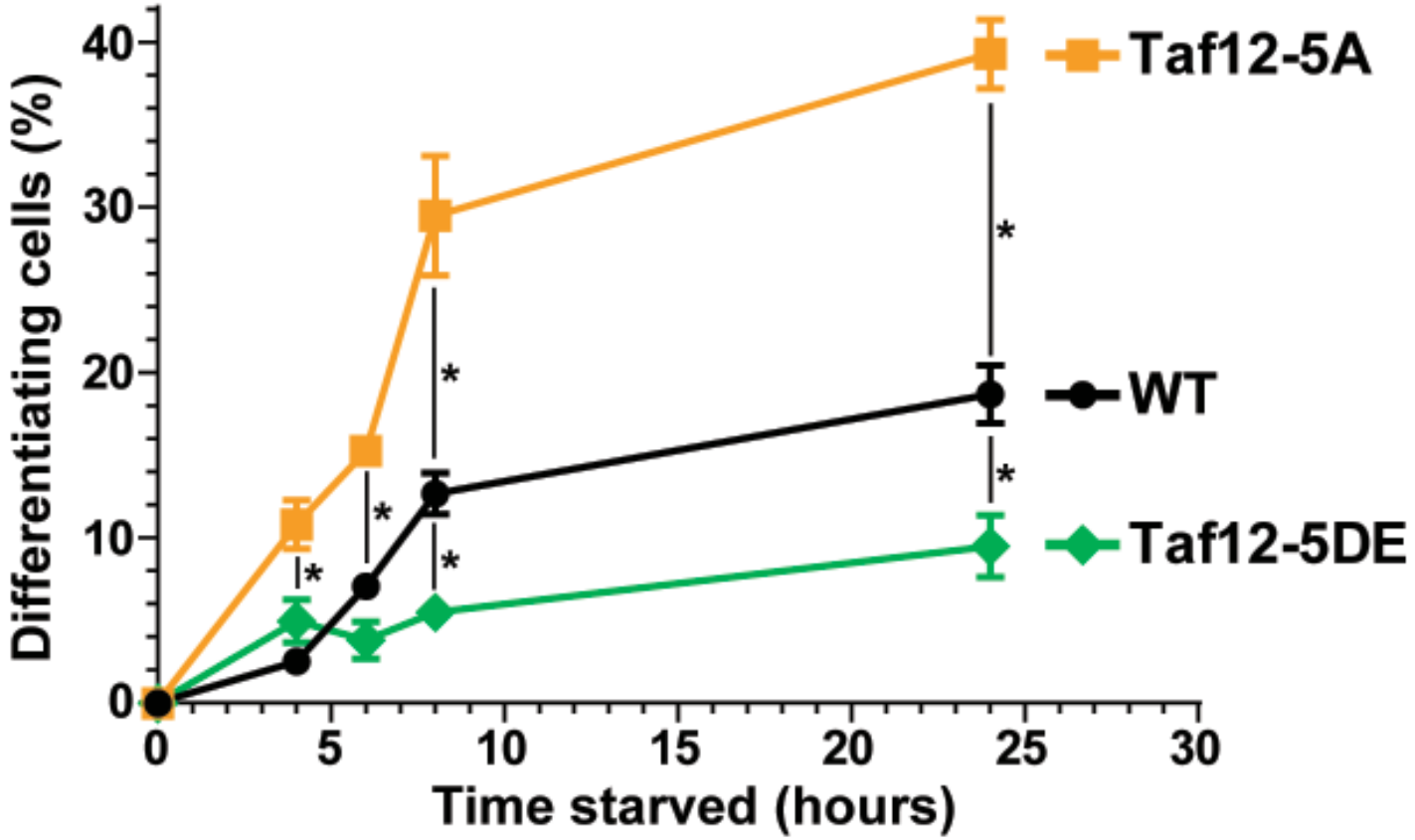


Effect of TORC2/AKT



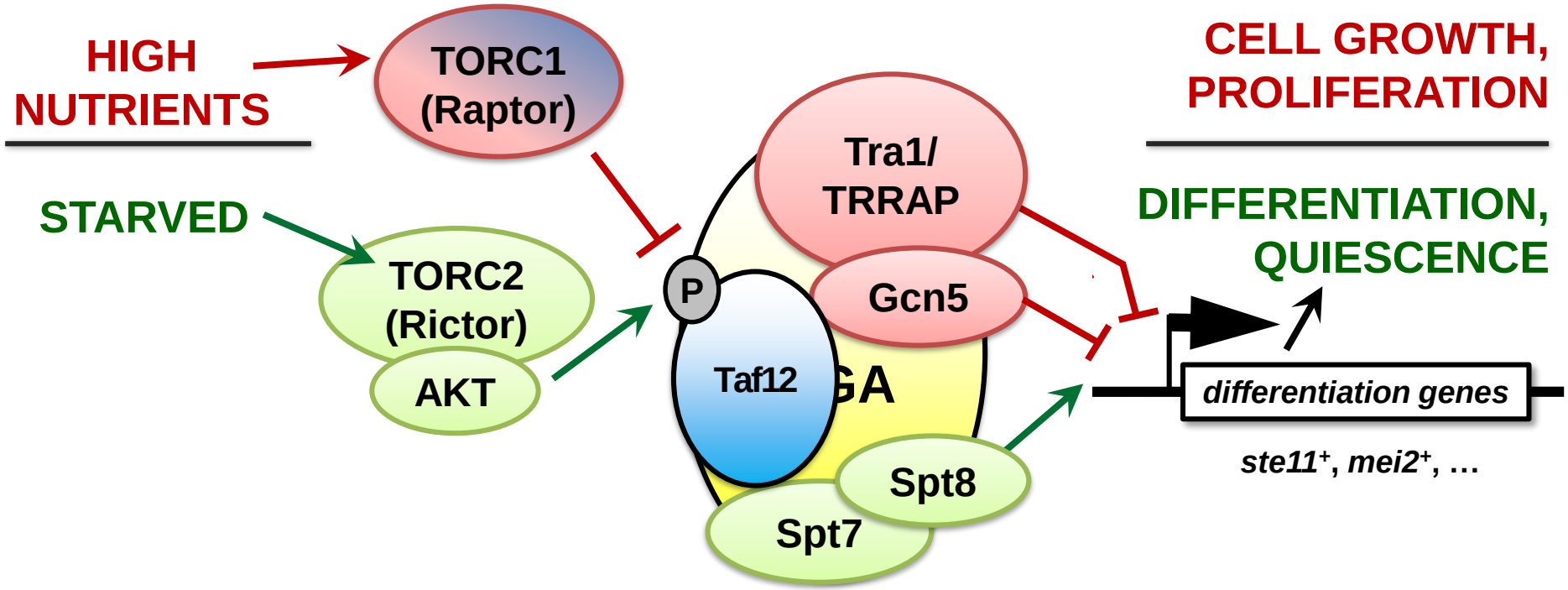


Role of Taf12-P in sexual differentiation



Conclusions

SAGA regulates differentiation genes in response to the nutrient-sensing TORC1 and TORC2 pathways, through Taf12 phosphorylation.



For further details

Published online: October 27, 2017

Article

TRANSPARENT PROCESS

EMBO reports

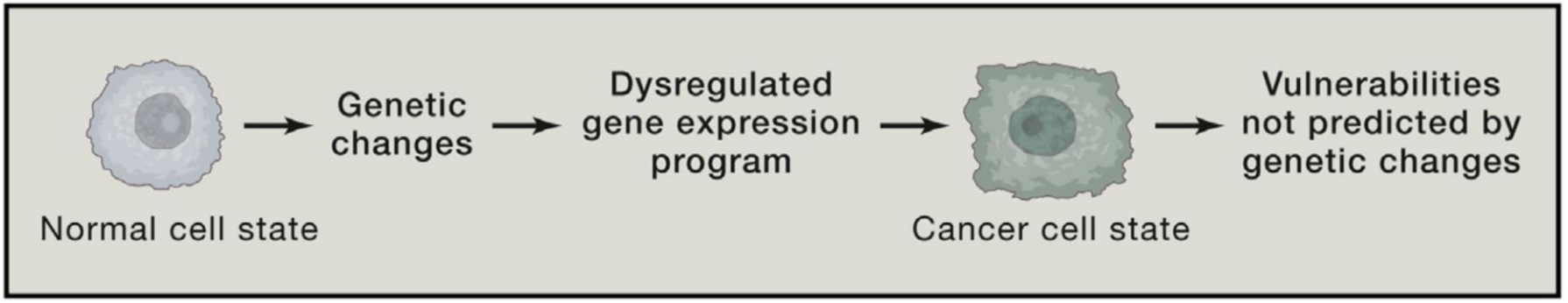
TORC1 and TORC2 converge to regulate the SAGA co-activator in response to nutrient availability

Thomas Laboucarié[1], Dylane Detilleux[1], Ricard A Rodriguez-Mias[2], Céline Faux[1], Yves Romeo[1,†], Mirita Franz-Wachtel[3], Karsten Krug[3], Boris Maček[3], Judit Villén[2], Janni Petersen[4] & Dominique Helmlinger[1,*]

Transcription (dys)regulation in cancer

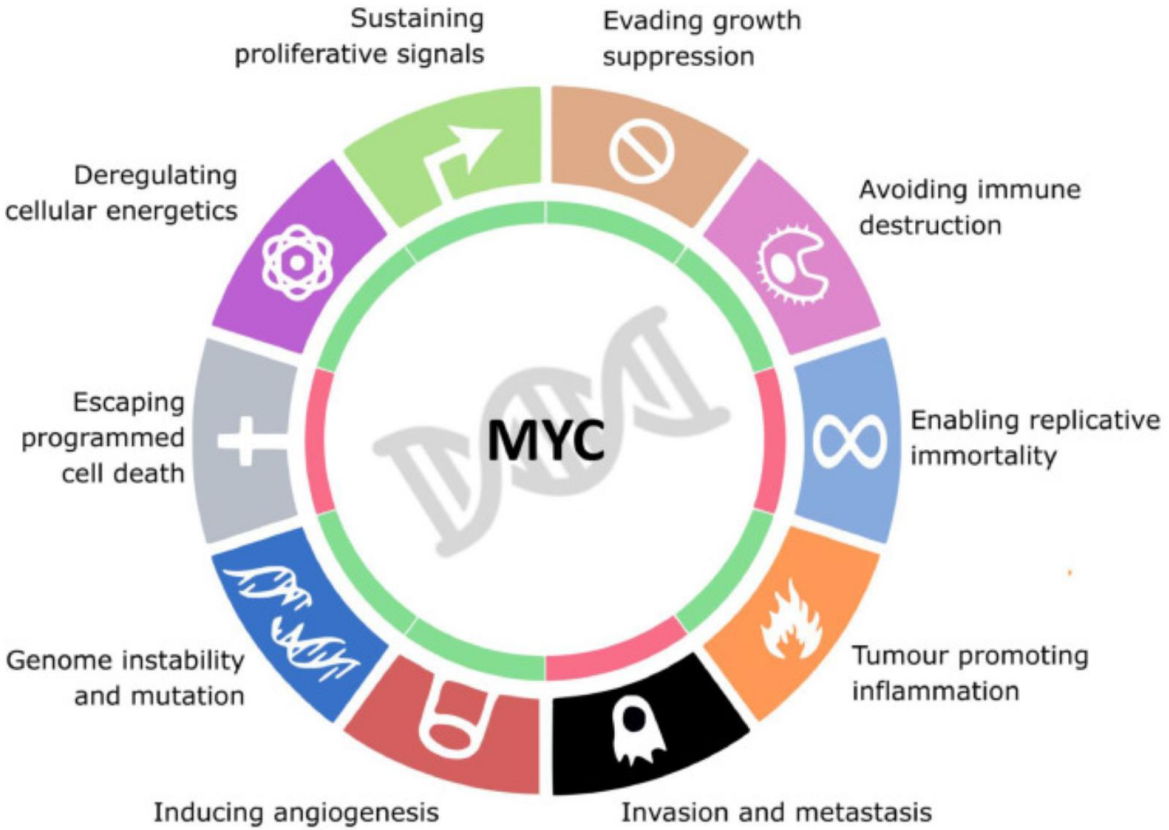
Trancriptional addiction in cancer

- **Genetic alterations can disrupt transcriptional control.**
- **Cancer cells become addicted to specific TFs.**
- **TFs require co-activators, which can be druggable.**

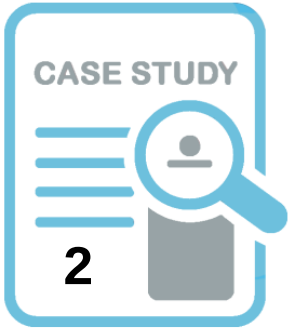


The example of MYC

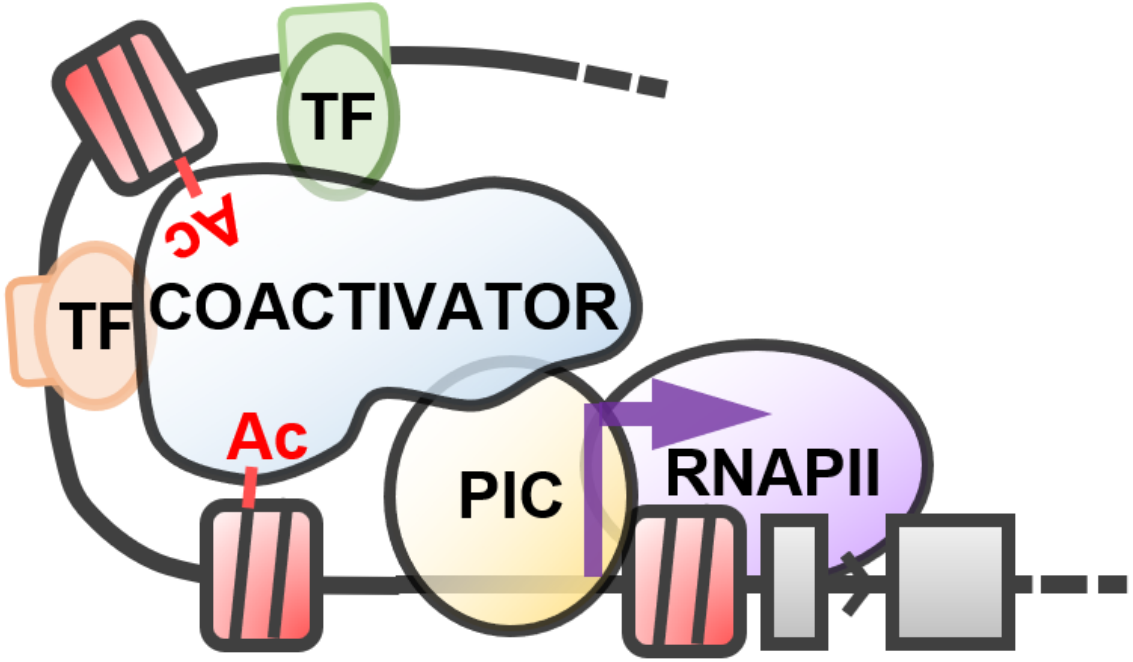
- **Major oncogene (~70% / cancers); difficult to target.**
- **First cofactor identified: TRRAP (SAGA and TIP60).**



Another project ongoing

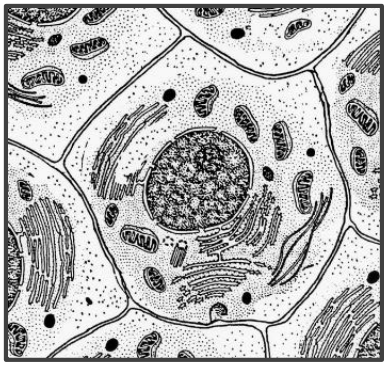


Which genes are directly controlled by SAGA/TIP60 and what is their contribution to MYC addiction in cancer cells?



Gene regulation by SAGA and TIP60

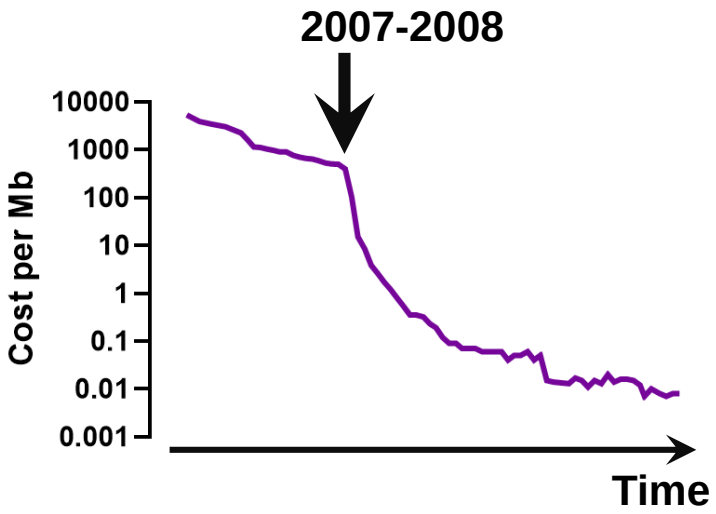
- **In yeasts: metabolic adaptation, ribosome biogenesis.**
- **In slower-growing eukaryotes (*aka* plants and metazoans):**
  - **Stress response.**
  - **Early development and differentiation.**
  - **Cell type-specific profiles.**



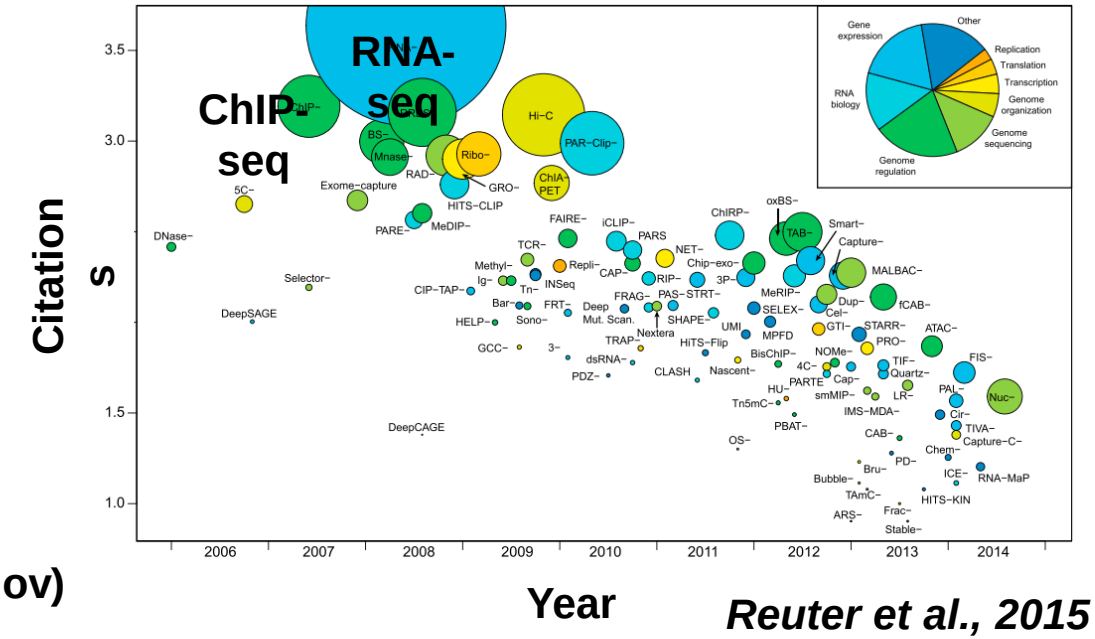
Workman, Winston, Berger, Côté, Struhl, Young, Green, Pugh, Holstege, Shore, Pillus, Helmlinger, Tora, Herceg, Dent, Aquea, Pineiro labs

Predictive, general models of action

Hopes from the NGS revolution.



Source: NIH (www.genome.gov)

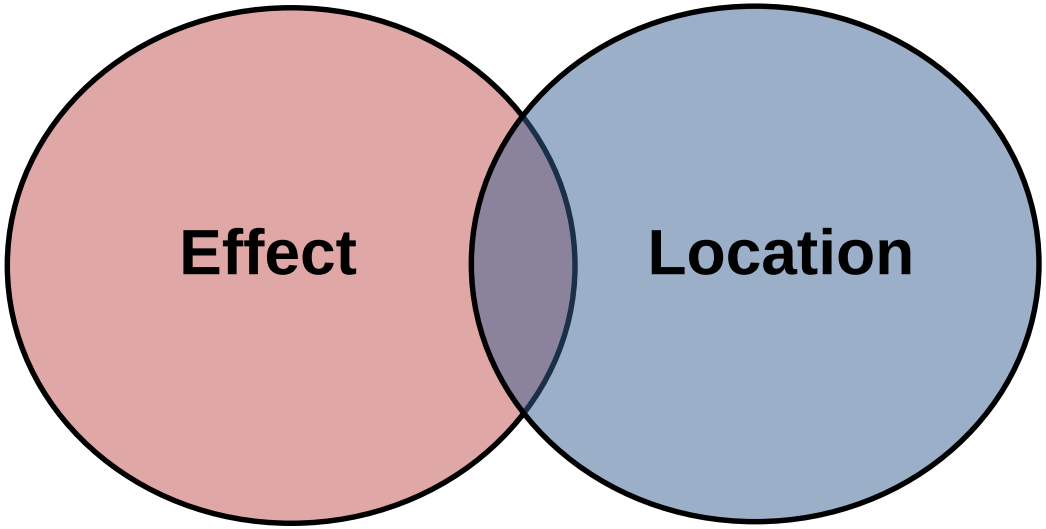


Reuter et al., 2015

Predictive, general models of action

RNA-seq in deletion mutant of a factor

ChIP-seq profile of that factor



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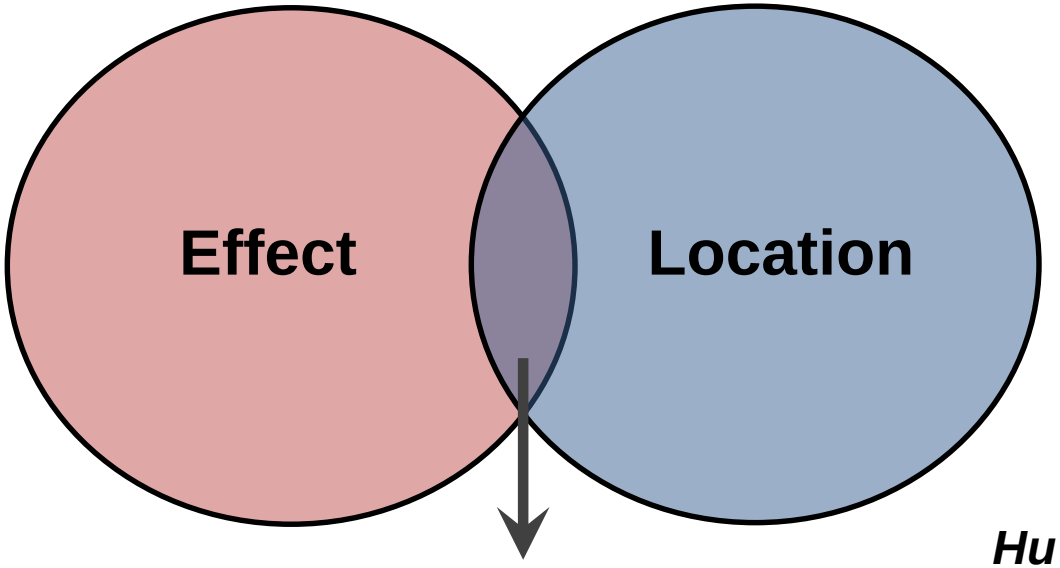

~3% overlap only!
Low number of direct targets

Hu et al., 2007
Venters et al., 2011
Lenstra et al., 2011

Location does not predict effects!

A major problem in the field

- **Biological explanations:**
  - **Experimental conditions.**
  - **Compensatory mechanisms: adaptation, functional redundancy.**
  - **Neutral evolution as null hypothesis (≠ adaptationist approach).**

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  - **Statistical significance ≠ biological meaning.**
  - **Static analyses.**
  - **Methodological limitations: indirect (RNA-seq), qualitative (ChIP-seq).**

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Which gene-specific properties can predict regulatory effects?

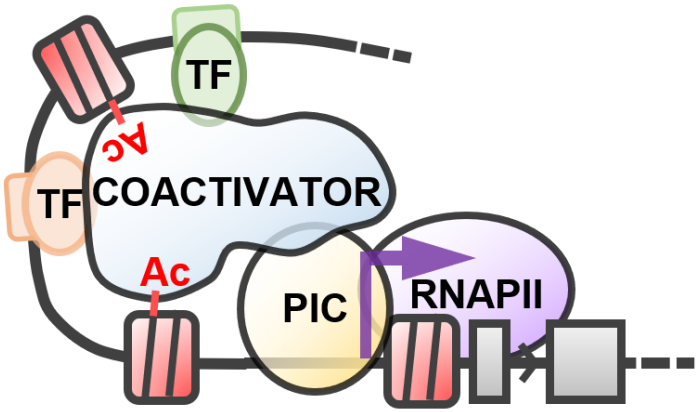
What we implement

Acute genetic perturbations

Nascent transcriptomics

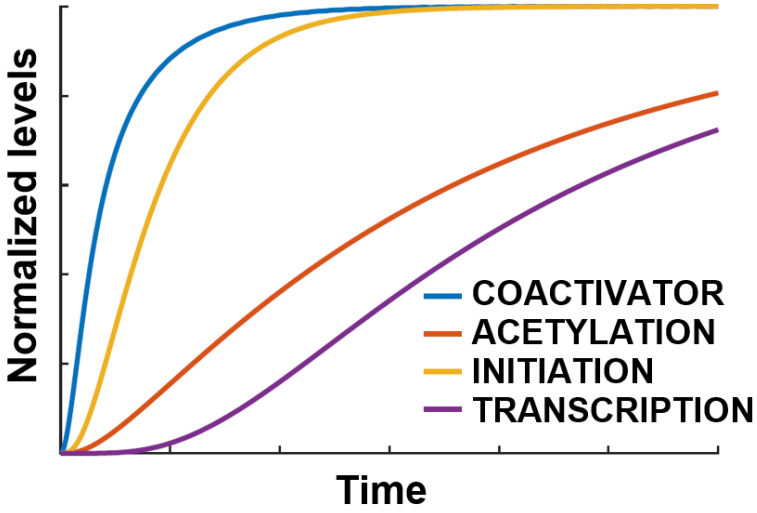
Quantitative chromatin profiling

Predictive biophysical model

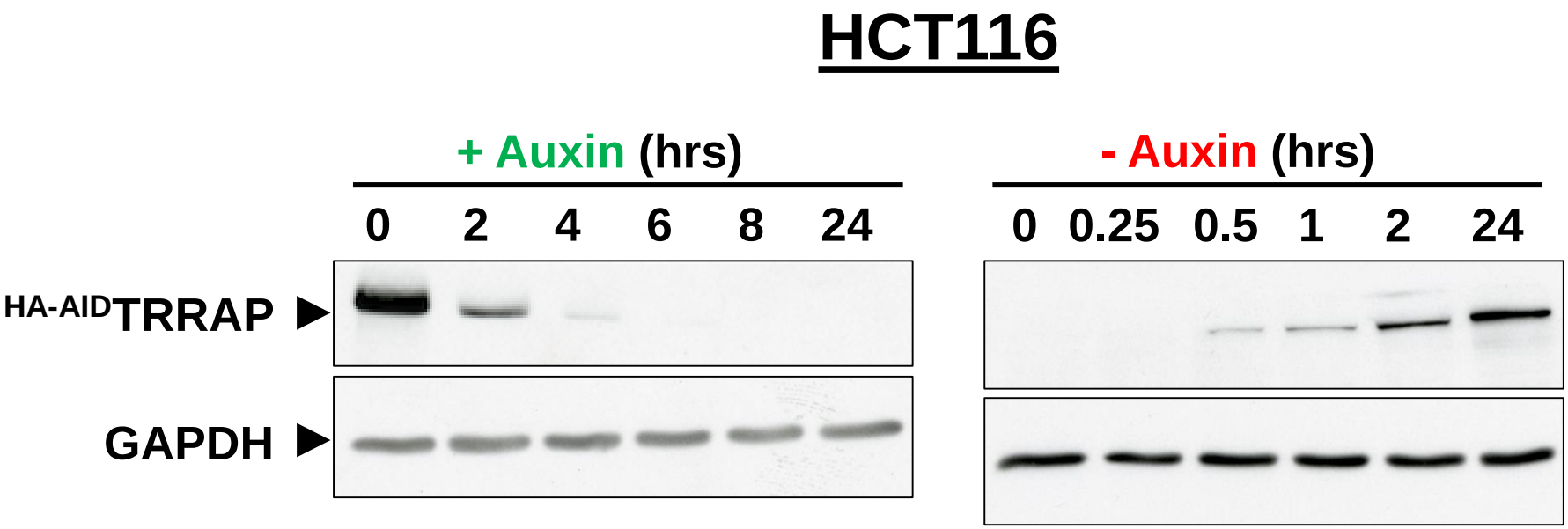


Model

Predict

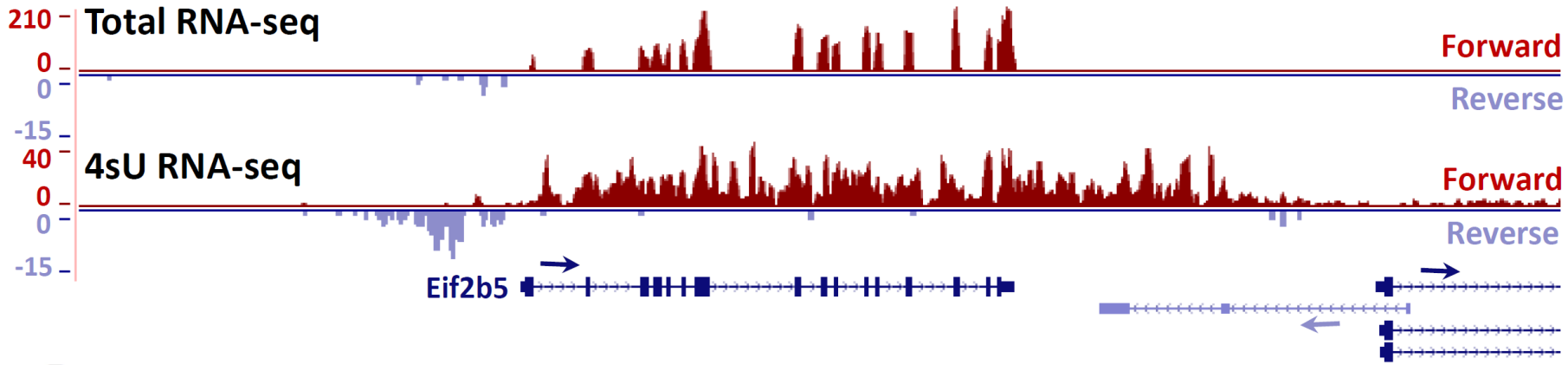


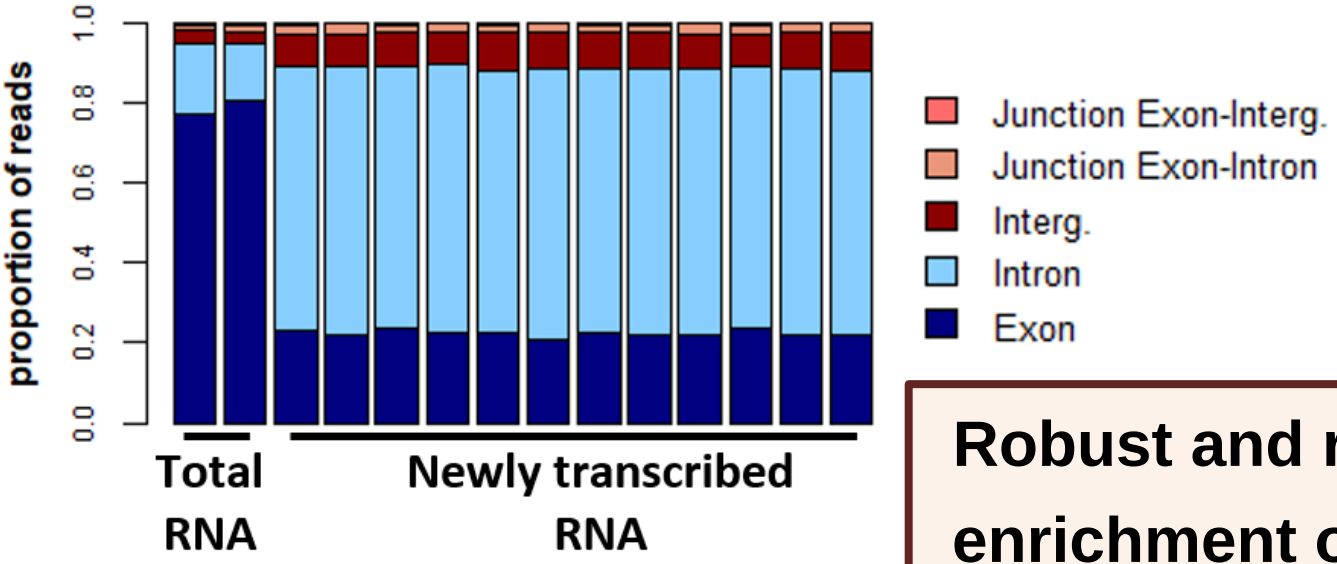
Kinetic analyses



Rapid and reversible depletion of endogenous SAGA and TIP60 subunits using an auxin-inducible degron.

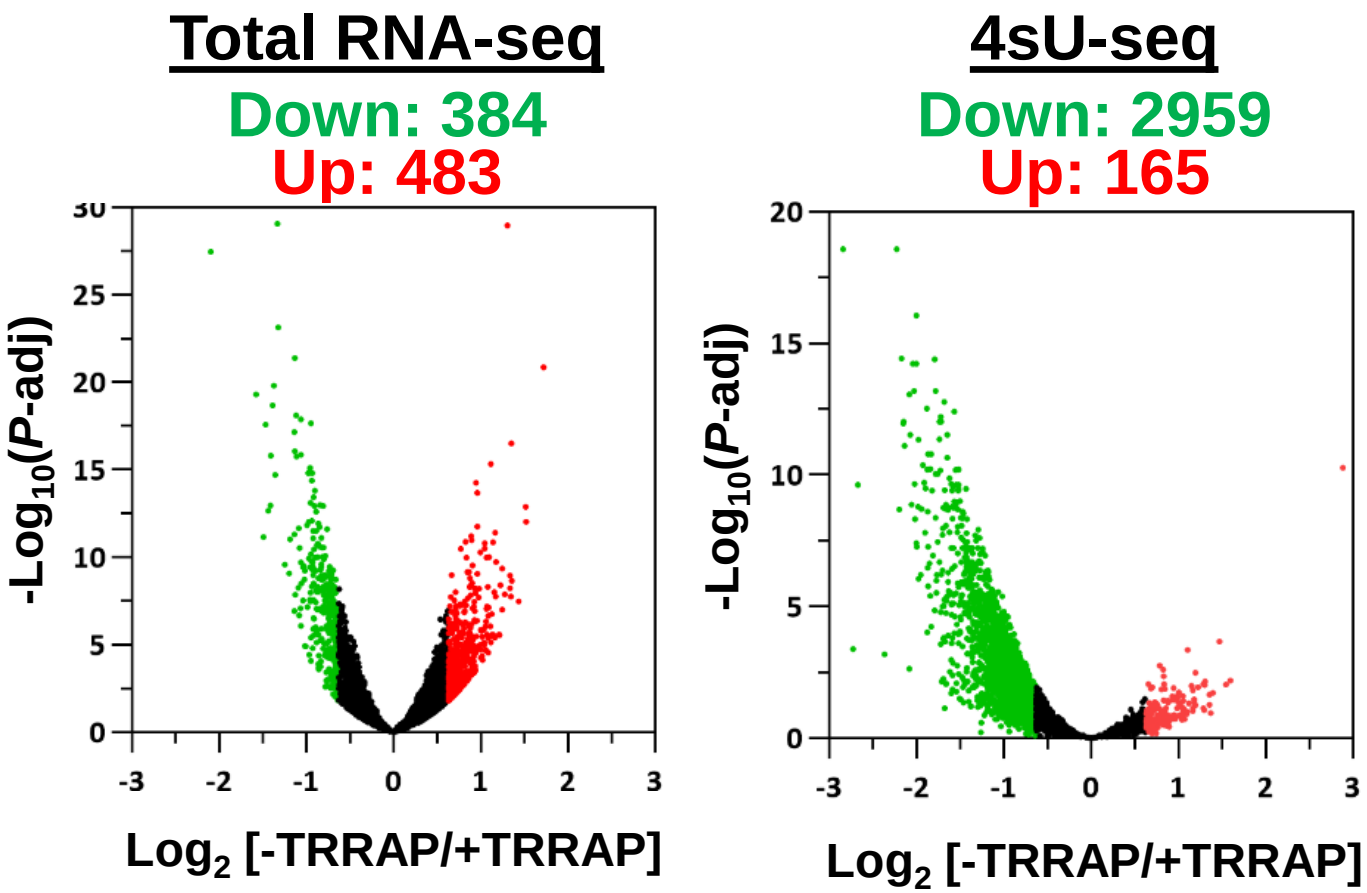
Measuring nascent transcript levels





Robust and reproducible enrichment of nascent RNAs by 4sU-seq.

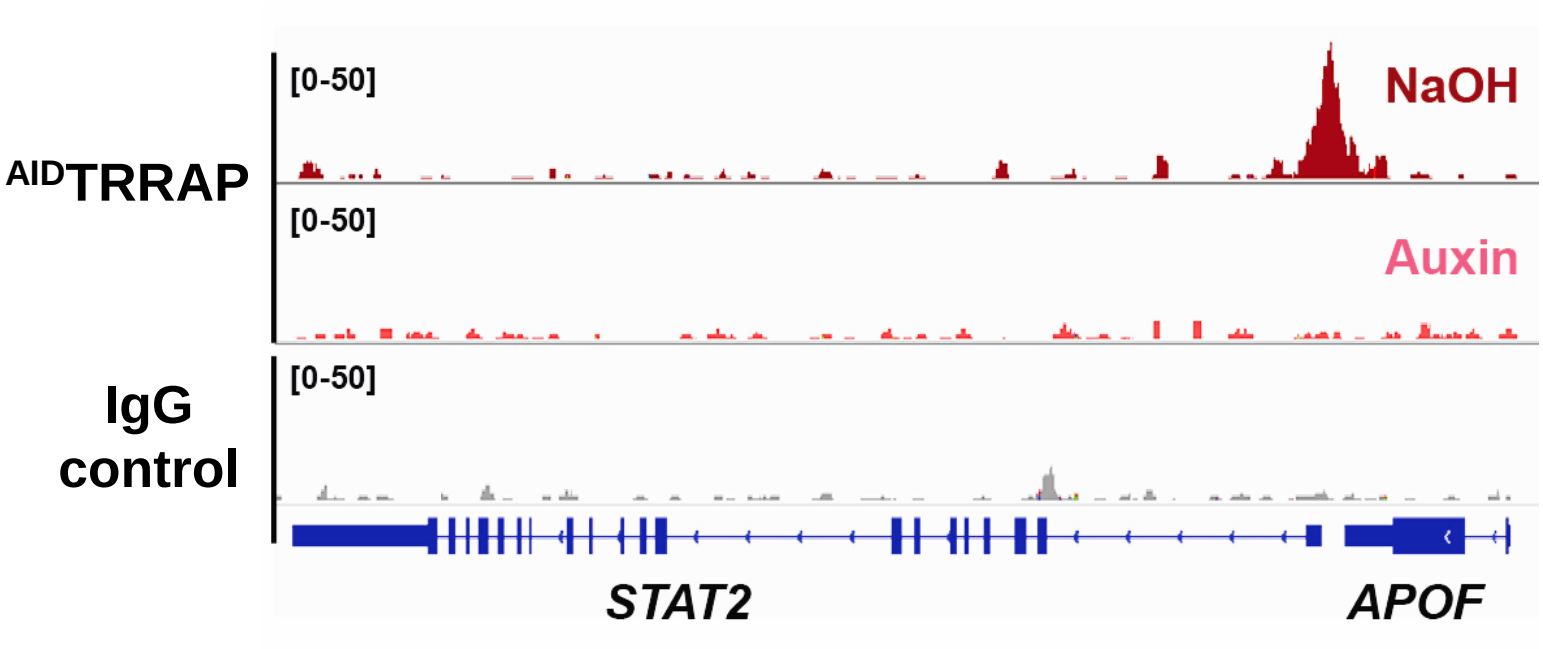
Nascent *vs* total transcriptomics upon acute TRRAP depletion



Stronger and less compensatory effects of TRRAP.

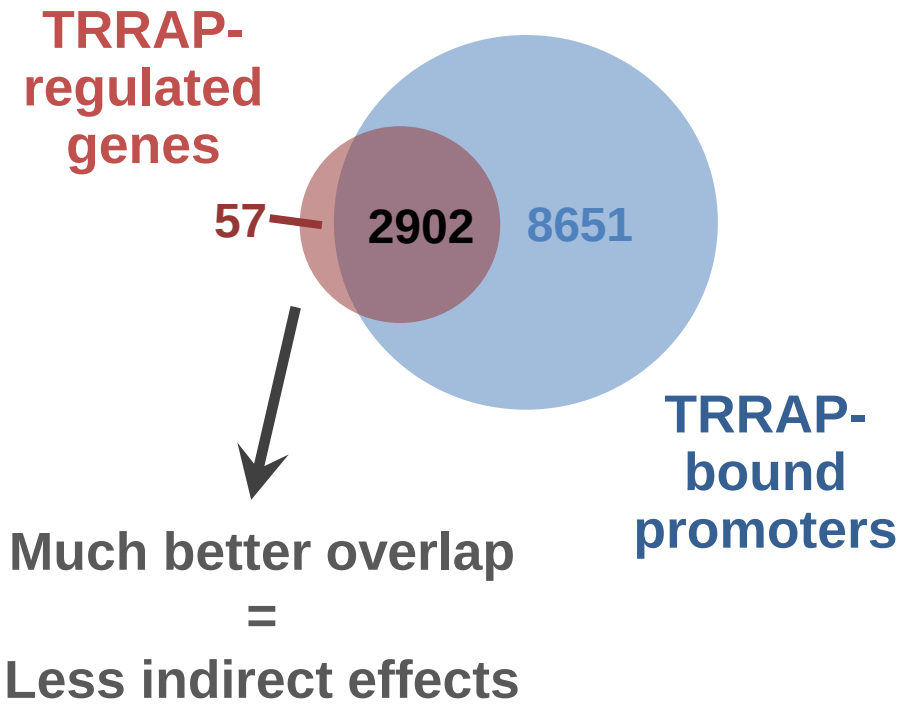
Quantitative native chromatin profiling

CUT&RUN-seq

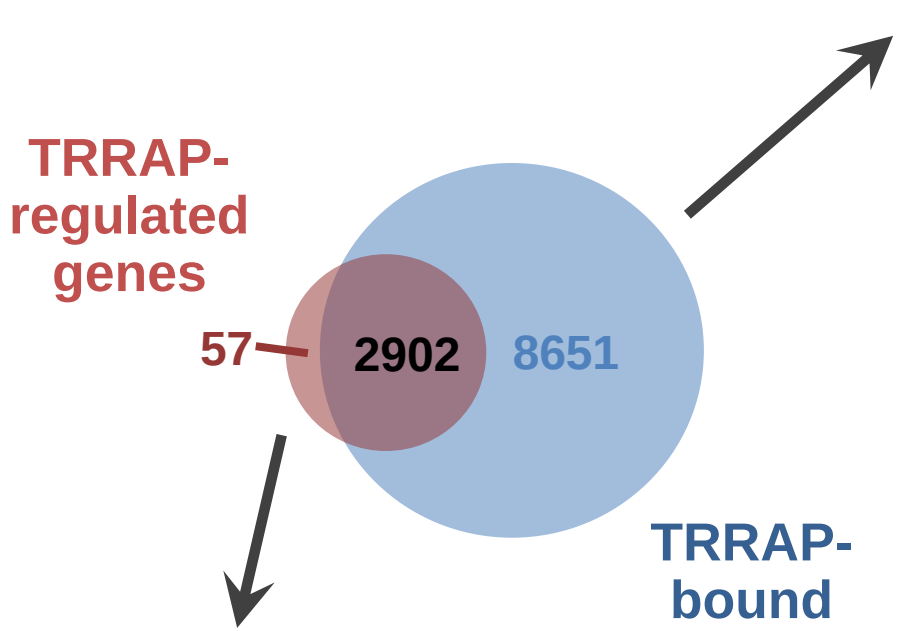


Robust and high-resolution profiling of TRRAP genomic occupancy.

Correlating location and effects

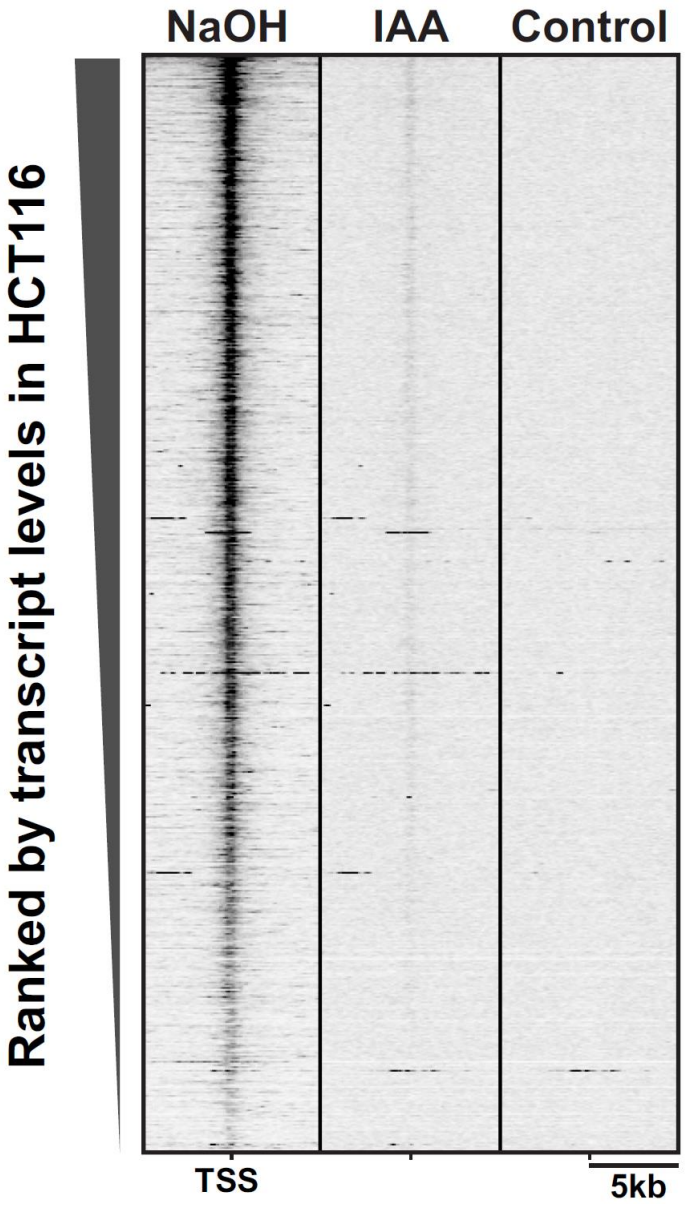


Correlating location and effects

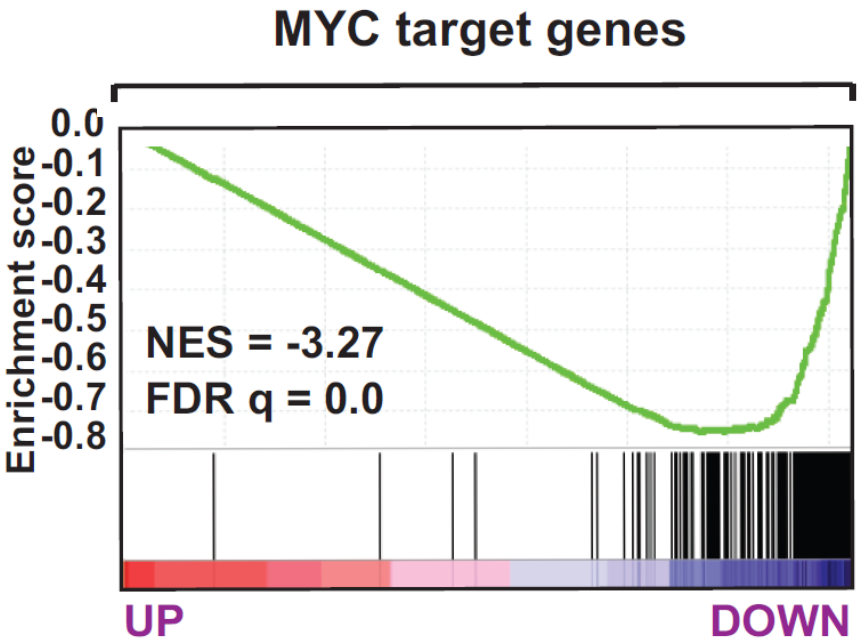


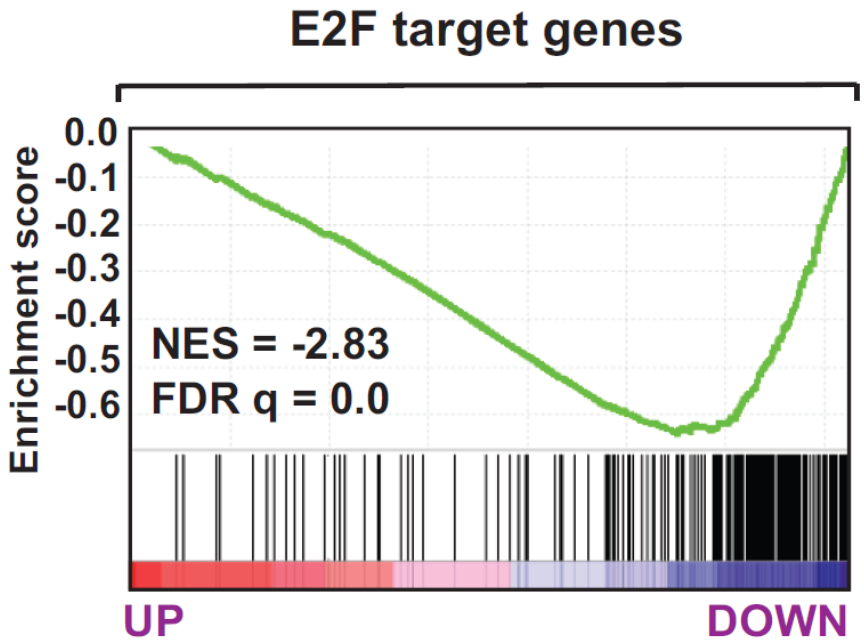
But:

Much better overlap
=
Less indirect effects



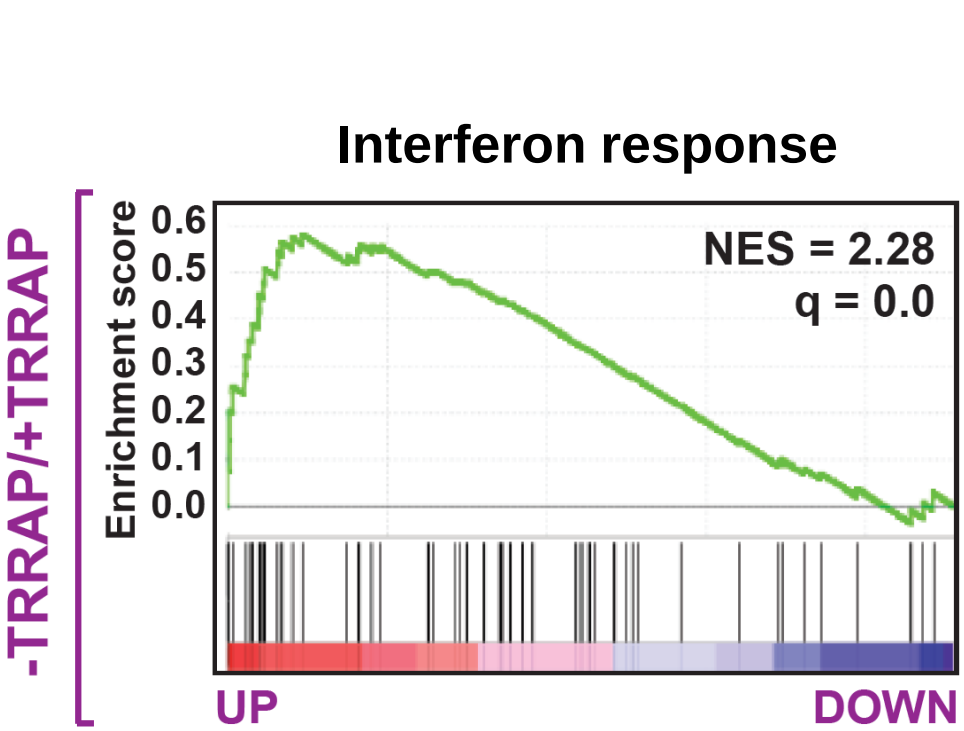
TRRAP direct target genes

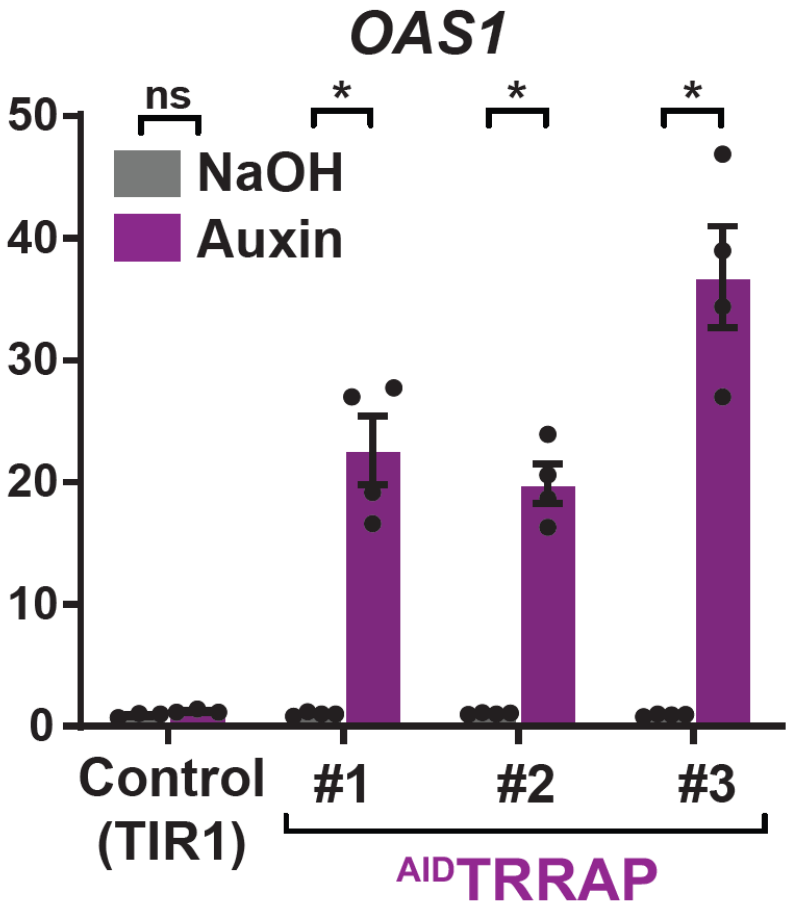




TRRAP is a co-activator of MYC and E2F in cycling colorectal cancer cells.

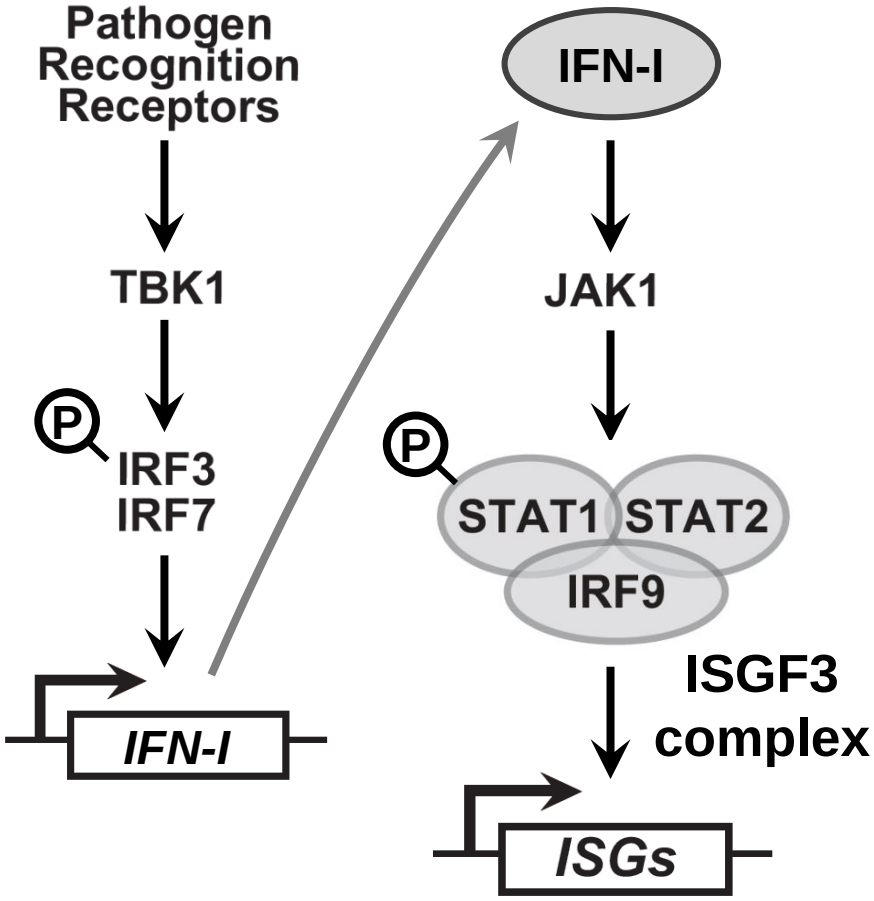
Unexpected repressive roles

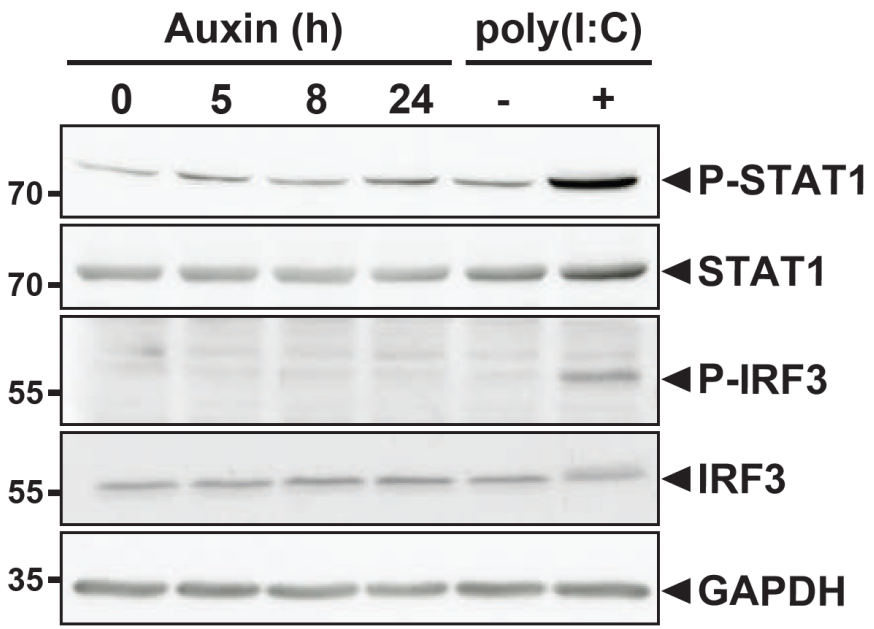




TRRAP depletion induces interferon-stimulated genes.

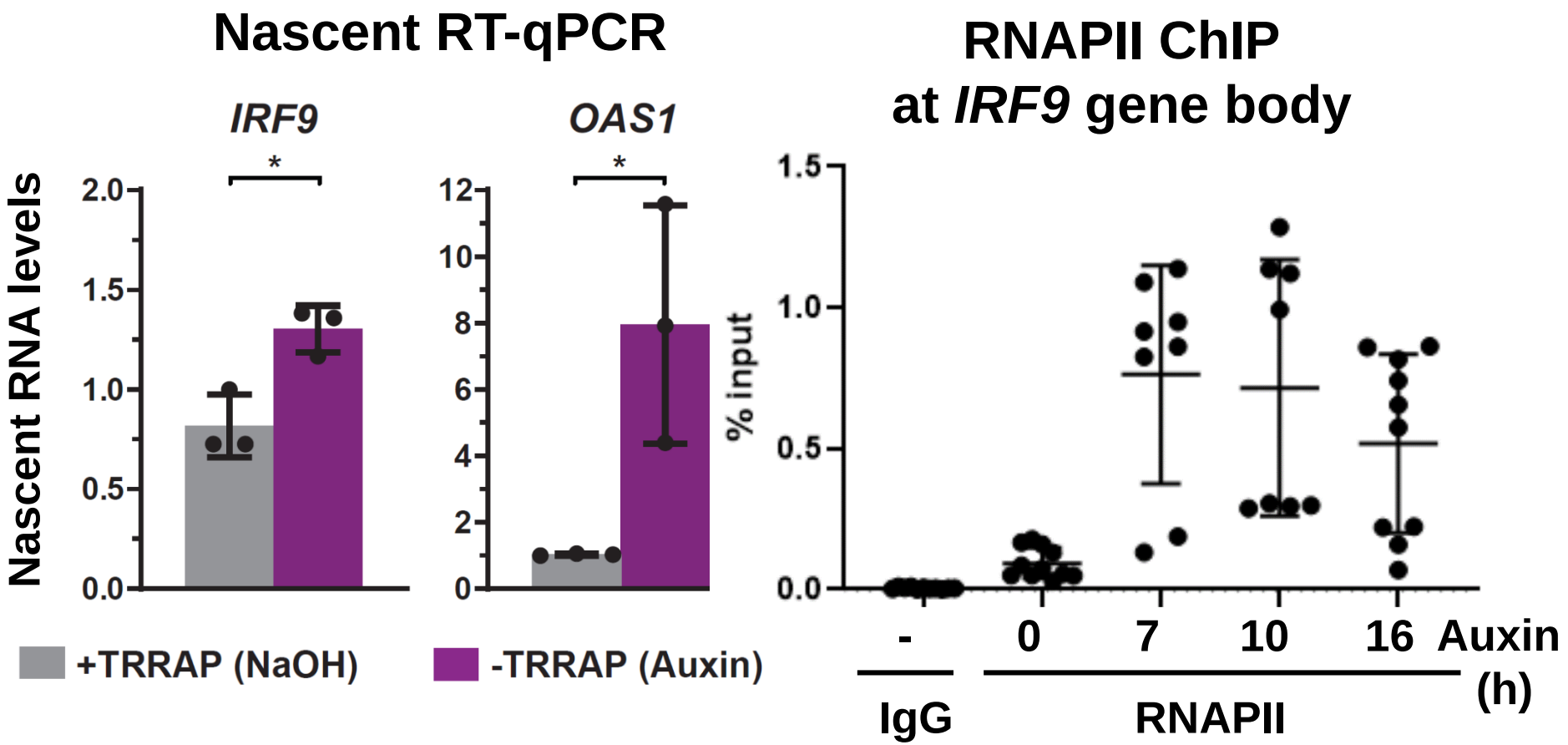
Innate immune pathway activation





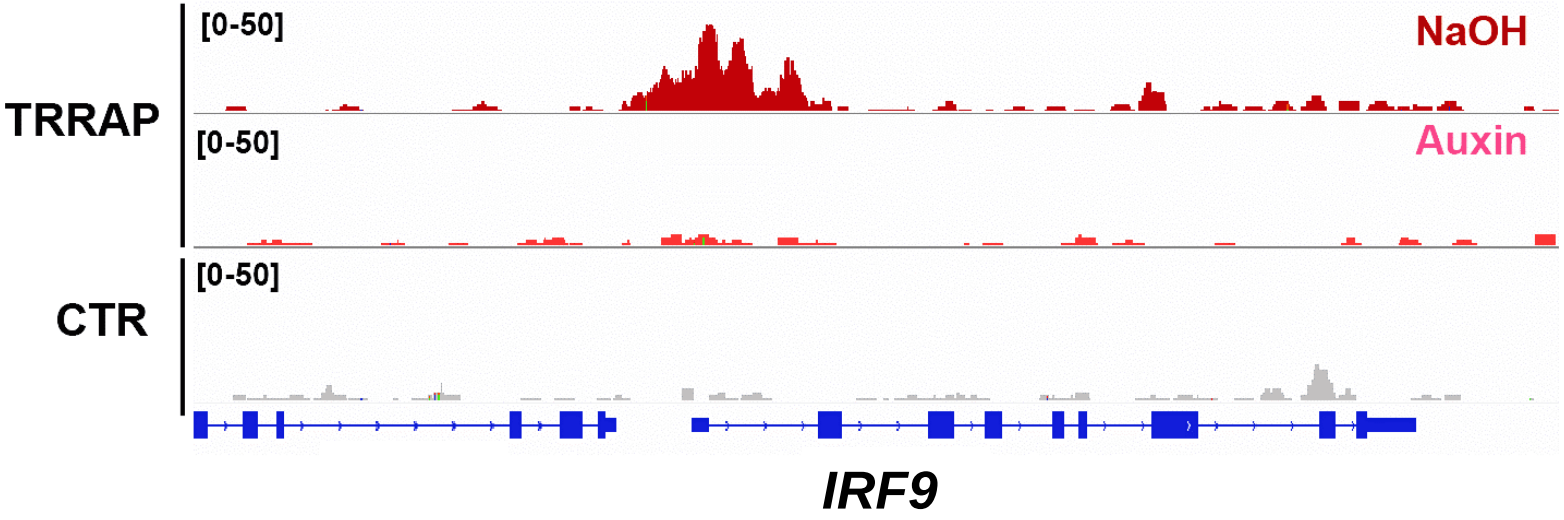
ISGs are induced without detectable pathway activation.

Effect on TRRAP on ISG transcription



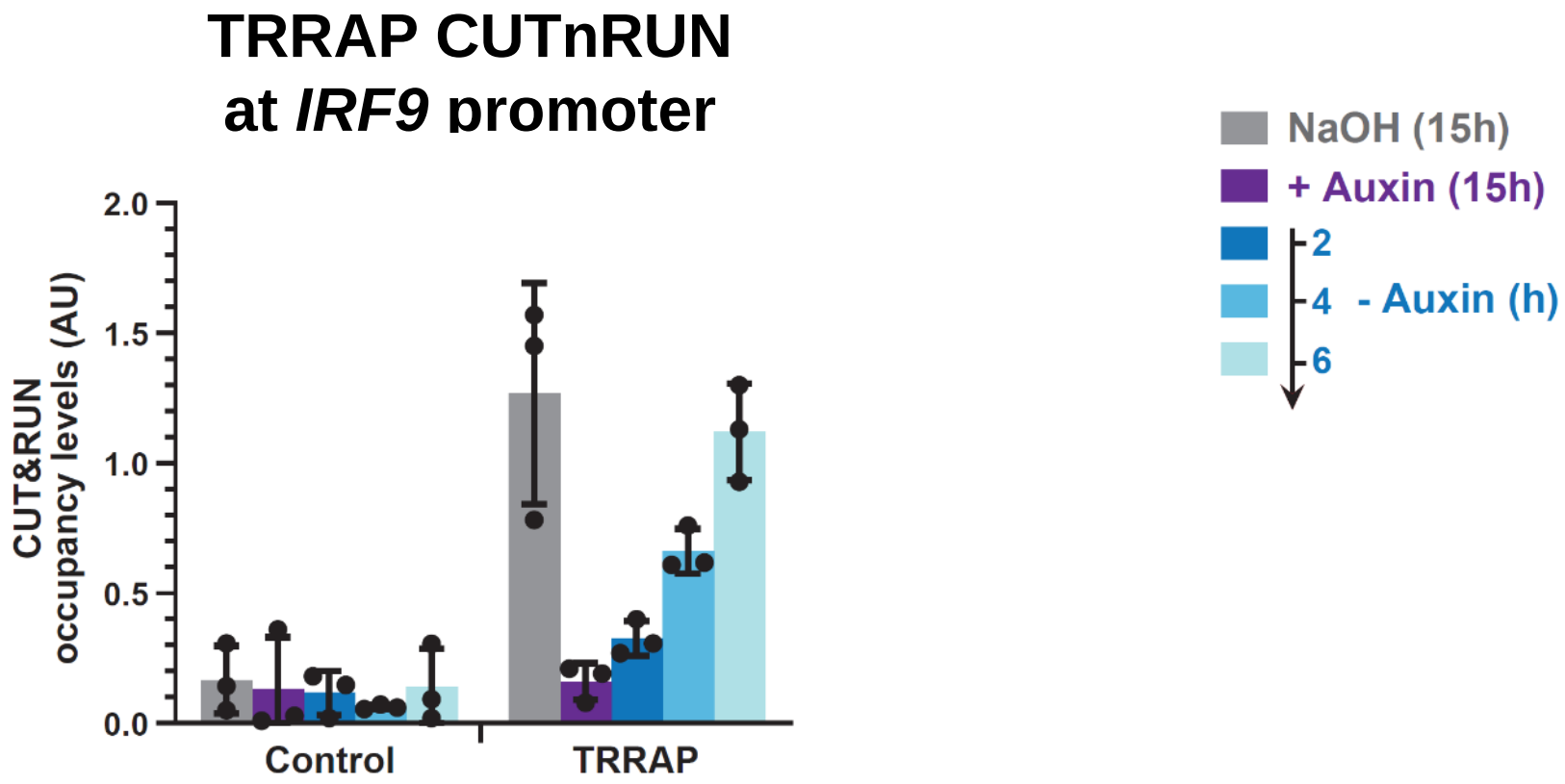
TRRAP contributes to transcriptional repression of ISGs.

TRRAP occupancy at ISG promoters

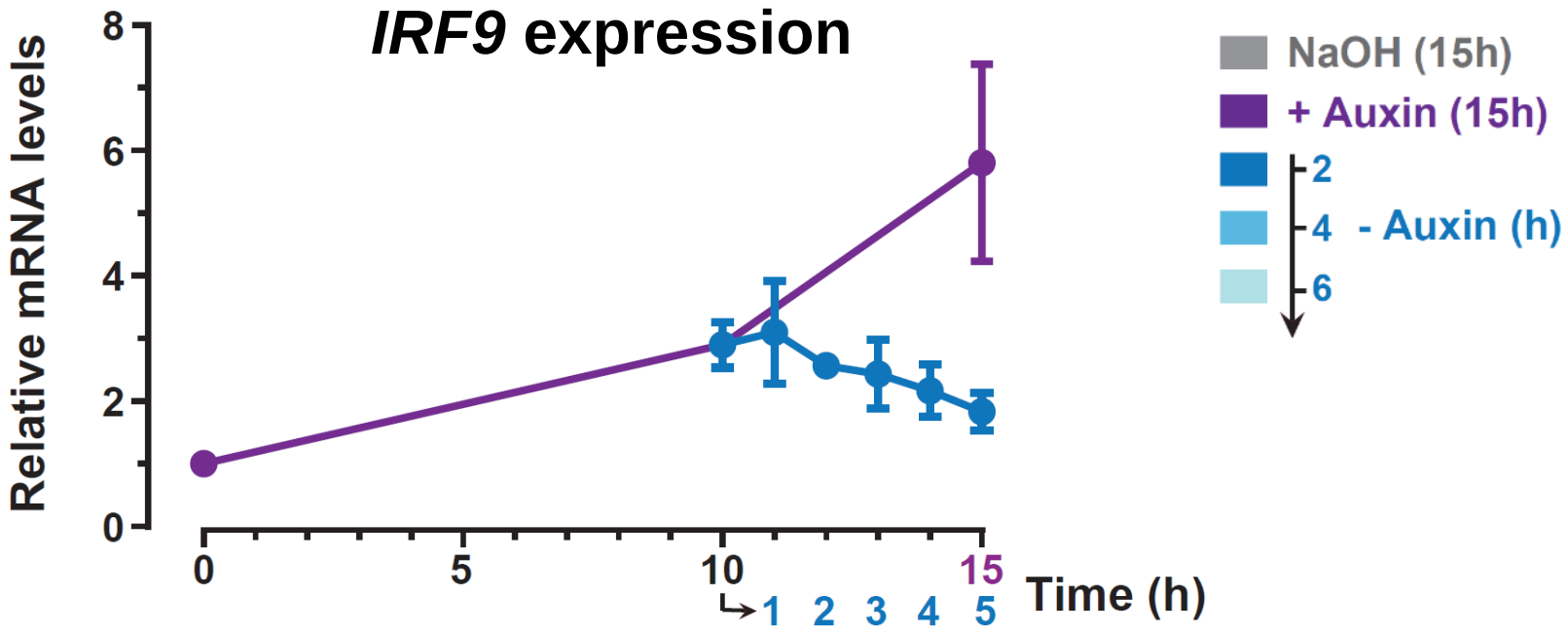


TRRAP binds the promoter of ISG master TFs.

Dynamics of ISG regulation by TRRAP

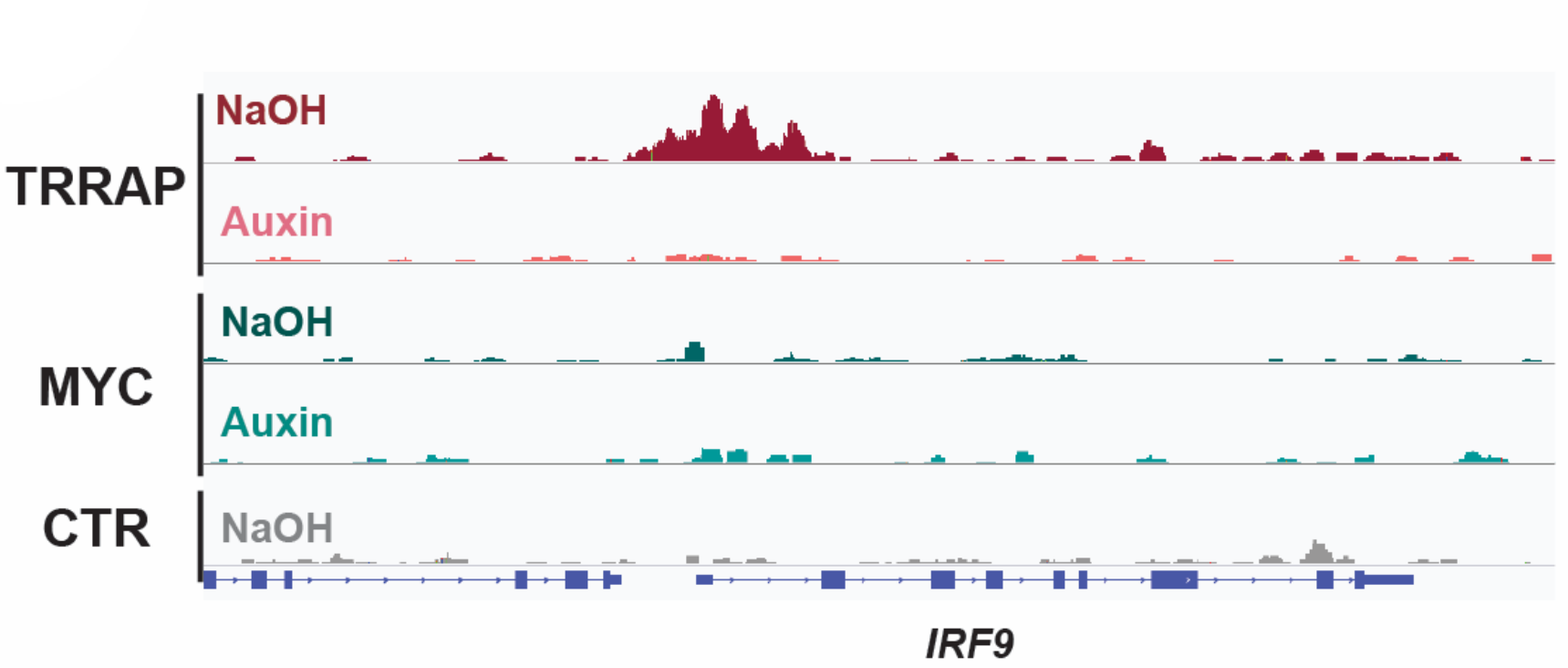


Dynamics of ISG regulation by TRRAP



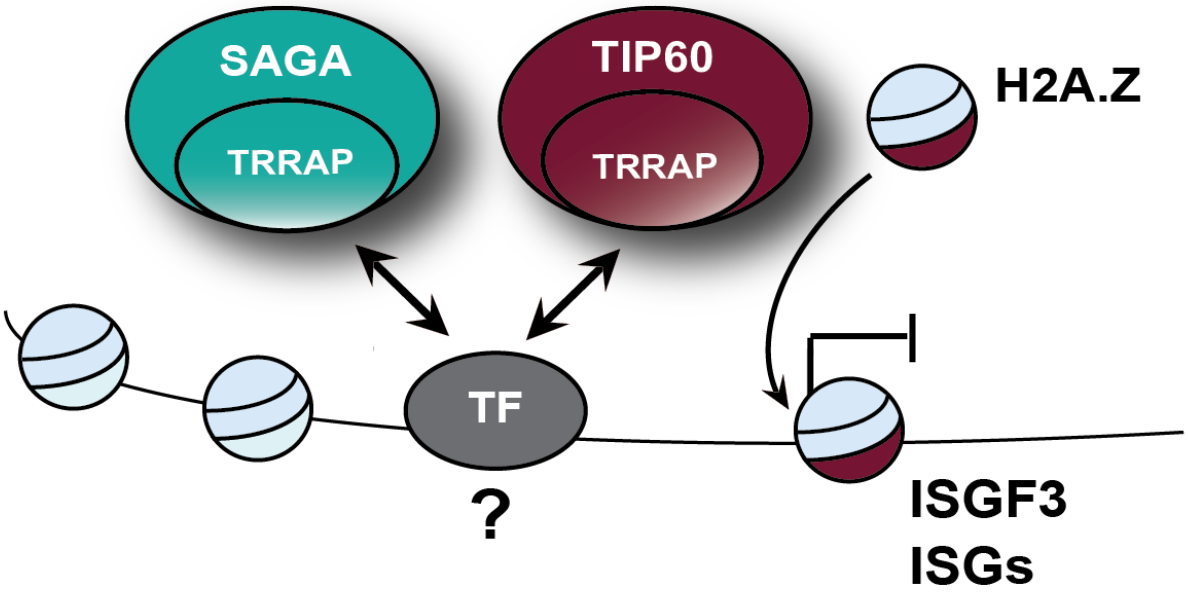
ISG expression is dynamically controlled by TRRAP.

Contribution of MYC



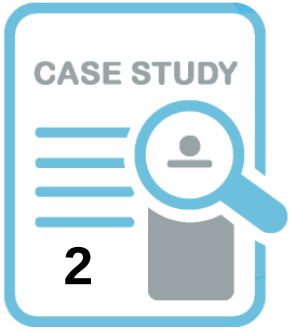
TRRAP binds to ISG promoters independently of MYC!

Conclusions & Perspectives



- **TRRAP directly represses interferon-responsive transcription factors in proliferating cancer cells.**
- **By which mechanisms? Recruited by which TFs?**

For further details:



eLife

RESEARCH ARTICLE

The TRRAP transcription cofactor represses interferon-stimulated genes in colorectal cancer cells

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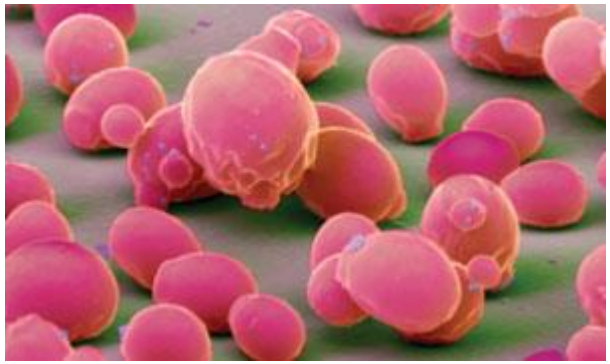
Yeast as model systems

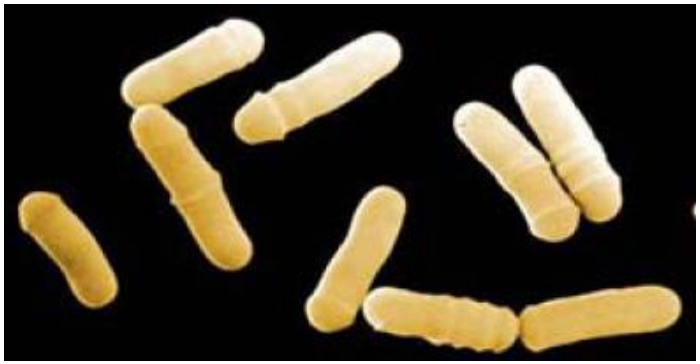
- **Unicellular Eukaryotes**
- **Version 1.0 of "*higher*" Eukaryotes**
- **Conservation of many factors / mechanisms**

Budding yeast
Saccharomyces cerevisiae



***cerevisiae* = beer in Latin**

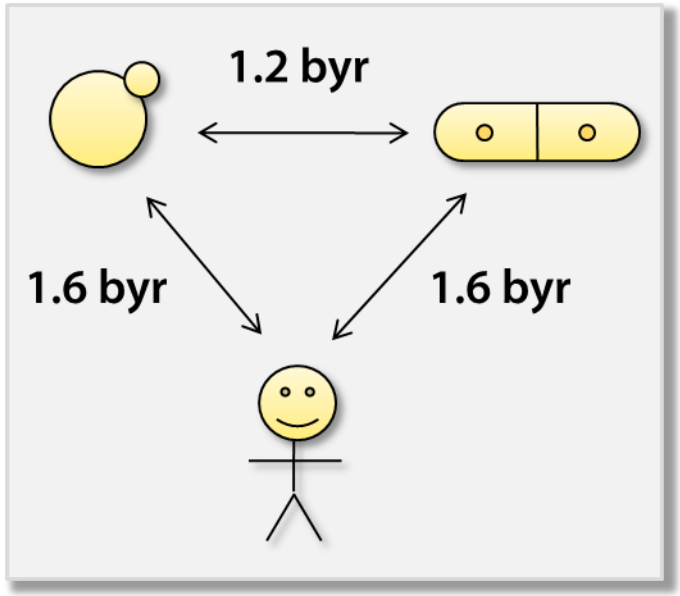
Fission yeast
Schizosaccharomyces pombe



***pombe* = beer in Swahili**

Some numbers

- Cell size: 4-12 mm
- Genome size: 12-14 Mb
- Gene numbers: 5000-6000
- Evolutionary distance:
- Timing:
  - Doubling time: few hours
  - Getting 10L culture: 24 hrs
  - Creating a novel mutant: 2 weeks



Their importance

- Bread, beer & wine (agriculture)
- Unicellular - very easy to manipulate
- Biomedical research:
  - Enzyme (1897)
  - Cell cycle (2001 Nobel)
  - Telomeres (2009 Nobel)
  - Trafficking (2013 Nobel)
  - Autophagy (2016 Nobel)
  - Epigenetics: histone-code, RNAi
  - Role of aneuploidy / genome instability in cancer
  - Diagnosis tool in breast cancer (BRCA1)
  - Gene therapy concept

The power of yeast genetics

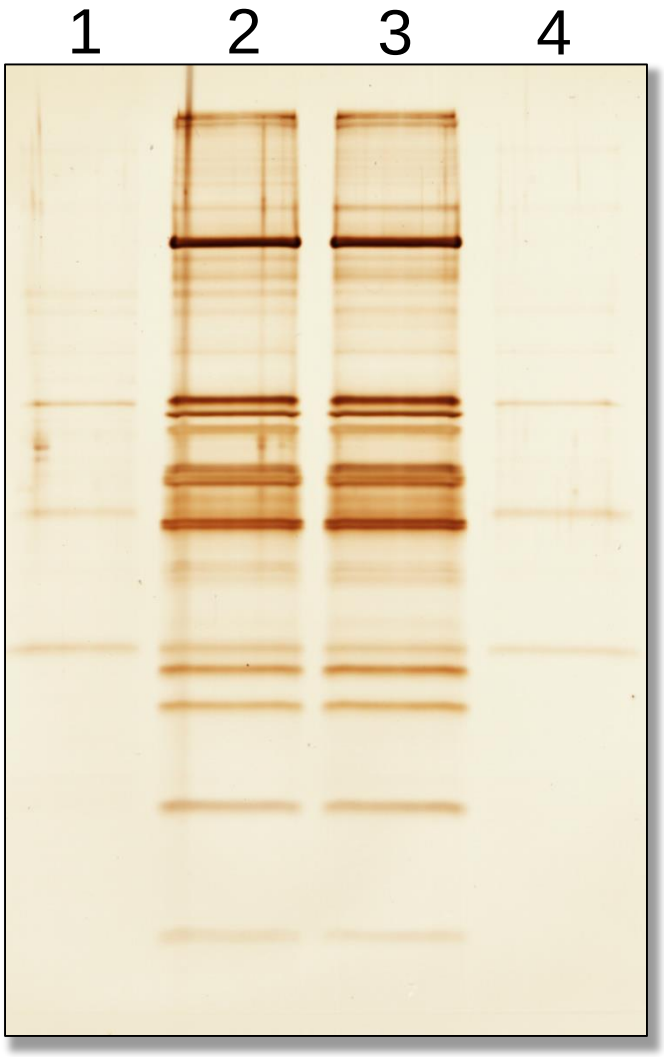
- **Conjugation of haploid yeast cells ➔ diploid zygote**
- **Diploids enter meiosis ➔ 4 haploid spores, physically kept within one bag (ascus)**
- **Each ascus has the 4 products of ONE meiosis (Mendel laws live!)**
- **Mutants are easy to create, propagate, and analyze**

This the power of yeast genetics!

Subunit composition depending on nutrient availability

Lane 1: no tag
Lane 2: *ada1-TAP*] Rich

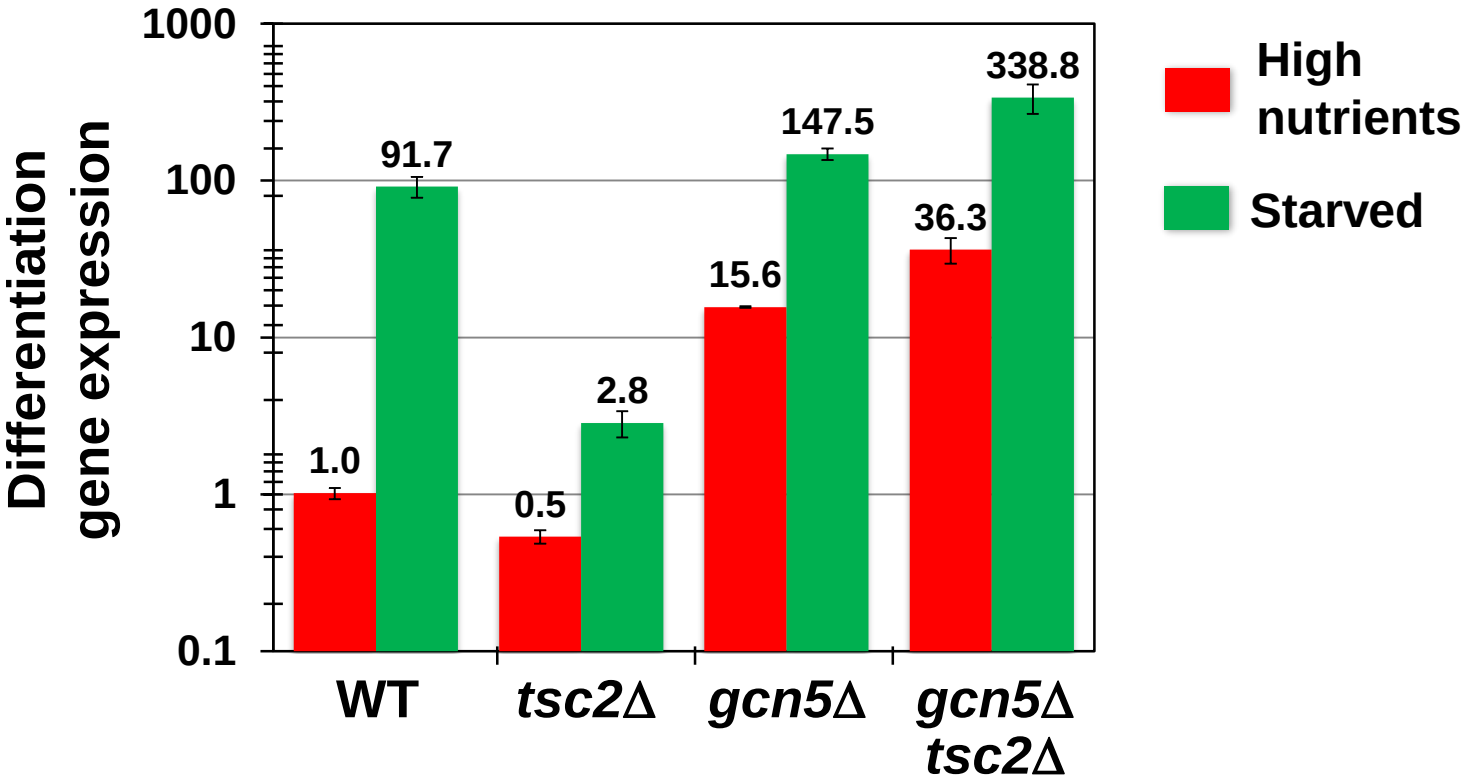
Lane 3: *ada1-TAP*
Lane 4: no tag] Starved



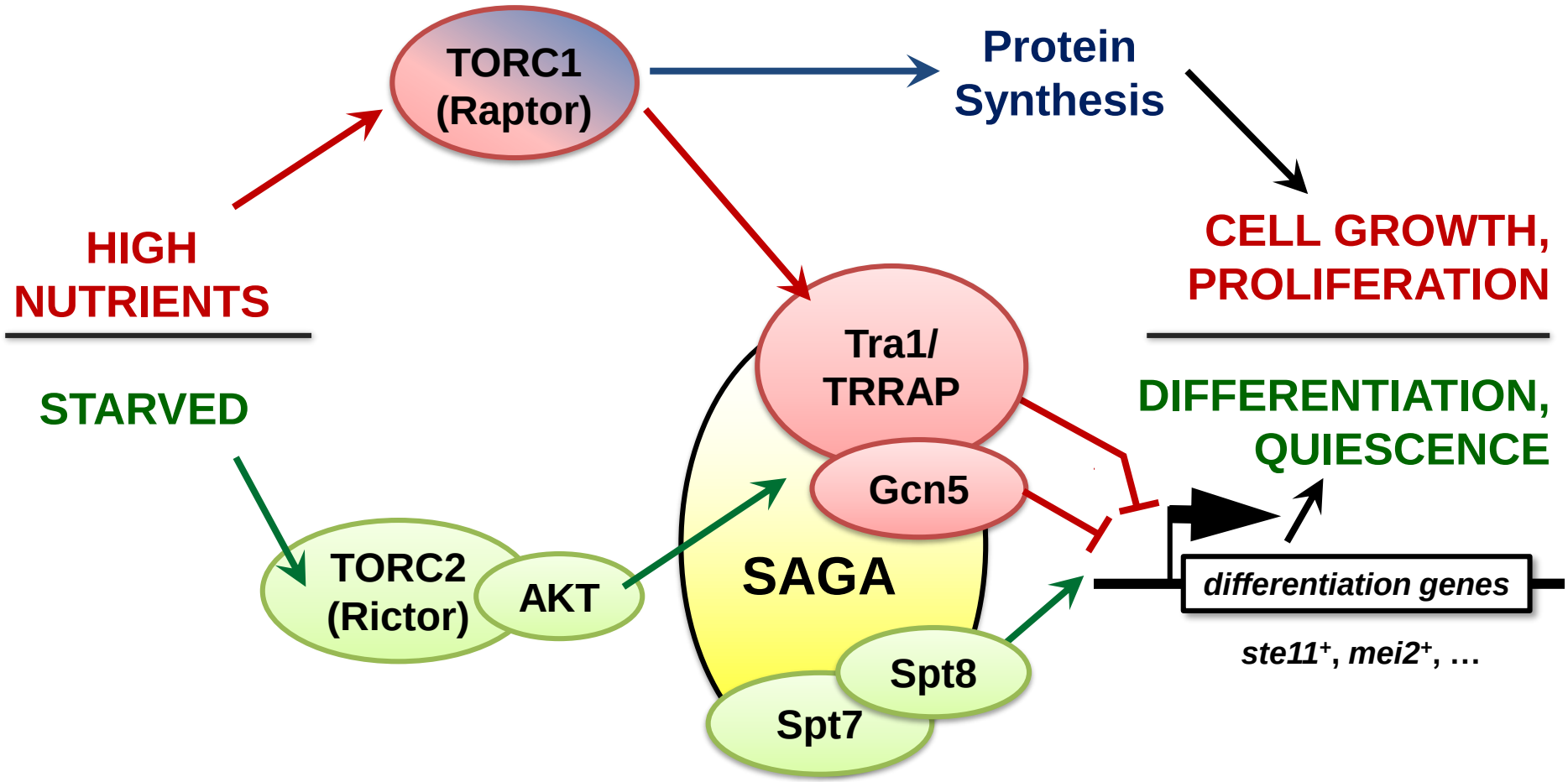
SAGA subunit composition is identical between rich and starved conditions.

Gcn5 functions downstream of TORC1 to repress differentiation

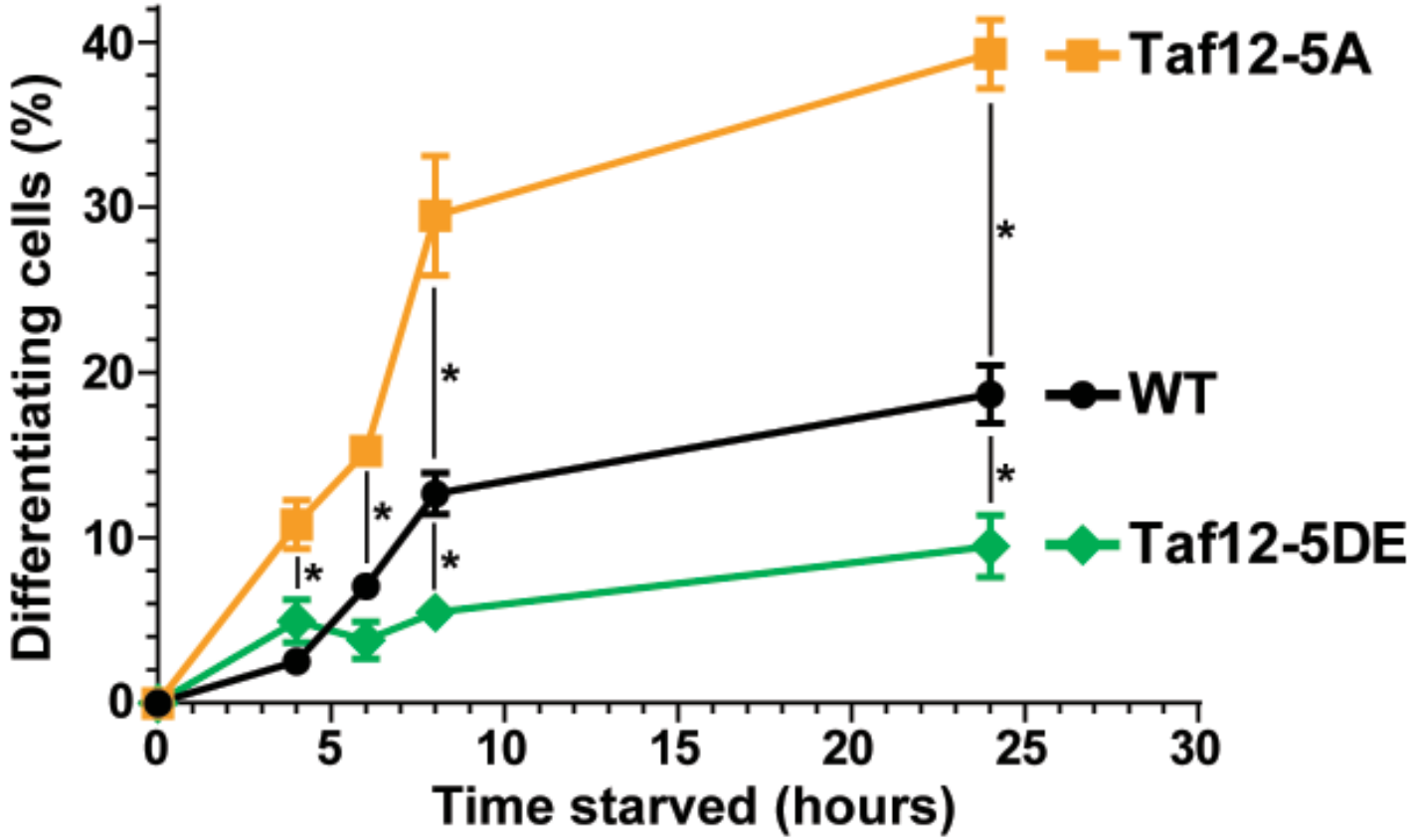
High nutrients → AKT ⊣ Tsc1 Tsc2 ⊣ Rhb1 → TORC1 ⊣ Differentiation



Functional interactions between TORC1, TORC2, and Gcn5



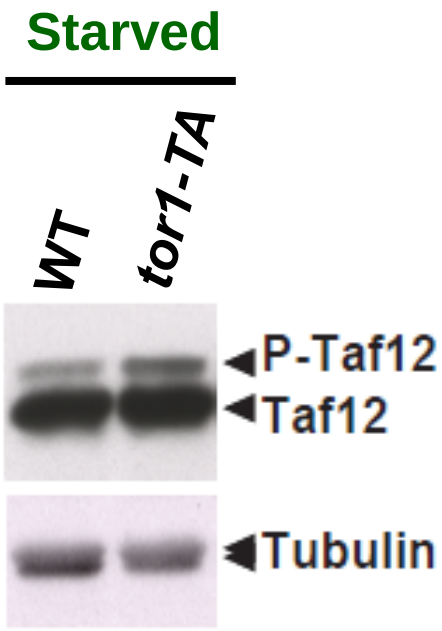
Taf12 phosphorylation inhibits differentiation upon nutrient starvation



Increased TORC2 activity negatively regulates differentiation, through Taf12 phosphorylation

Both loss of Tor1 AND higher Tor1 activity reduces differentiation.

(Halova D *et al*., J. Cell Biol., 2013)



Genotype	Differentiating cells
WT	39 ± 1%
tor1-T1972A	25 ± 2%
taf12-5A	50 ± 4%
tor1-T1972A taf12-5A	55 ± 2%

Conclusions

1. **SAGA functions downstream of both the TORC1 and TORC2 signaling pathways to regulate differentiation.**

2. **Taf12 phosphorylation is tightly and rapidly controlled by the opposing activities of TORC1-PP2A and TORC2-AKT.**

3. **Suggest thats TORC2 both activates and inhibits differentiation, reminiscent of an INCOHERENT feed-forward loop.**

TRRAP occupancy at ISG promoters