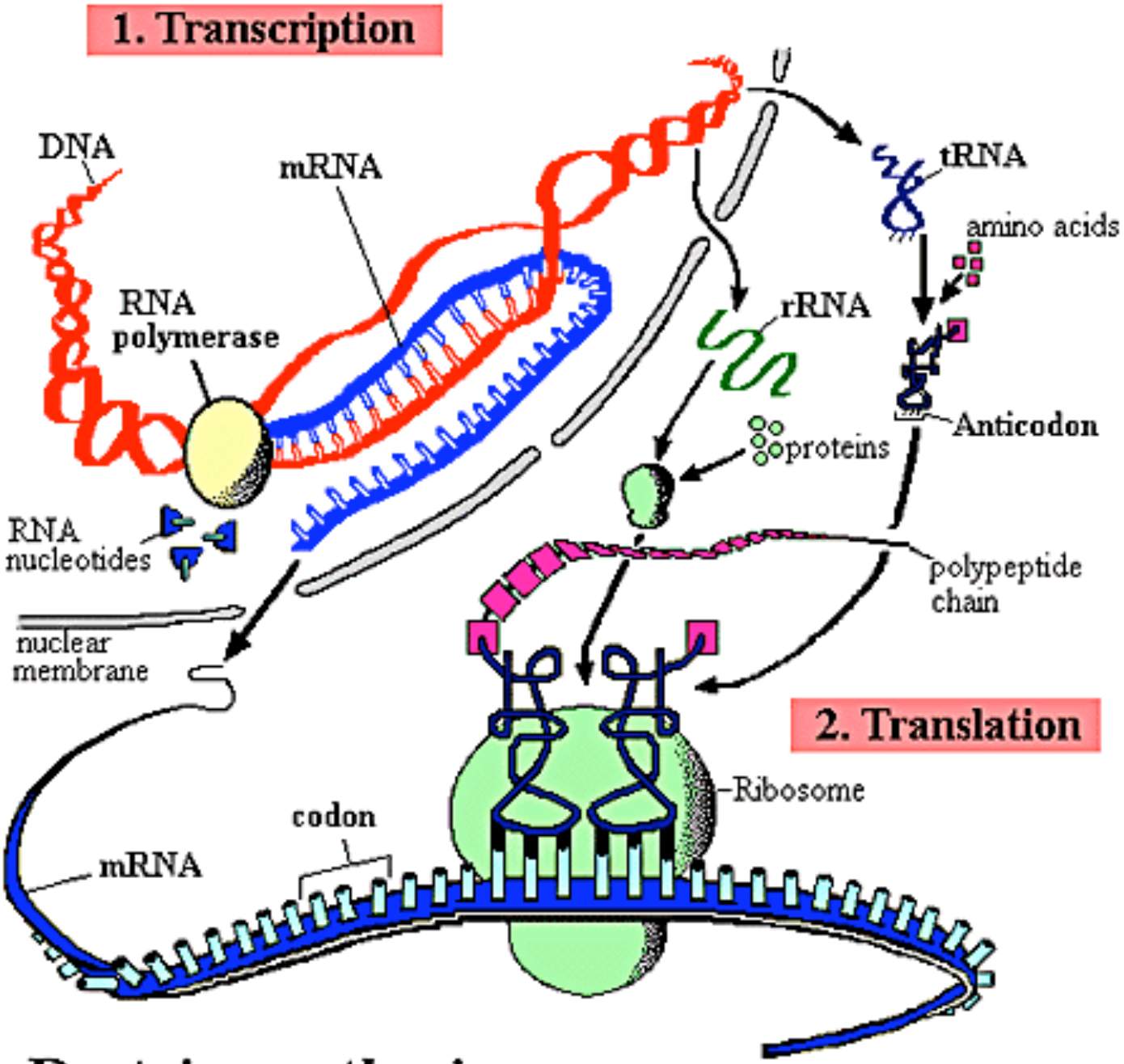
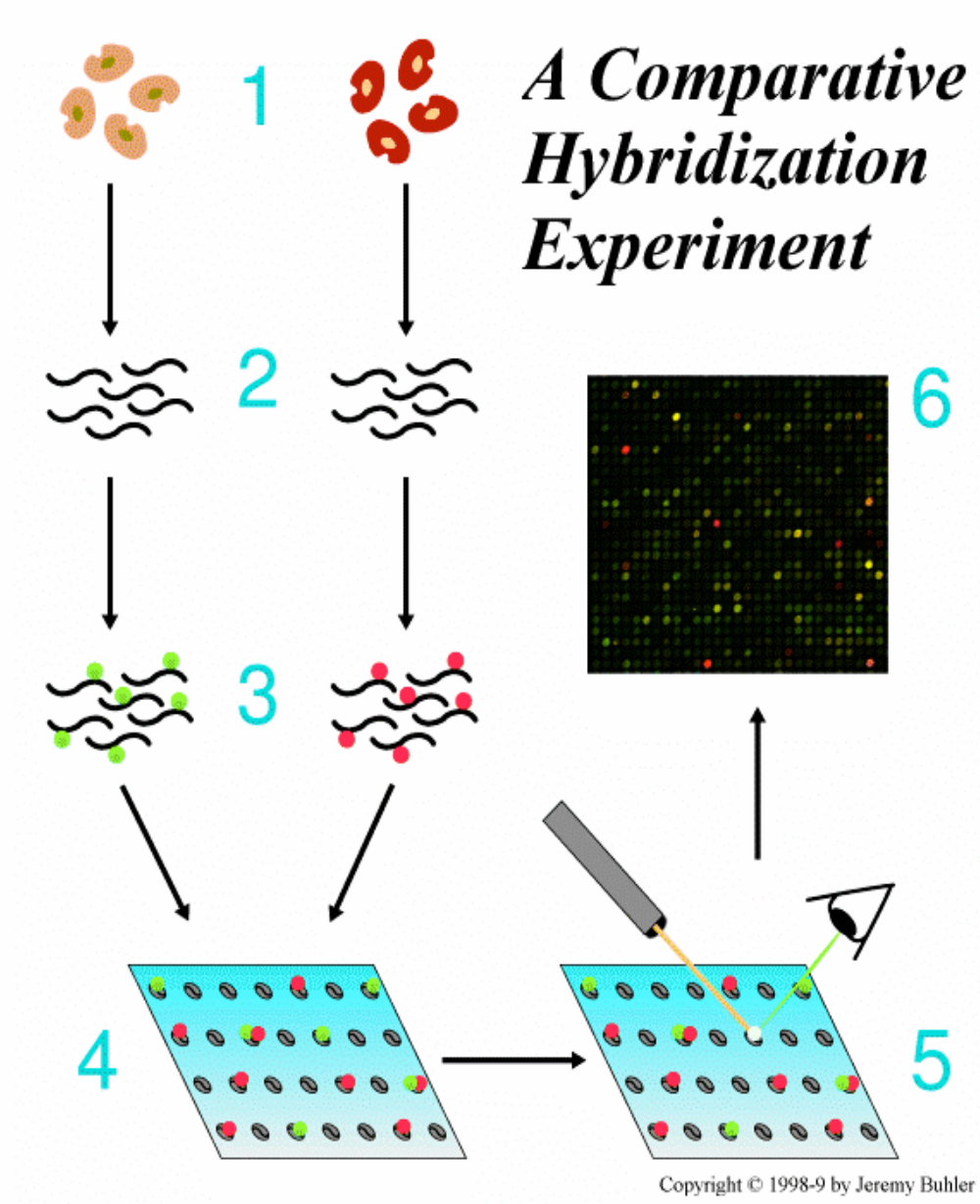
Condition-specific regulation of mRNA stability in yeast

Harmen J. Bussemaker

Department of Biological Sciences & C2B2
Columbia University, New York City



Protein synthesis


A Comparative Hybridization Experiment
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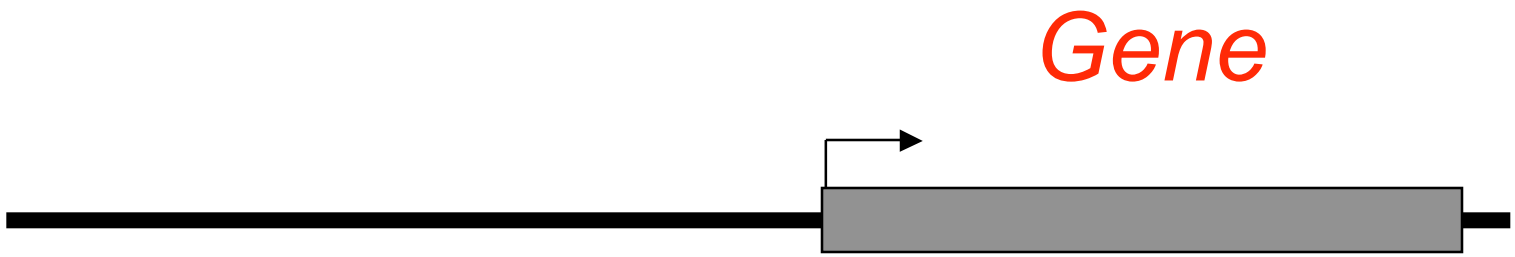
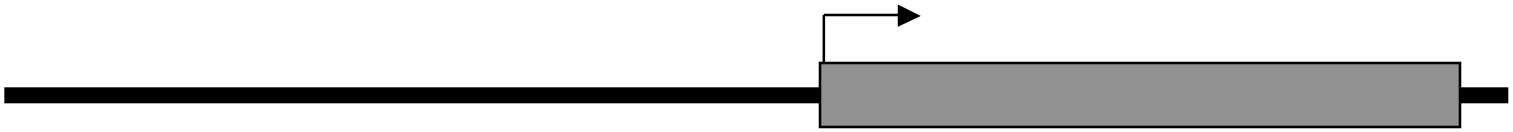
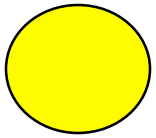

Gene

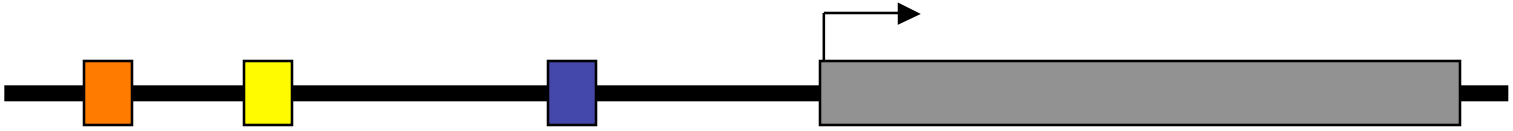
Upstream Region

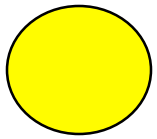


Cis-Regulatory Elements



Trans-Acting Factor
(DNA-binding protein)

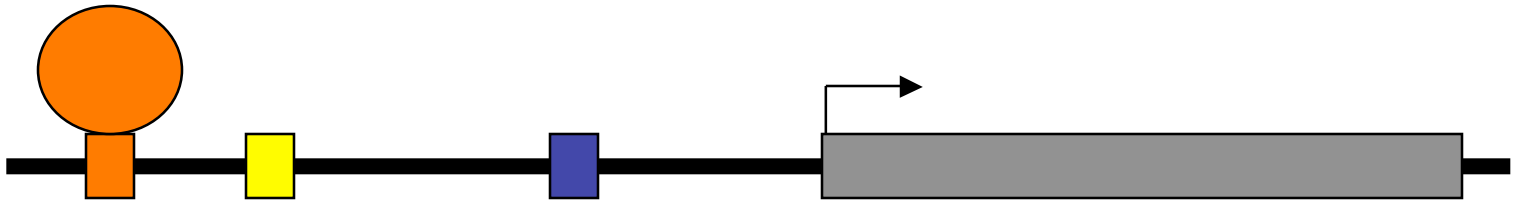




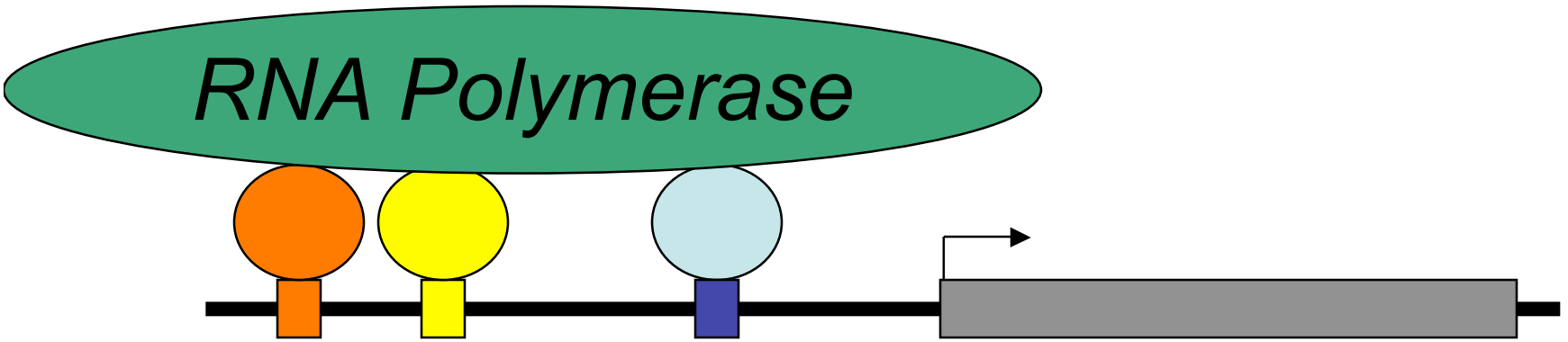
Cytoplasm

Nucleus

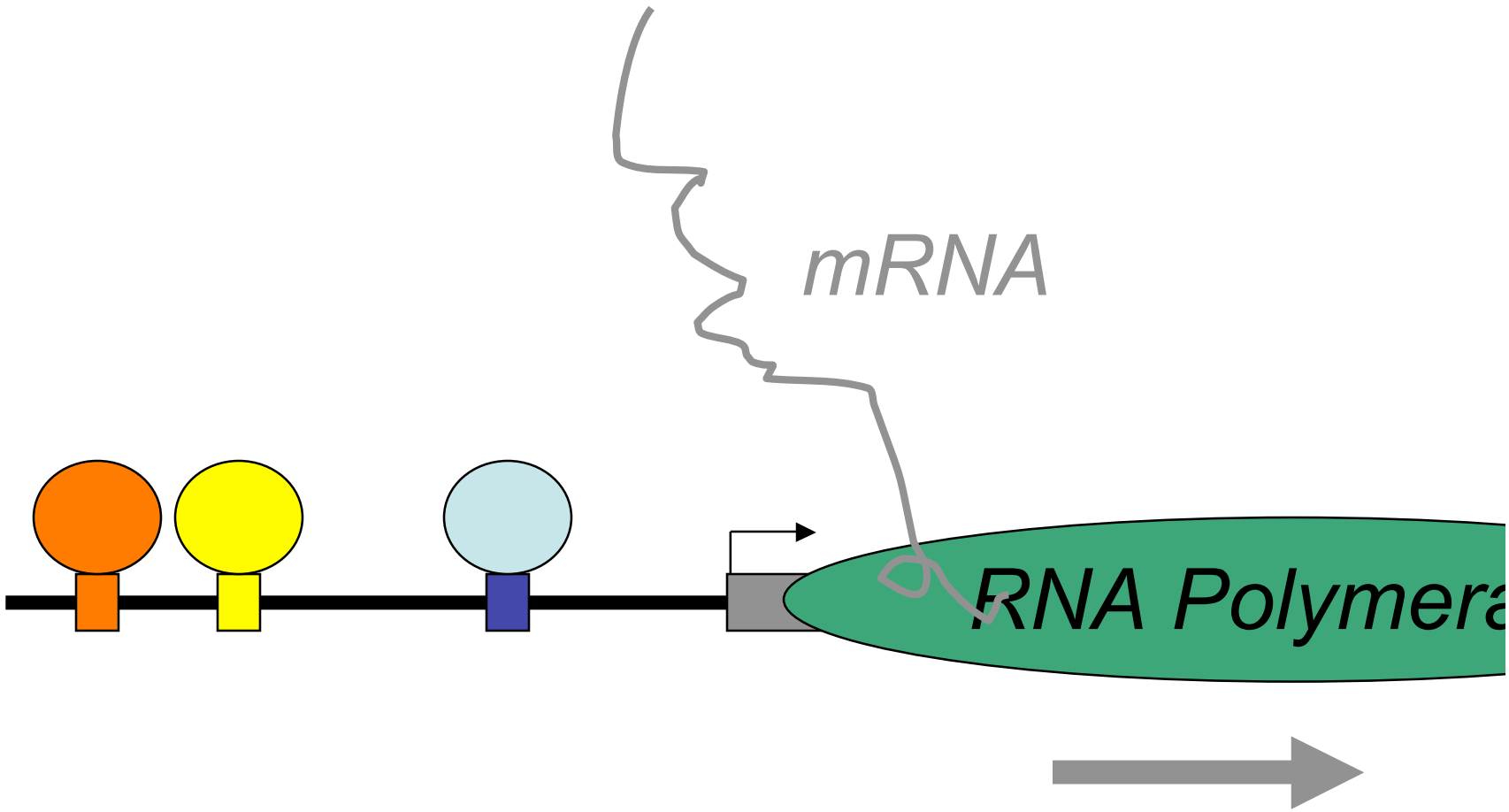
Occupancy by Transcription Factors



Recruitment of RNA Polymerase II



Transcriptional Activation

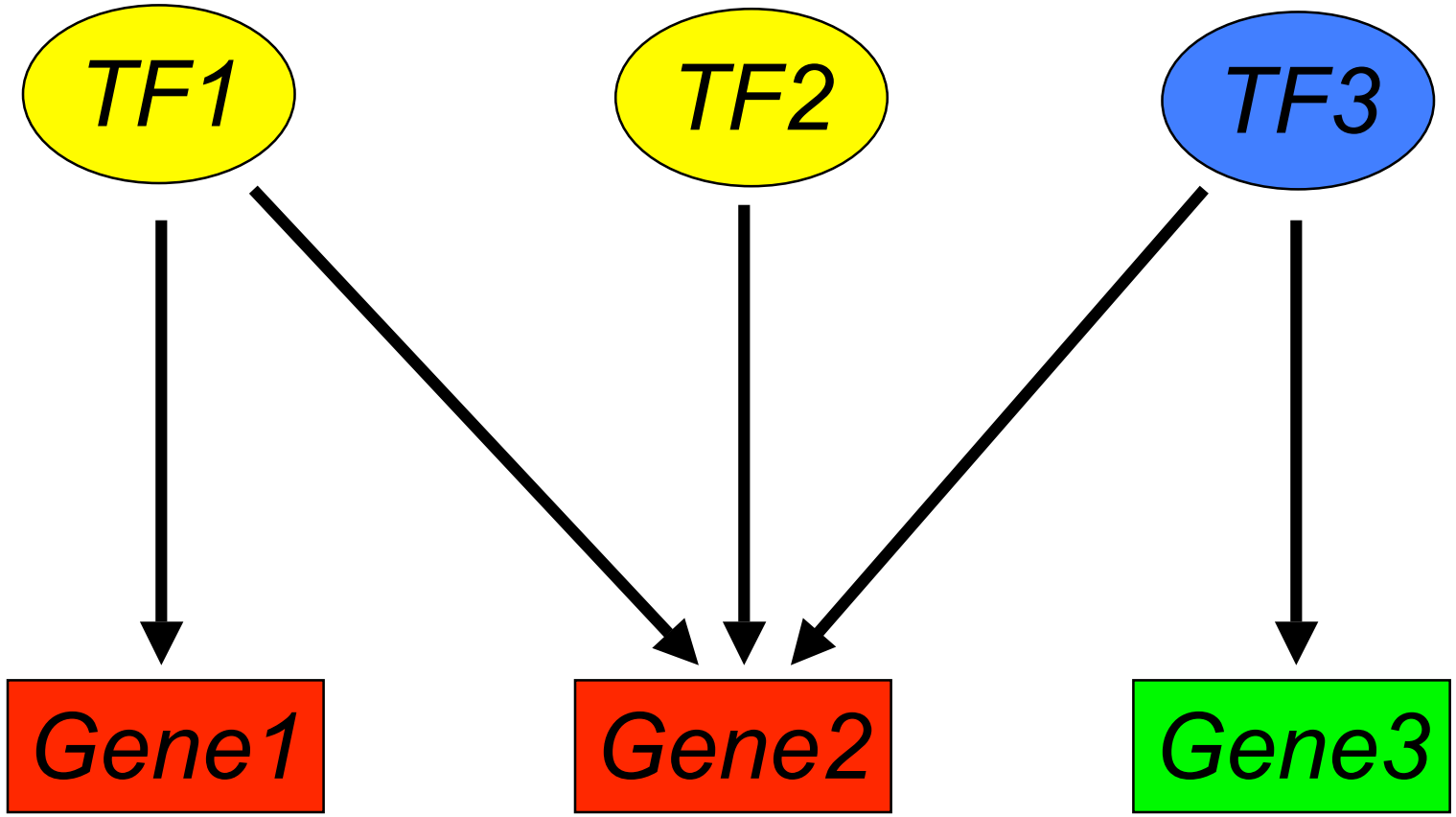


Our Main Goal:

- Can we predict the effect that mutating any non-coding sequence has on the mRNA expression levels in a gene-specific as well as a condition-specific manner?

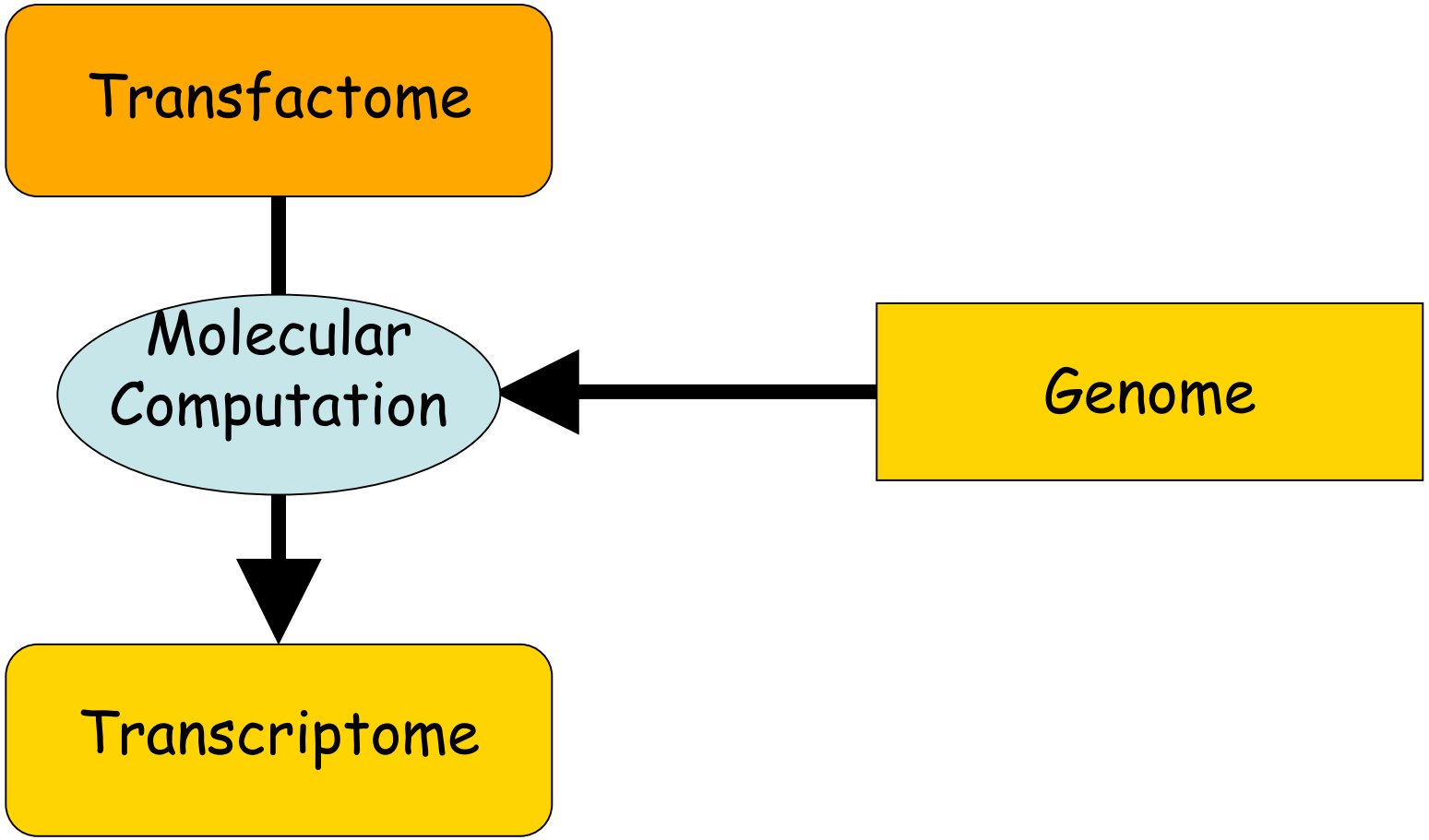
- Ingredients of our modeling:
  - Sequence specificity of DNA/RNA binding TFs
  - Gene-specific promoter region & 3'-UTR
  - Condition specific "hidden" TF activities

Hidden, protein-level TF activities

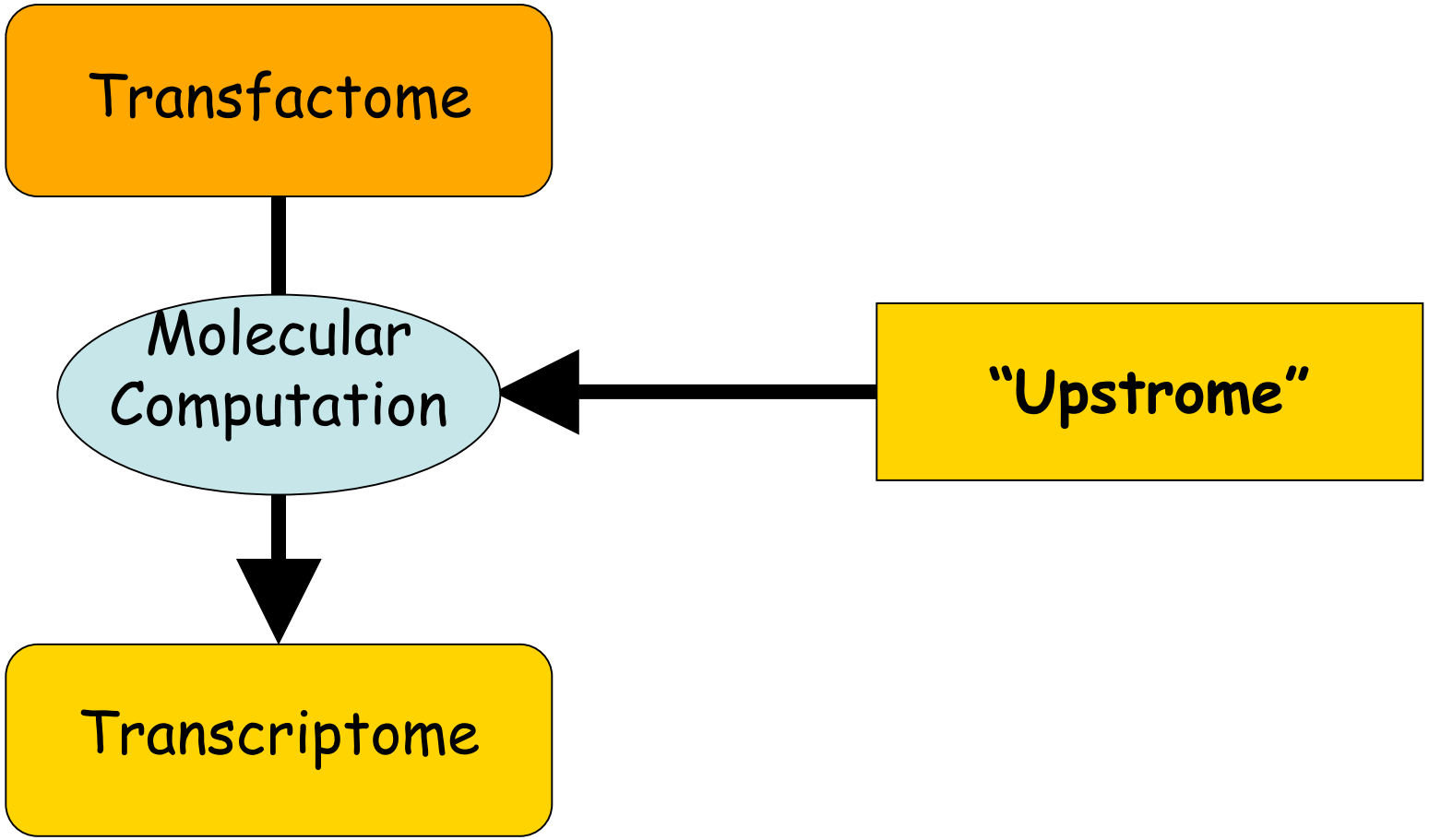


Measured mRNA abundances

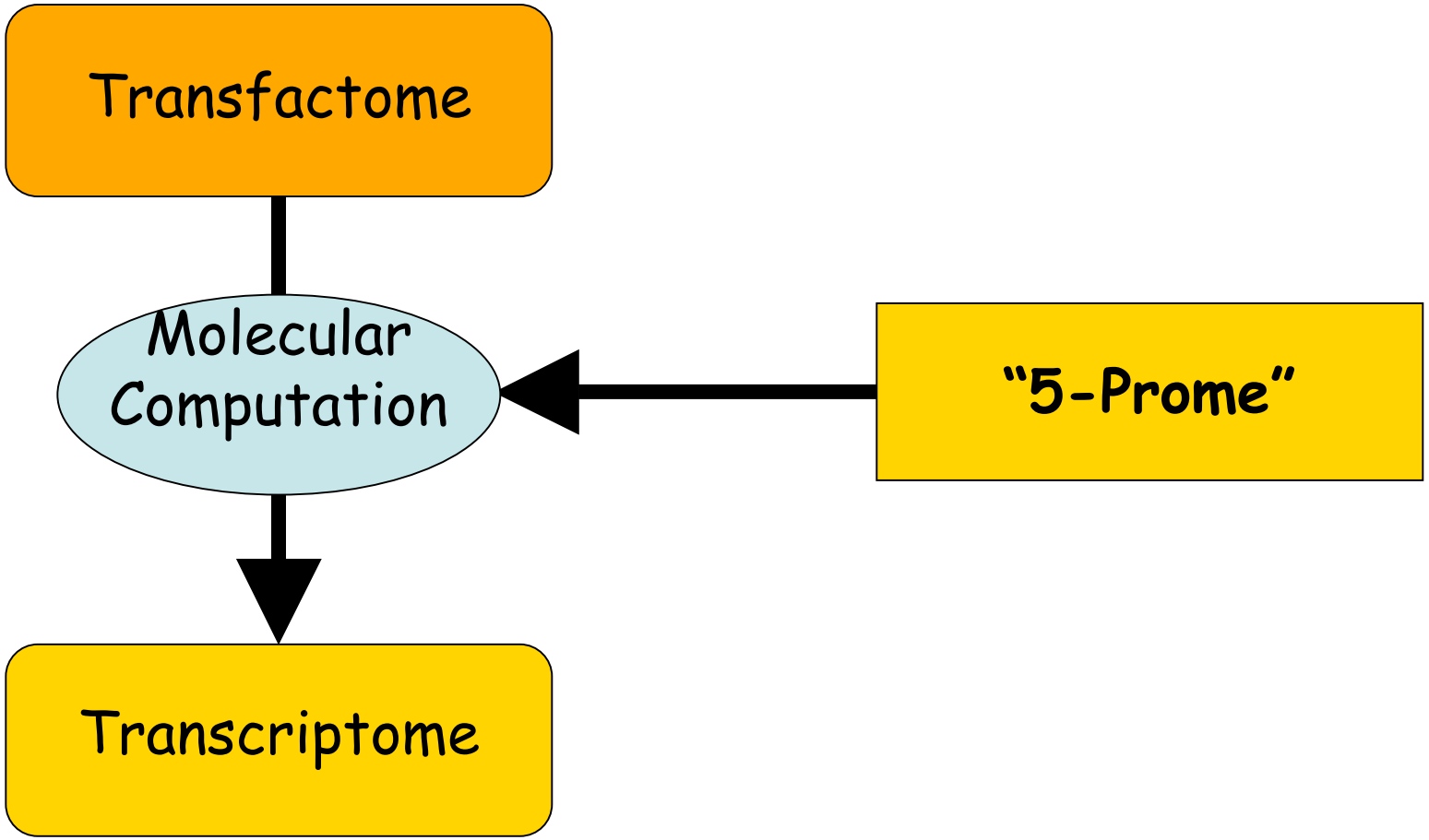
"Transfactome": condition-specific activities of all TFs



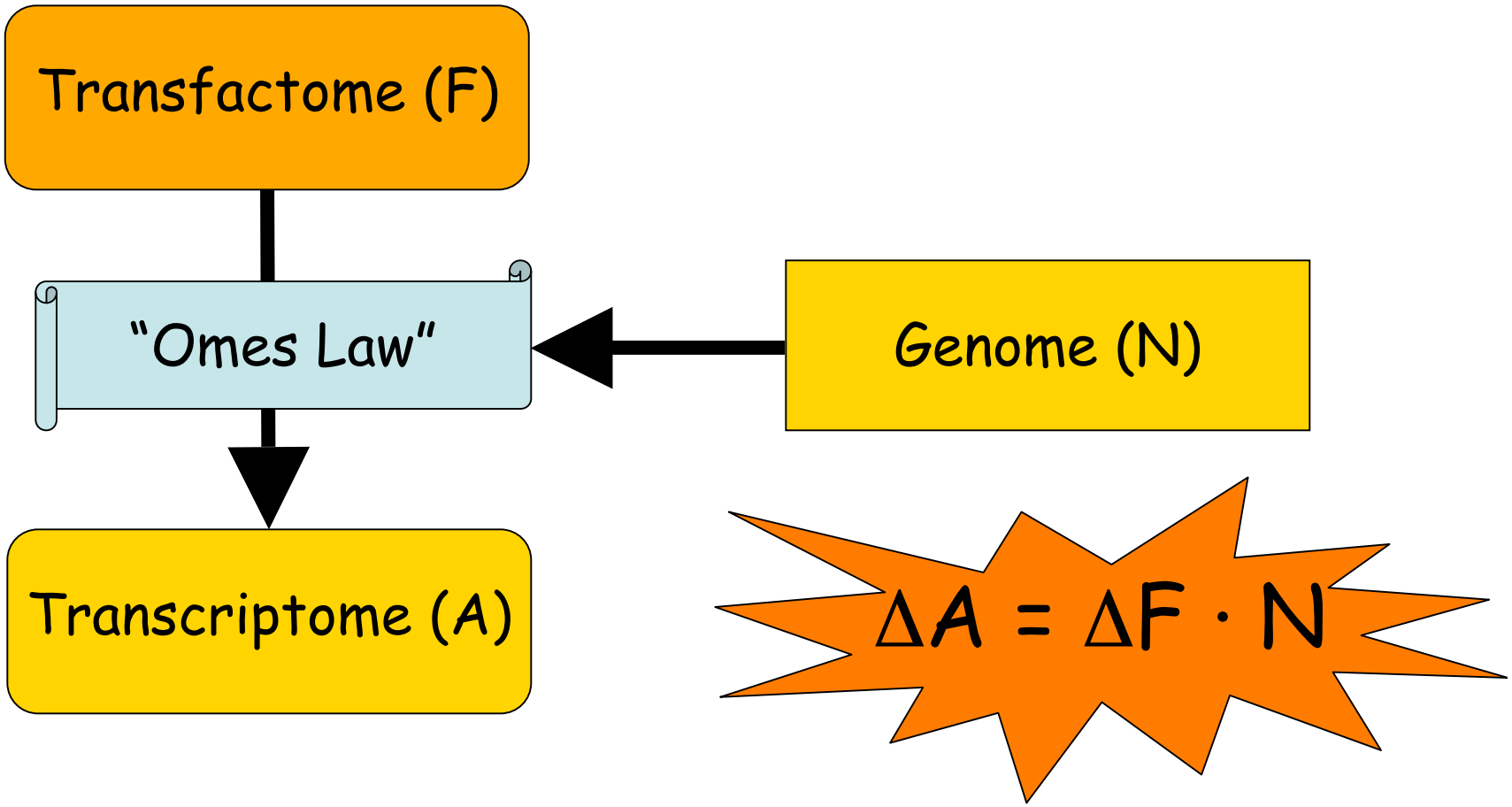
"Transfactome": condition-specific activities of all TFs



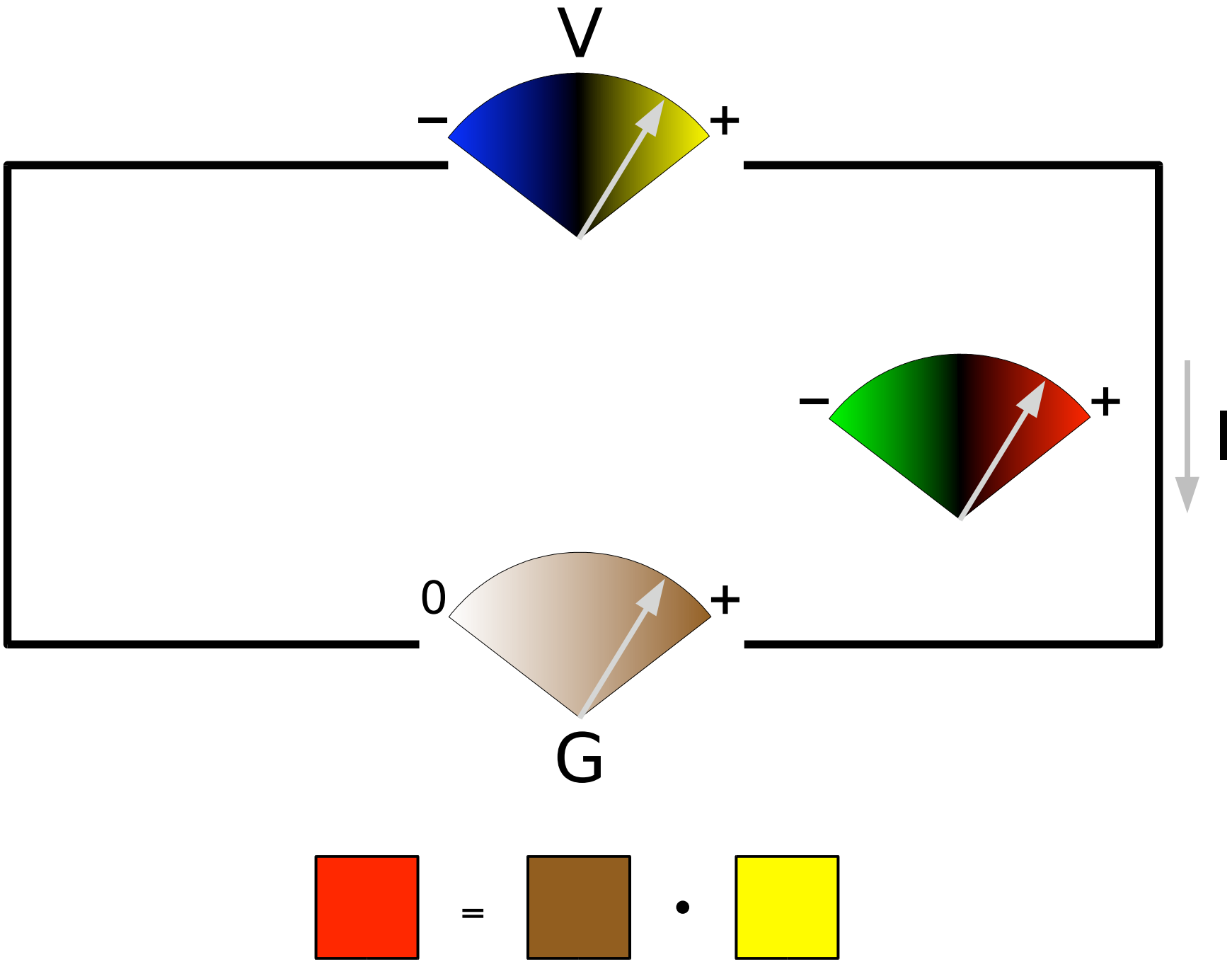
"Transfactome": condition-specific activities of all TFs



Linear Response Theory for Cells: "Omes Law"



"Electromics"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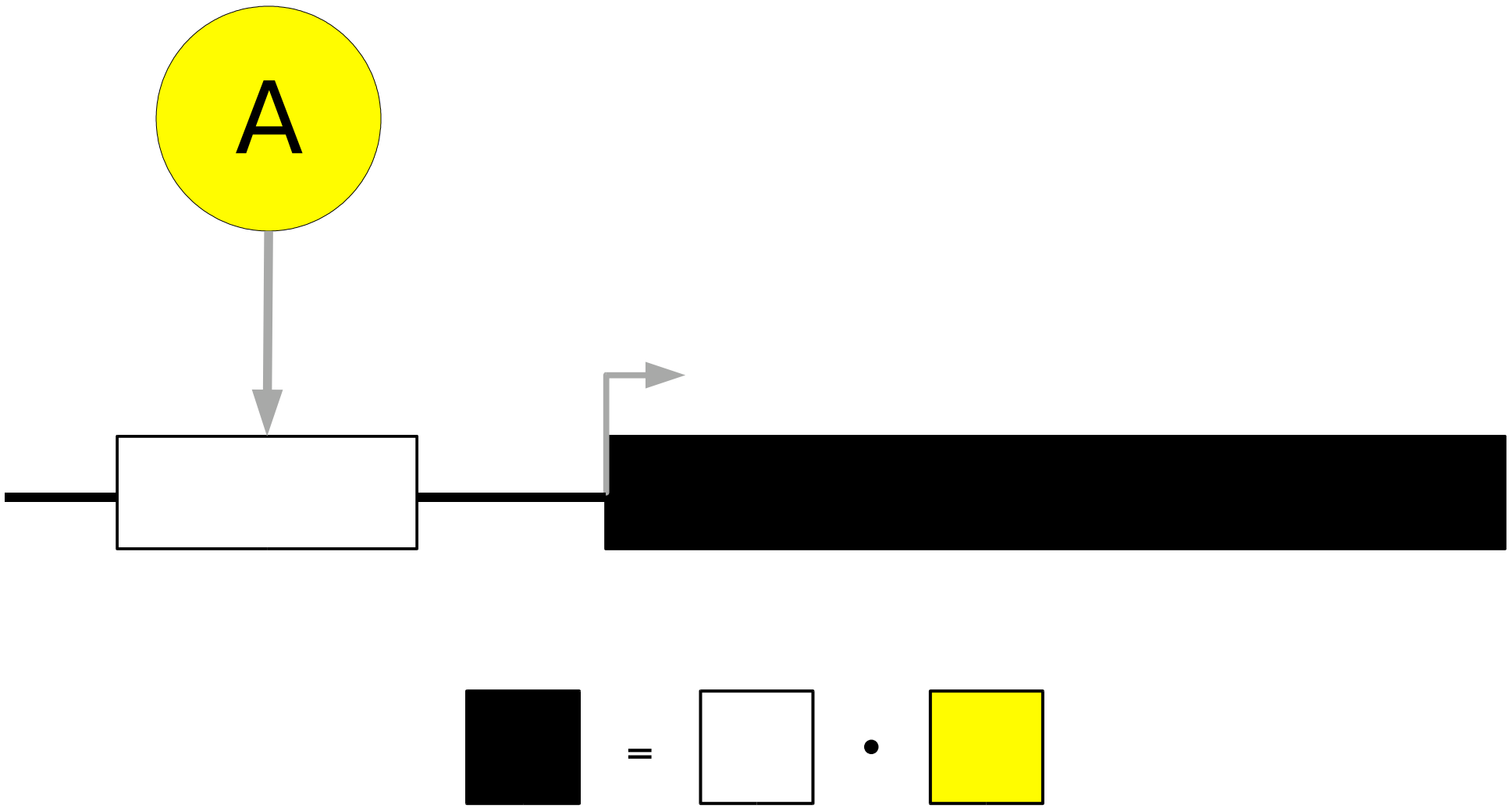
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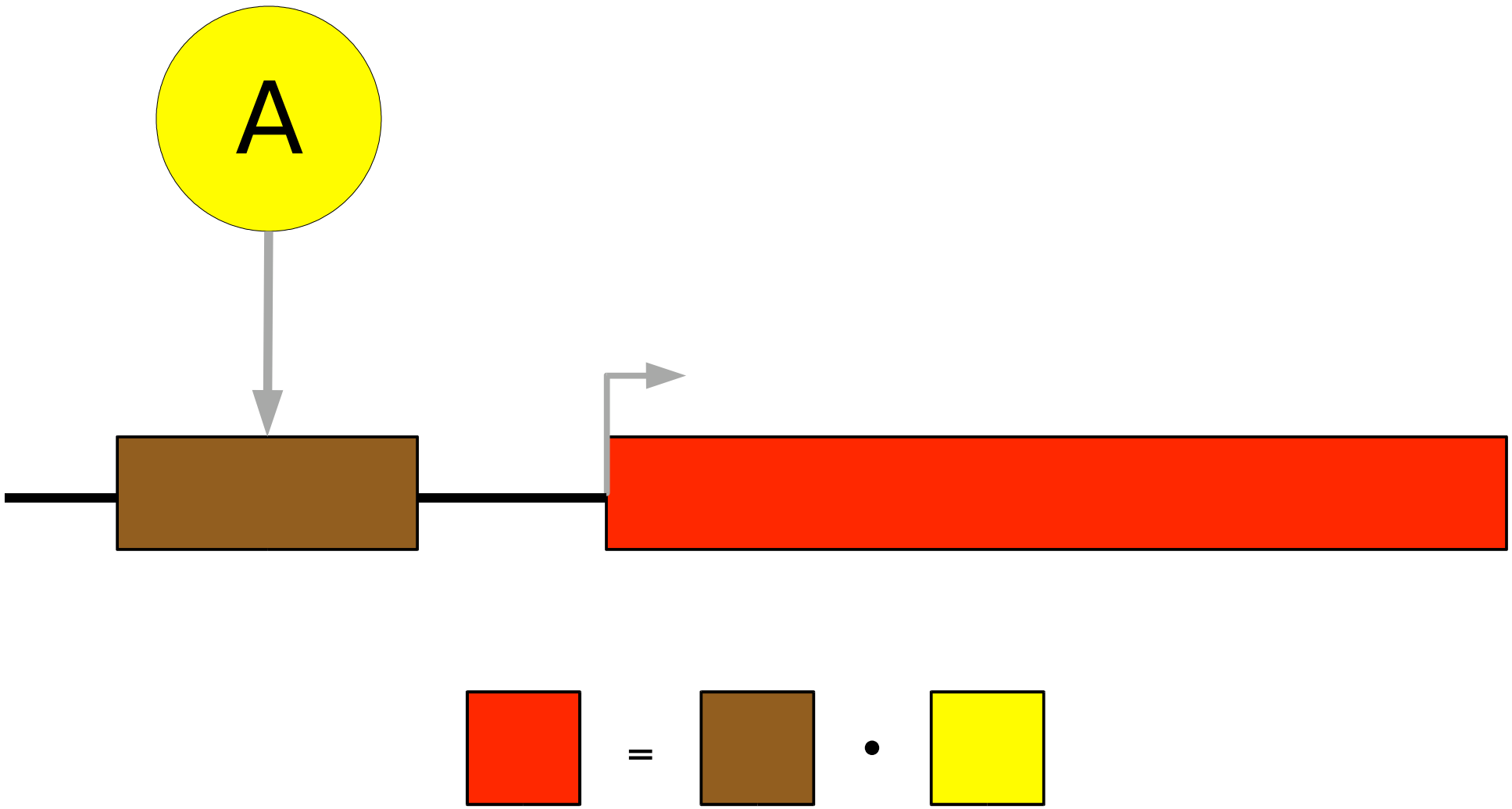
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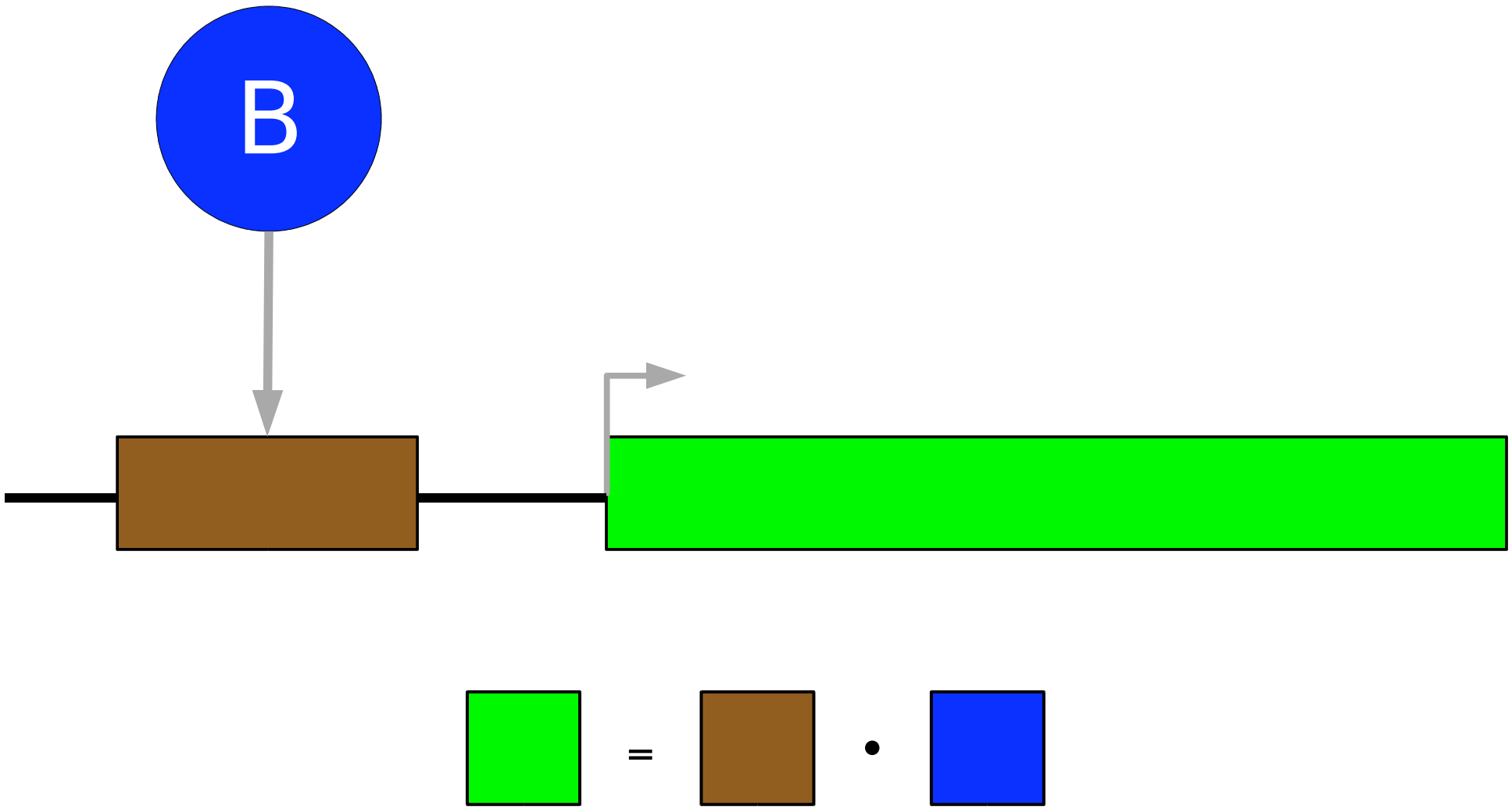
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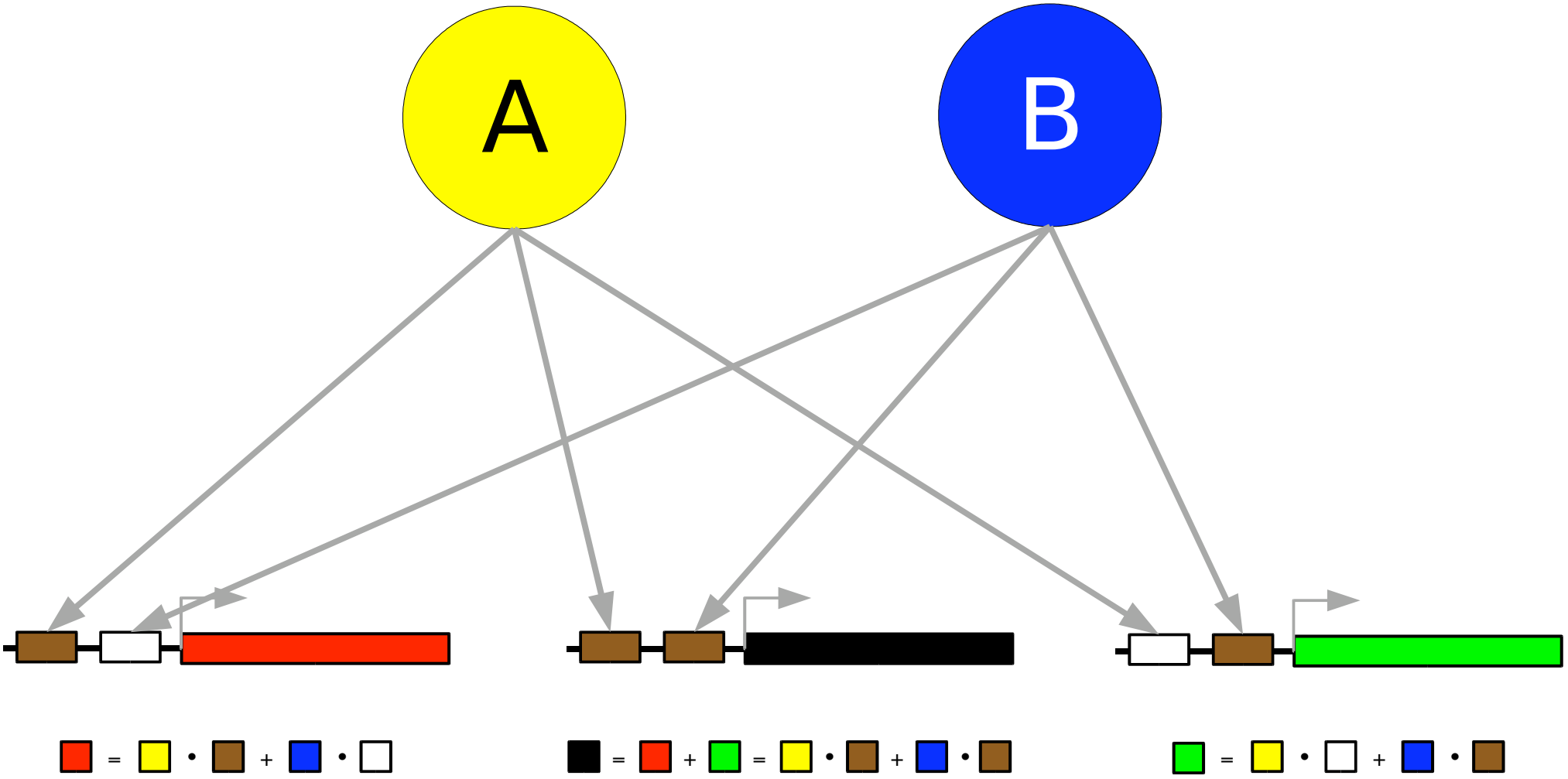
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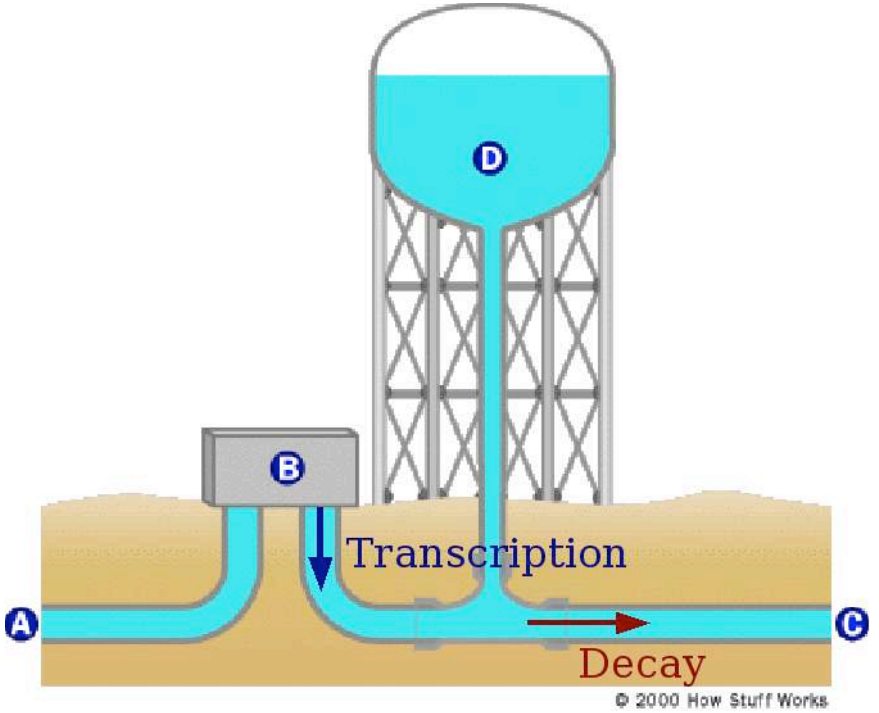
Modeling Condition-Specific Regulation of mRNA Stability in Yeast



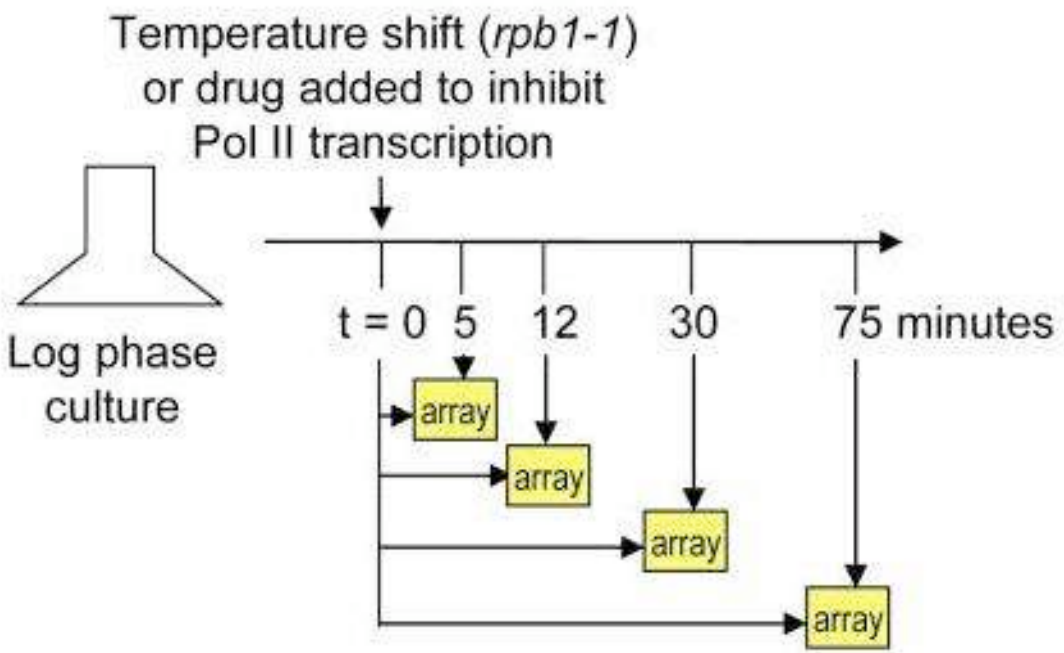
"Hydromics"

"H_2Omics"

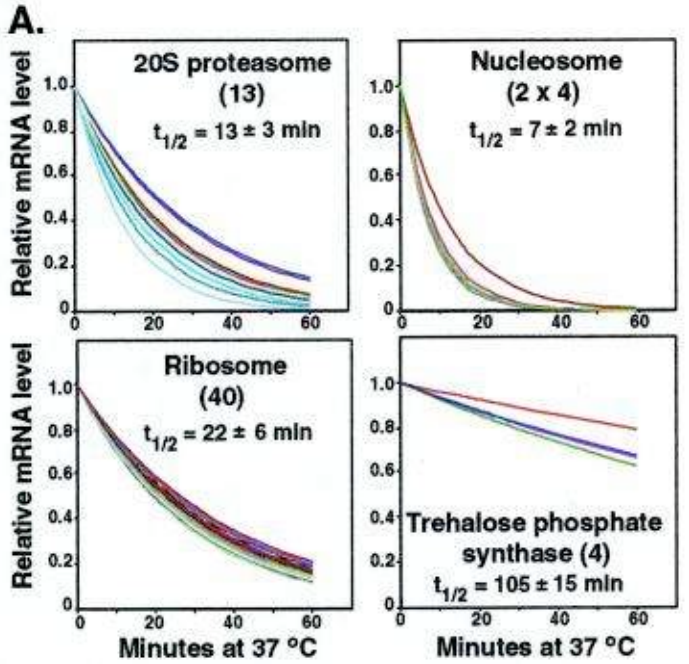
"Eaumics"

Transcriptional Arrest Microarray Experiments

Eight studies in various organisms (two in yeast) measured steady state decay rates via a transcriptional arrest microarray experiment.



Grigull, J., *et al.* (2004) Mol. Cell Biol. 24, 5534-5547.

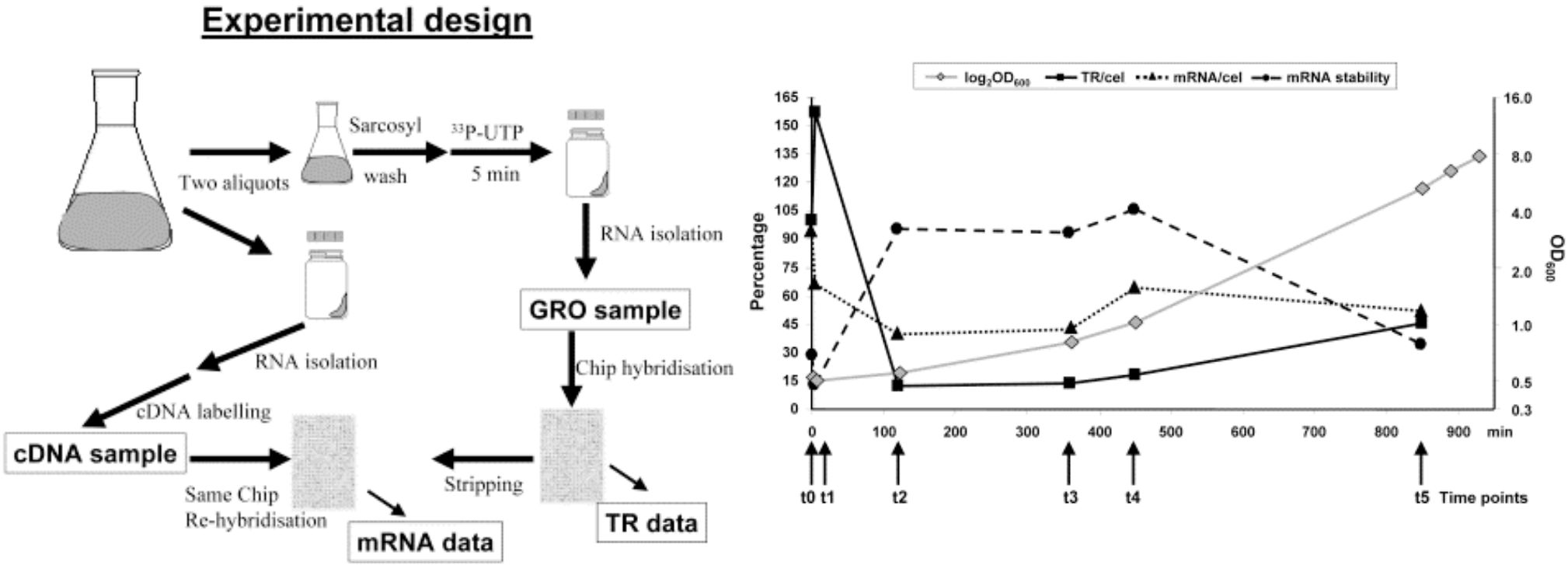


Wang, Y., *et al.* (2002) Proc Natl. Acad. Sci. USA 99, 5860-5865.

They commonly show that decay rates are coordinated among functionally related genes.

Genomic Run-On Microarray

Garcia-Martinez *et al.* inferred decay rates from discrepancies between transcription rate and steady state mRNA abundances.

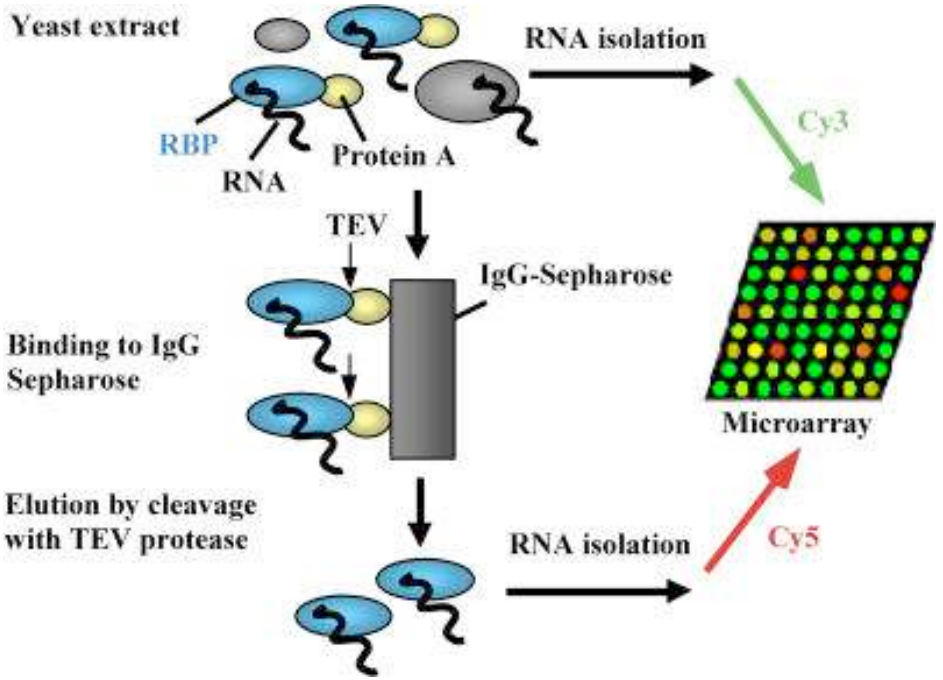


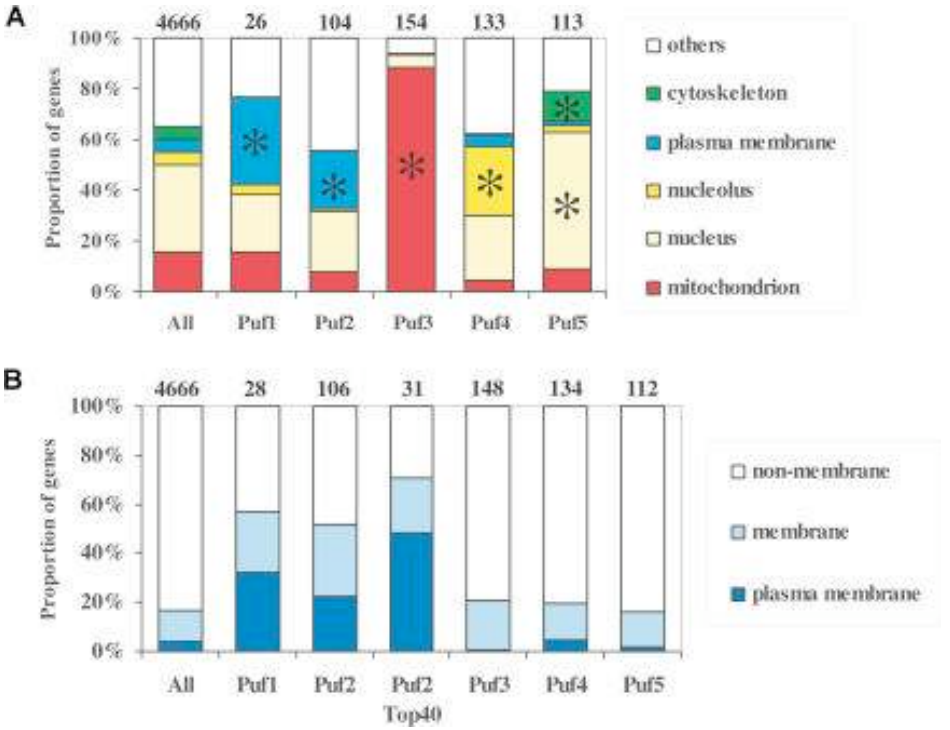
Garcia-Martinez, J., Aranda, A. & Perez-Ortin, J.E. (2004) Mol. Cell 15, 303-313.

The authors provided evidence for dynamic regulation of mRNA stability but did not identify any responsible cis-regulatory elements or trans-factors.

Puf Protein Affinity Selection Microarray Experiment

Gerber *et al.* performed an affinity selection microarray experiment (analogous to ChIP-chip) on the five yeast Puf proteins to identify target mRNAs.





Gerber, A.P., Herschlag, D. & Brown, P.O. (2004) PLoS Biol. 2, E79.

They showed functional biases in the targets of the Pufs, but provided no evidence for regulation of mRNA stability.

Barrett Foat

Finding More Evidence for mRNA Stability Regulation

$$\frac{d}{dt}[mRNA] = \alpha - \tau[mRNA] = 0$$

$$\log_2\left(\frac{[mRNA]_g^{\text{red}}}{[mRNA]_g^{\text{green}}}\right) = \log_2\left(\frac{\alpha_g^{\text{red}}}{\alpha_g^{\text{green}}}\right) - \log_2\left(\frac{\tau_g^{\text{red}}}{\tau_g^{\text{green}}}\right)$$

Biophysical motivation

$$N = \frac{[TF]}{[TF] + K} \approx \frac{[TF]}{K}$$

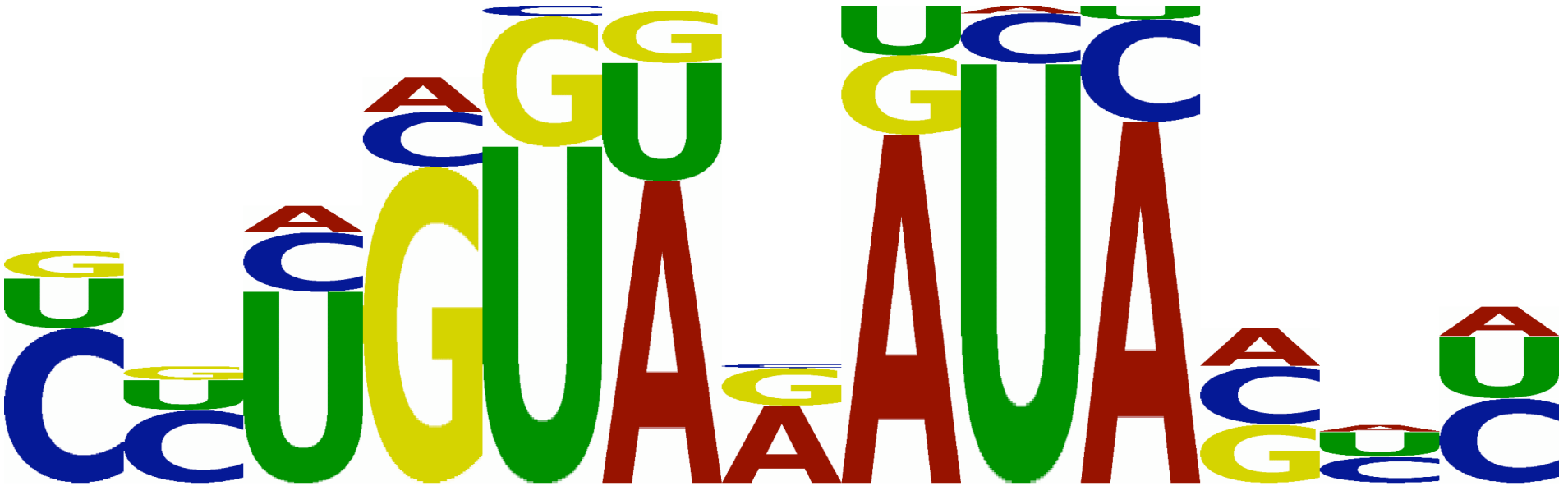
$$\frac{K(S_{optimal})}{K(S)} = \sum_{j=0}^{L-1} w_{j\sigma_j} \qquad S = \sigma_0 \sigma_1 \cdots \sigma_{L-1}$$

$$0 < w \leq 1$$

N = occupancy of specific site on DNA/RNA
K = dissociation constant
[TF] = trans-factor concentration

Position-Specific Affinity Matrices (PSAMs)

	0	1	2	3	4	5	6	7	8	9	10	11	12
A	0.28	0.55	0.32	0.16	0.00	1.00	1.00	1.00	0.02	1.00	0.85	0.85	0.66
C	1.00	1.00	0.45	0.22	0.03	0.00	0.56	0.00	0.12	0.28	1.00	1.00	1.00
G	0.41	0.64	0.21	1.00	0.39	0.18	0.76	0.23	0.00	0.00	1.00	0.80	0.47
U	0.51	0.74	1.00	0.05	1.00	0.39	0.54	0.14	1.00	0.04	0.58	0.99	0.86



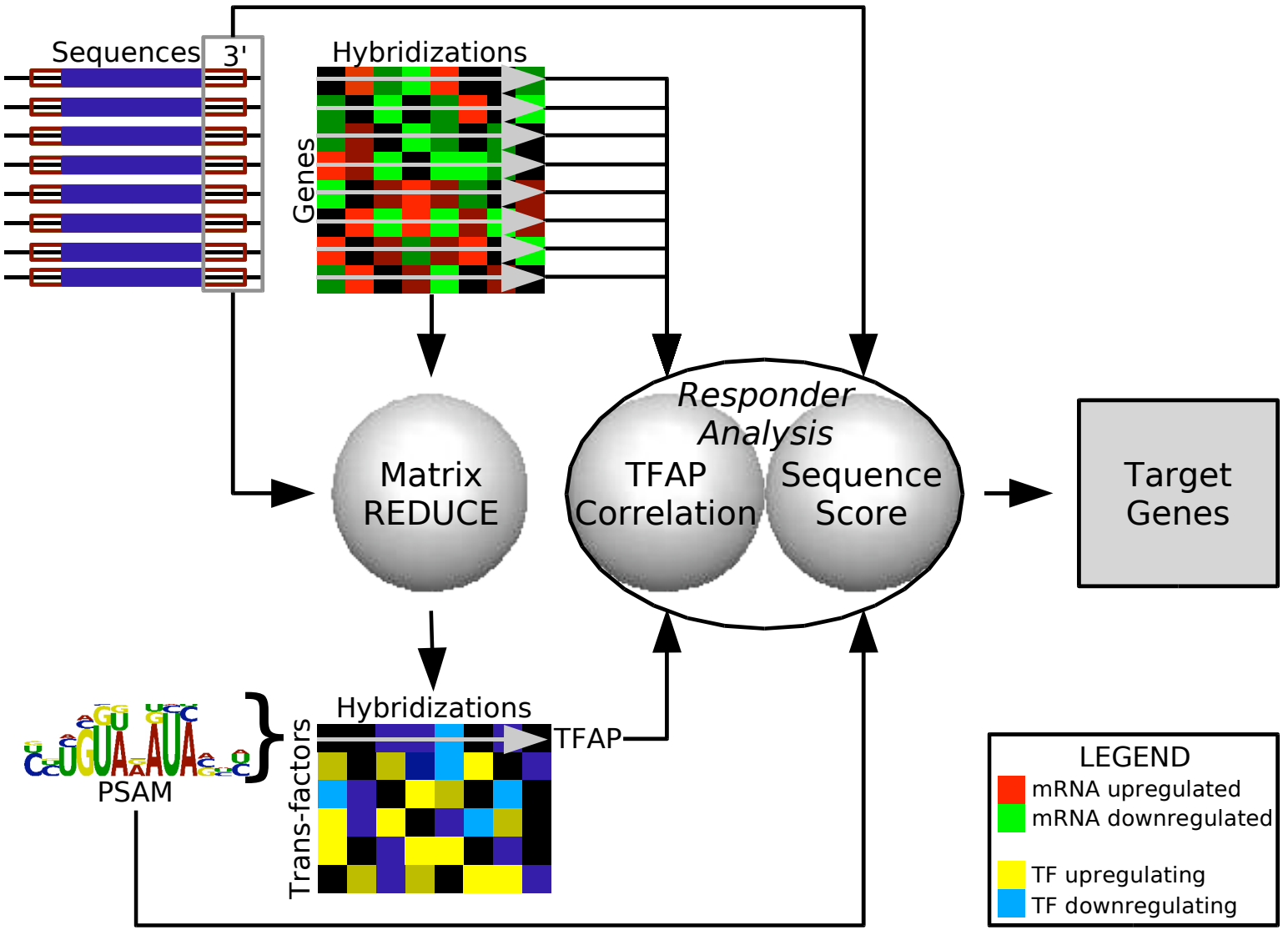
MatrixREDUCE

$$\log_2\left(\frac{[mRNA]_g^{\text{red}}}{[mRNA]_g^{\text{green}}}\right) = A_{0h} + \sum_{\phi \in M} F_{\phi h} N_{\phi g} + \epsilon_{gh}$$

$$N_{\phi g} = \sum_{i=1}^{L_g} \prod_{j=0}^{L_\phi - 1} w_{\phi j b_{(i+j,g)}}$$

$$X^2_{\phi h} = \sum_g \left(A_{gh} - C_h - F_{\phi h} N_{\phi g} \right)^2$$

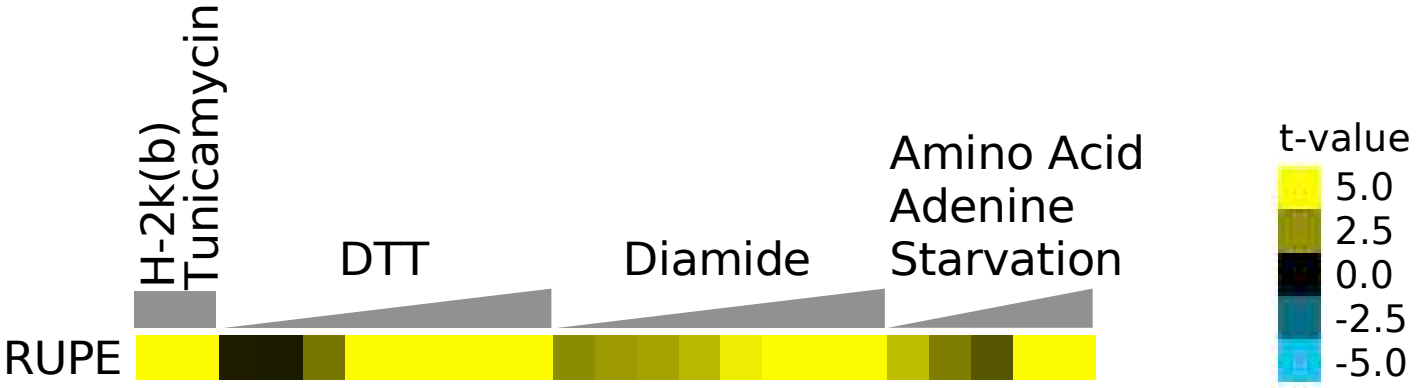
Discovery Process Summary



RUPE

Response to Unfolded Protein Element

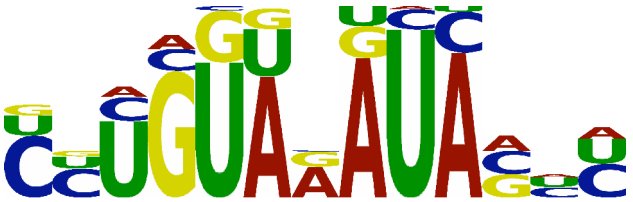
RUPE

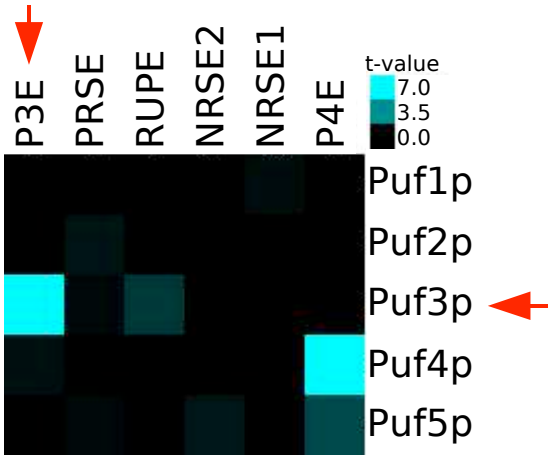


P3E and PRSE

Puf3p element

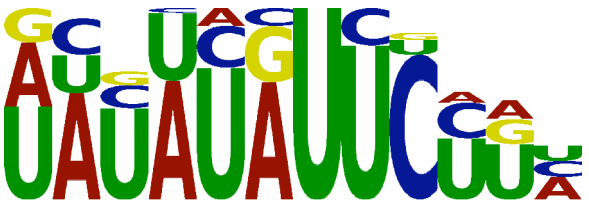
P3E





Positive Response to Starvation Element

PRSE

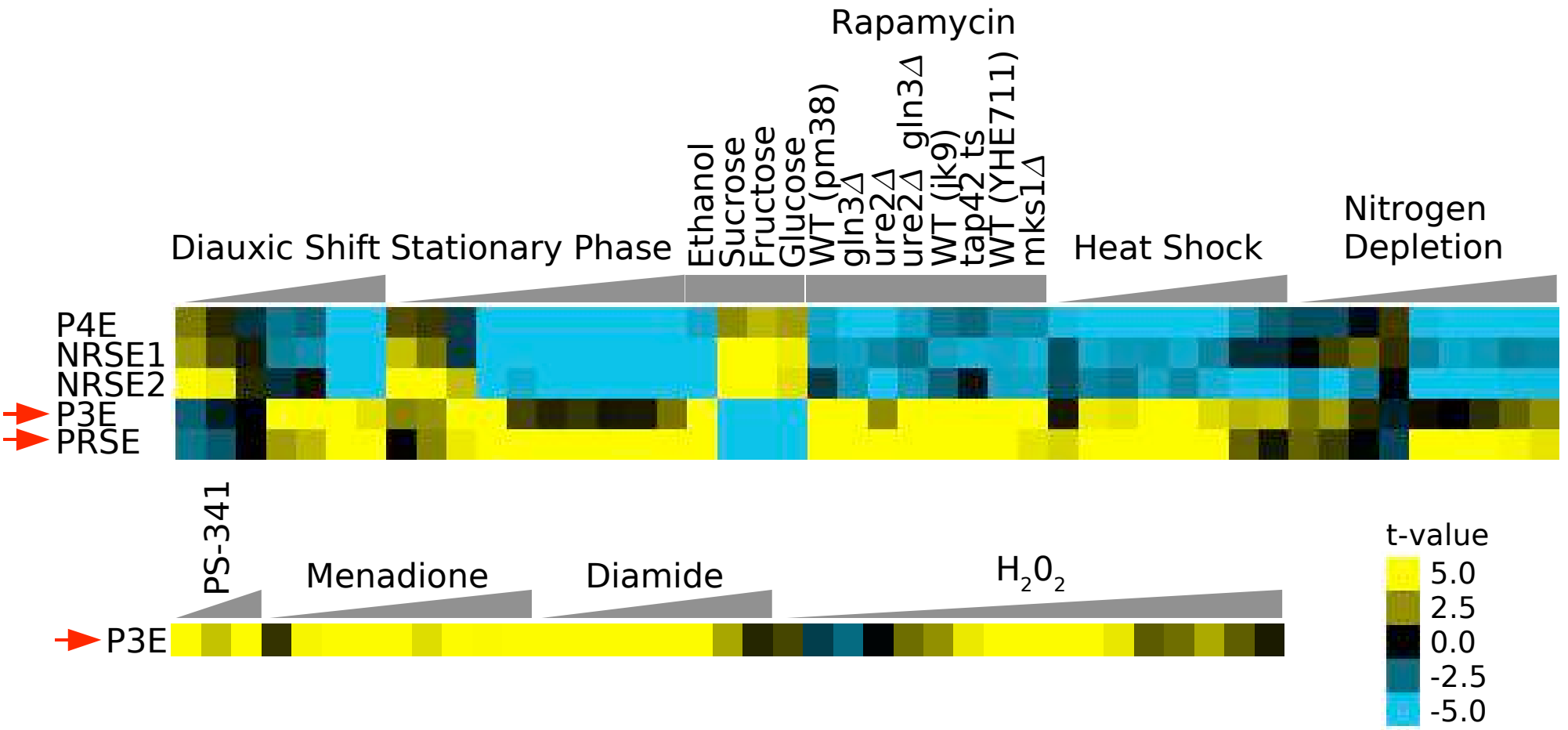


P3E and PRSE Target mRNA Functions

Gene Ontology Category Description	P3E	PRSE
C mitochondrion		
C mitochondrial ribosome		
C mitochondrial matrix		
C mitochondrial large ribosomal subunit		
C mitochondrial small ribosomal subunit		
F tRNA ligase activity		
P aerobic respiration		
C mitochondrial inner membrane		
P electron transport		
P oxidative phosphorylation		
P ATP synthesis coupled electron transport		
C mitochondrial electron transport chain		

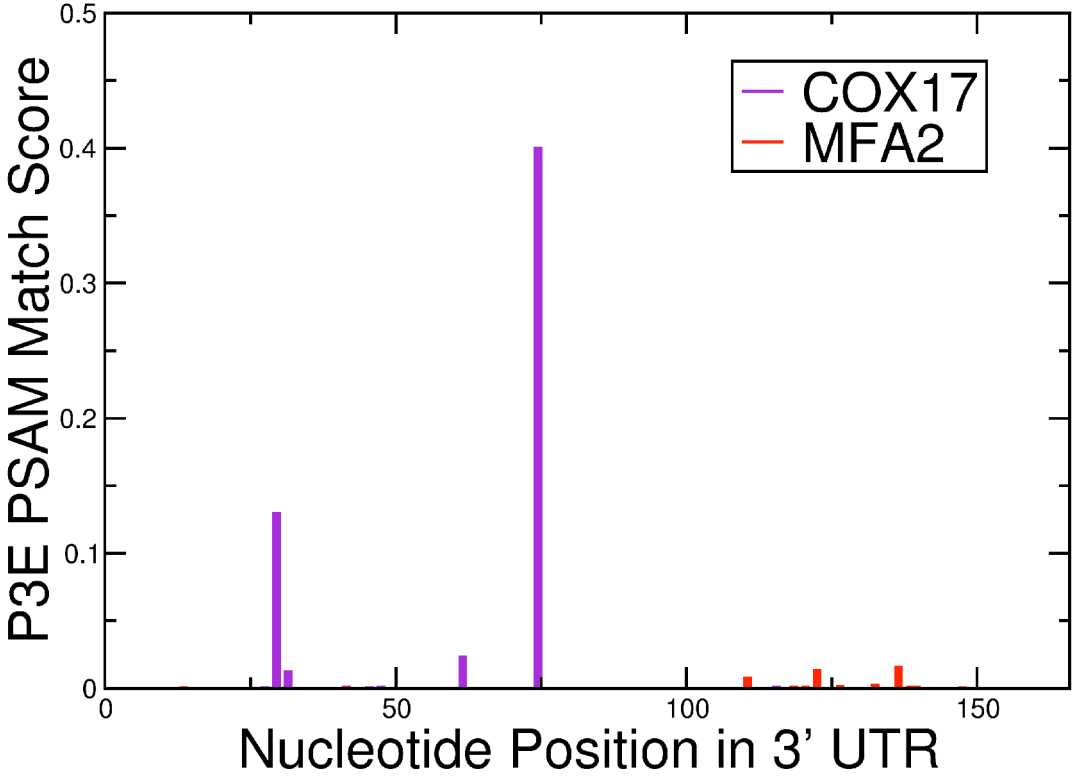
P-value
$10^{-7} < P \leq 10^{-3}$
$10^{-15} < P \leq 10^{-7}$
$P \leq 10^{-15}$

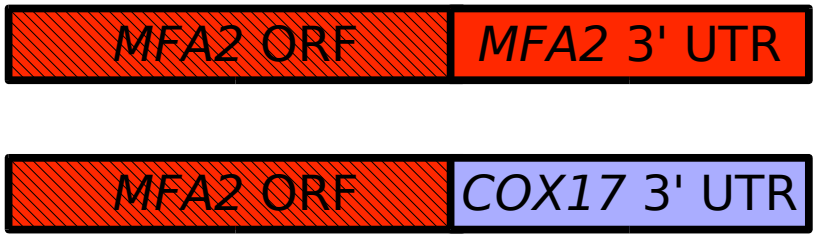
P3E and PRSE TFAPs



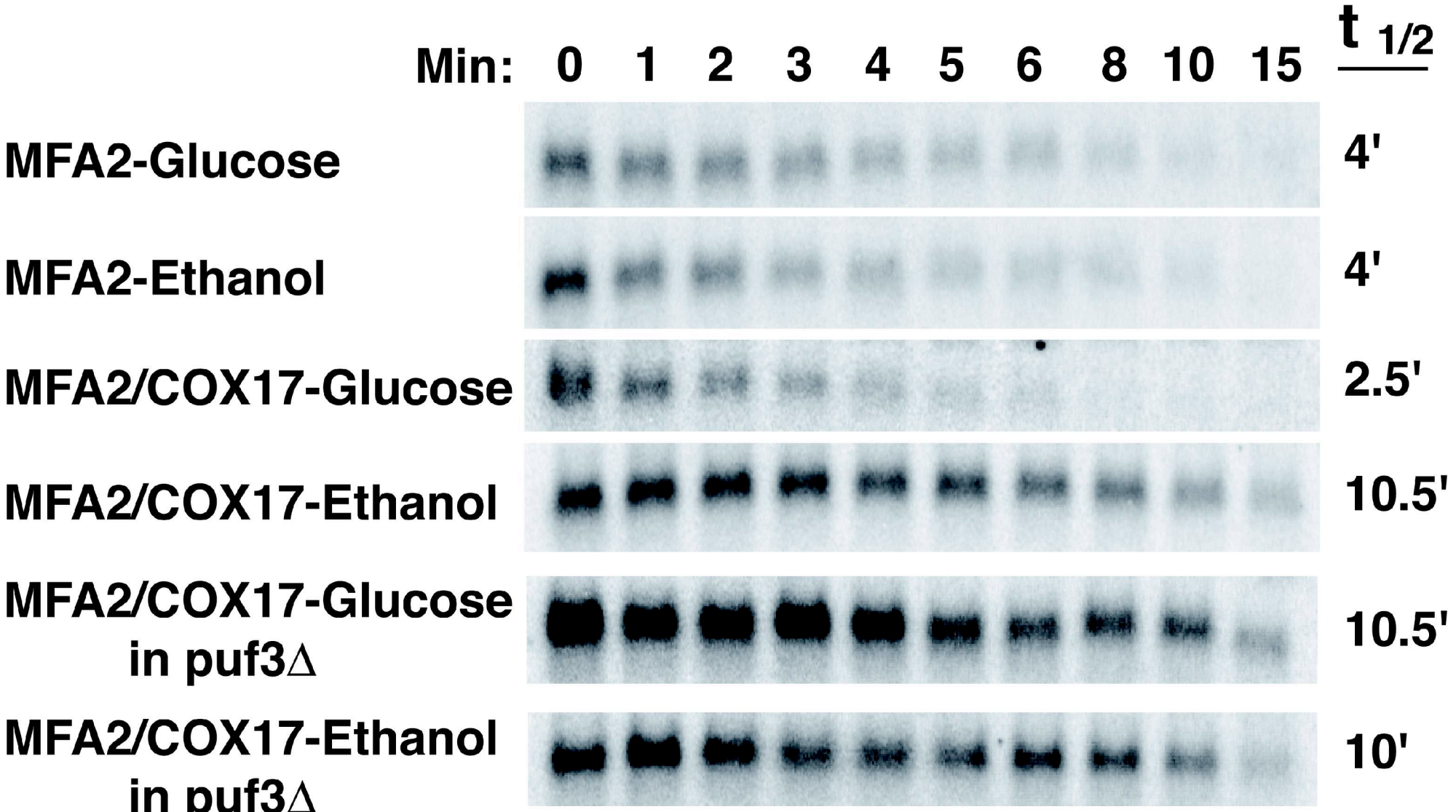
COX17, a Puf3p Target mRNA

- Puf3p binds directly to the *COX17* 3'-UTR and promotes rapid deadenylation and decay.
- The *COX17* 3'-UTR is sufficient to direct Puf3p decay regulation when attached to the ORF of *MFA2*.

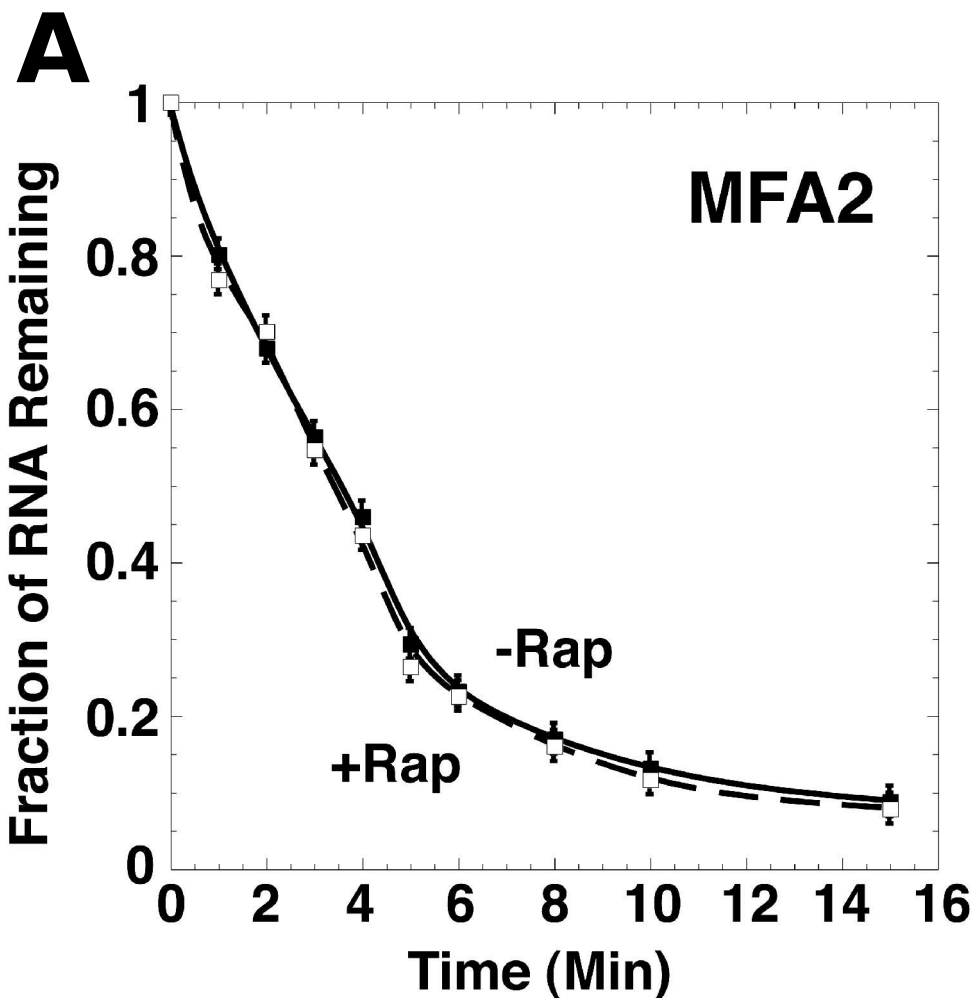


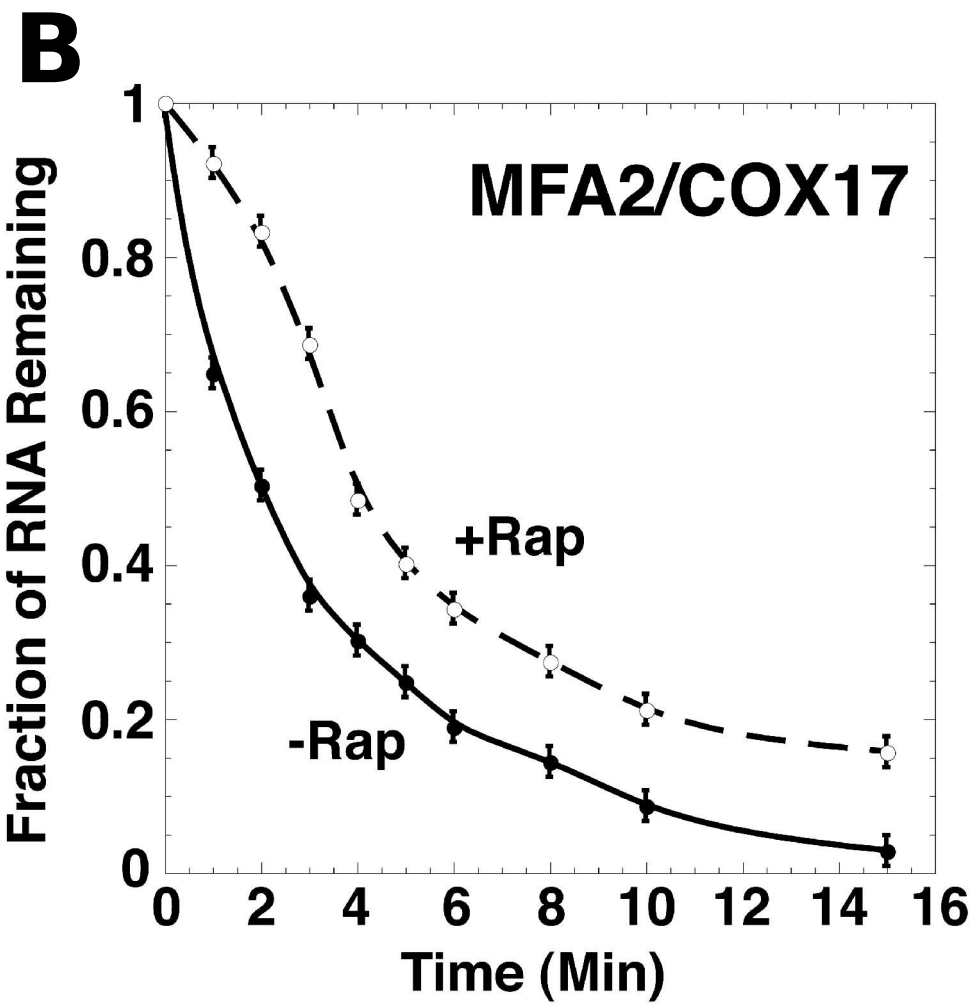


Puf3p Activity in Response to Carbon Source

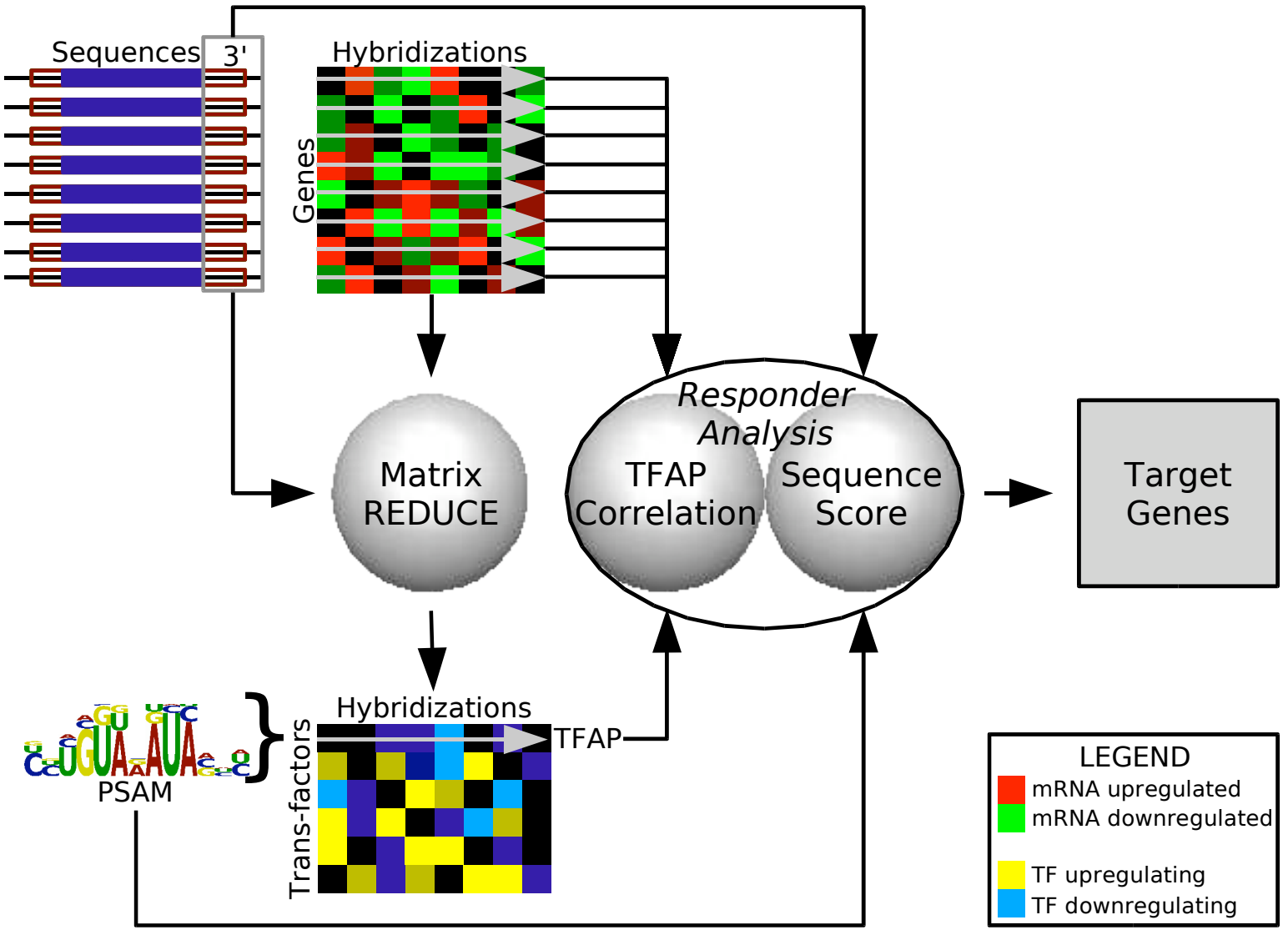


Puf3p Activity in Response to Rapamycin





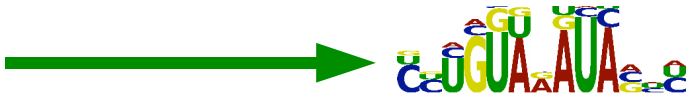
Discovery Process Summary



Responder Analysis

For Every Gene...

Sequence Score



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UGUAUAUAUAAGAAUAGGUAUUCACAAACUA

UAGUAUAUACUACCUGUAAAUAUGUGCGAUG

CACAAUUAACAUUACCUCAUCACUACUACACC

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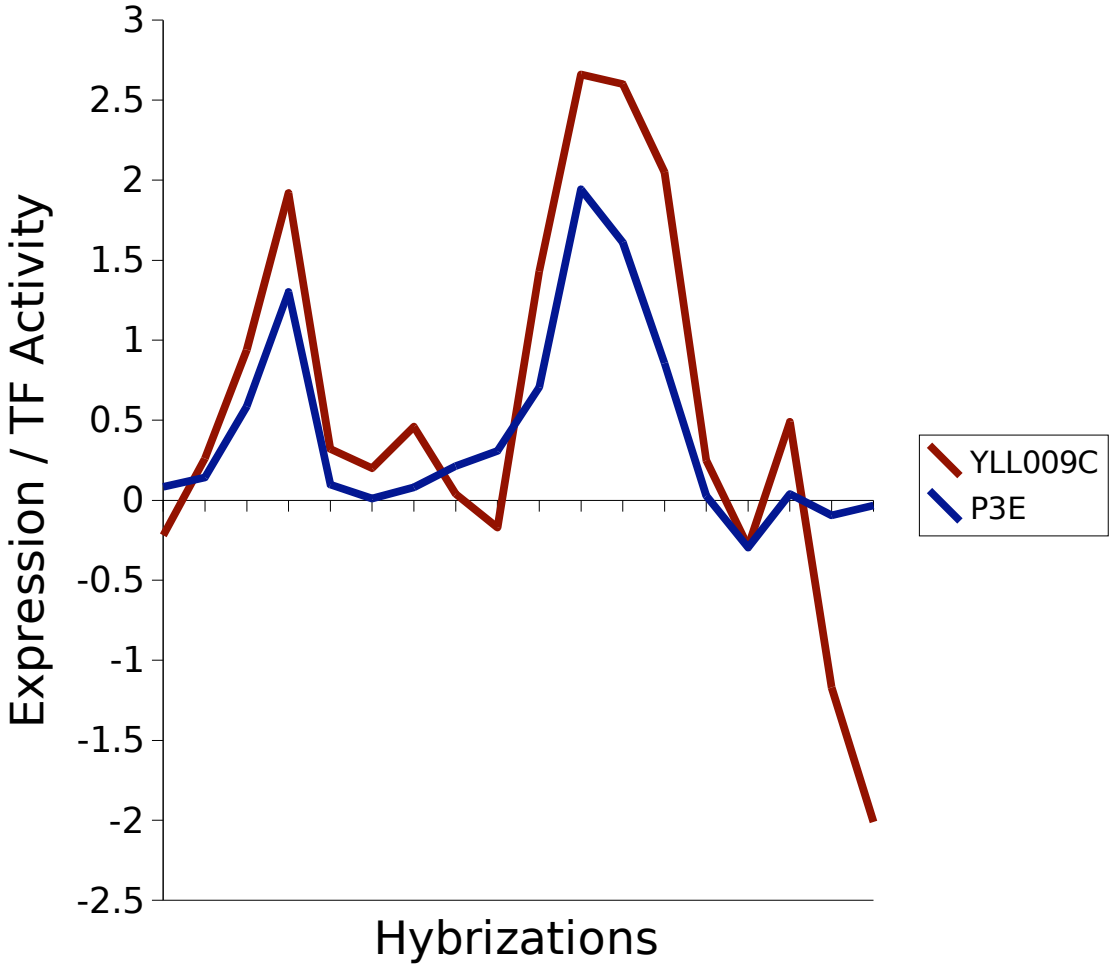
UGGCCCCUUAAGAGUGUUCUAAG

TFAP Correlation



"MA-Networker": Integrating mRNA expression and ChIP data

F Gao, BC Foat, and HJ Bussemaker, BMC Bioinformatics (2004)

- ChIP data for >100 TFs (Lee et al., *Science* (2002))
- mRNA expression data for ~800 conditions

- TF activity profiles from regression (mRNA~ChIP)
- Response of individual genes to TF activity profile

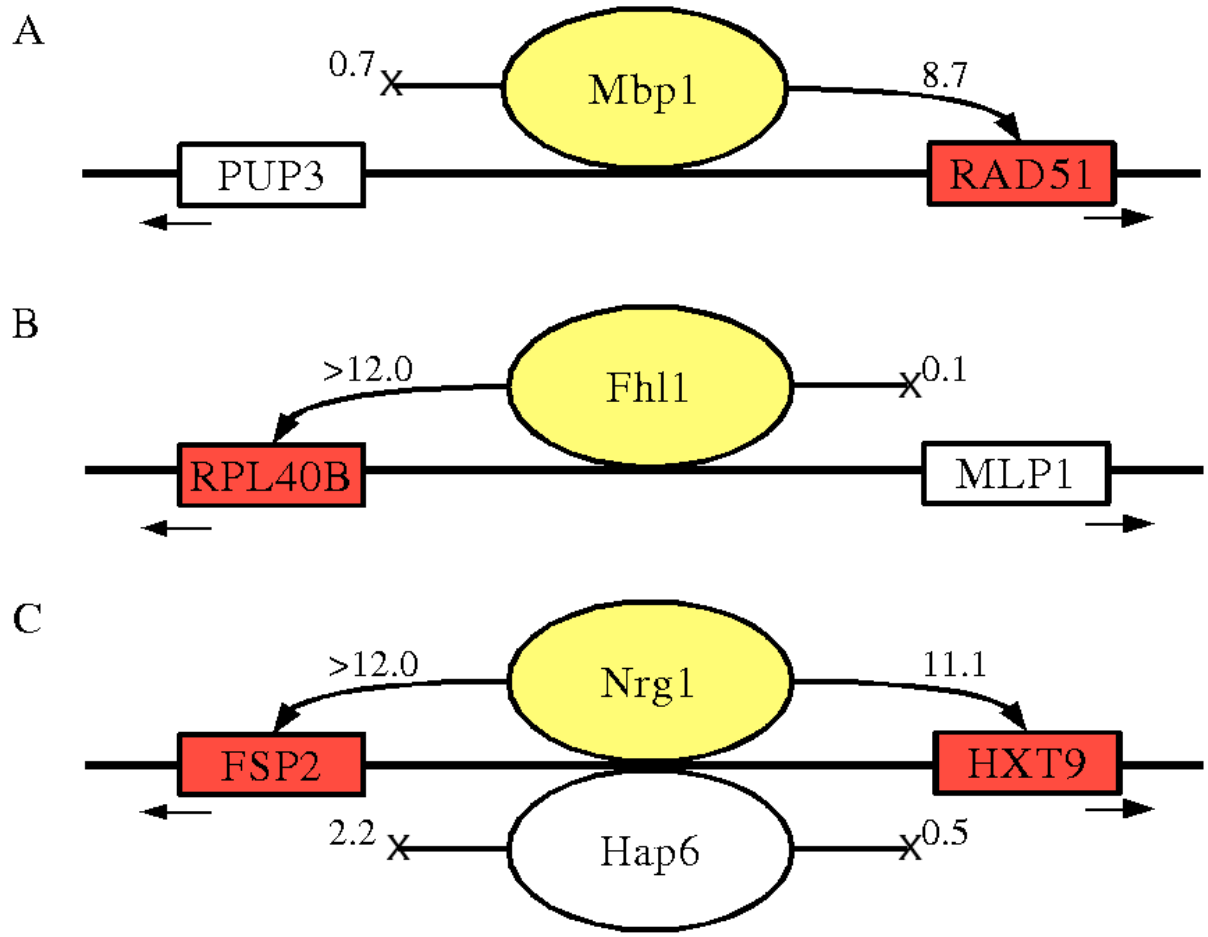
- Increase specificity of TF target prediction
- Overcome context dependence of TF deletion experiments related to combinatorial control

MA-Networker results

- Only 37 out of 113 transcription factors are statistically significant predictor of gene expression in one or more conditions
- On average only 58% of significantly bound genes are **functional** targets

F Gao, BC Foat, and HJ Bussemaker, BMC Bioinformatics (2004)

Divergently transcribed gene pairs



F Gao, BC Foat, and HJ Bussemaker, BMC Bioinformatics (2004)

Can it be done more simply?

Why not define functional, direct targets of TF as those genes that satisfy these conditions:

- Promoter bound by TF according to ChIP-chip experiment
- Change in mRNA expression in TF deletion vs. wild-type experiment

Turns out the answer is "no":

- many target genes are missed this way!
- deletion experiment also condition-specific

Conclusions (methodology)

- Using a **single** mRNA expression profile as input, **MatrixREDUCE** is able to infer:
  - Weight matrices (**PSAMs**) that model the sequence-specific binding constant K of trans-factors, **without using a background model**
  - Change in **post-translational TF activity** between any pair of conditions for which mRNA expression data is available (>1000 conditions)

Conclusions (methodology)

- **Using Responder Analysis** we can distinguish between functional and non-functional binding sites:
  - No comparative genomics needed, but does **not** provide any **mechanistic** explanation for context dependence
  - Requires a **large** set of hybridizations

Conclusions (biology)

- **Dynamic** regulation of mRNA stability can be inferred from **steady-state** expression data:
  - Several novel, well-characterized cis-elements discovered in 3' UTR's
  - Screened a large set of conditions
  - Successful **experimental validation**!

Acknowledgements

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- Sean Housmandi

Frans Klis Lab (Amsterdam)
- Andre Boorsma

Kevin White Lab (Yale)
Bas van Steensel Lab (NKI)
Frank Holstege Lab (Utrecht)

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