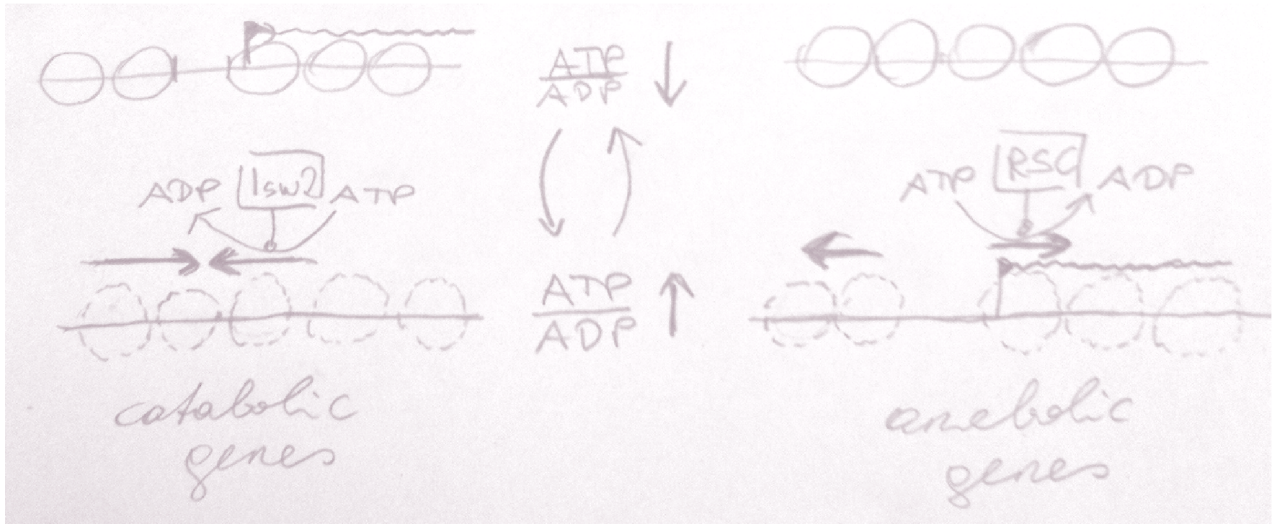
Chromatin Structure as a Metabolic Sensor and Global Regulator

Rainer Machné

May 15, 2024



A Tale of Cell Biology, Told by Budding Yeast (and a Cyanobacterium)

A lecture series beyond the **known knowns** of (cell) biology, exploring the **known unknows**, the **unknown unknowns**, . . . and some **unknown knowns** 👋.

0. Quantitative Microbiology: Exponential growth is rarely balanced.

I. Pervasive transcription during the low energy phase of respiratory oscillations.
II. Transcription at LTR retrotransposons. **TRANSCRIPTION**

III. DNA as a metabolic sensor, and
IV. Chromosomal domains and mobile elements. **GENOME HOMEOSTASIS**

V. Protein homeostasis by a transcriptional oscillator, and
VI. Pulse-width modulation of gene expression. **PROTEOME HOMEOSTASIS**

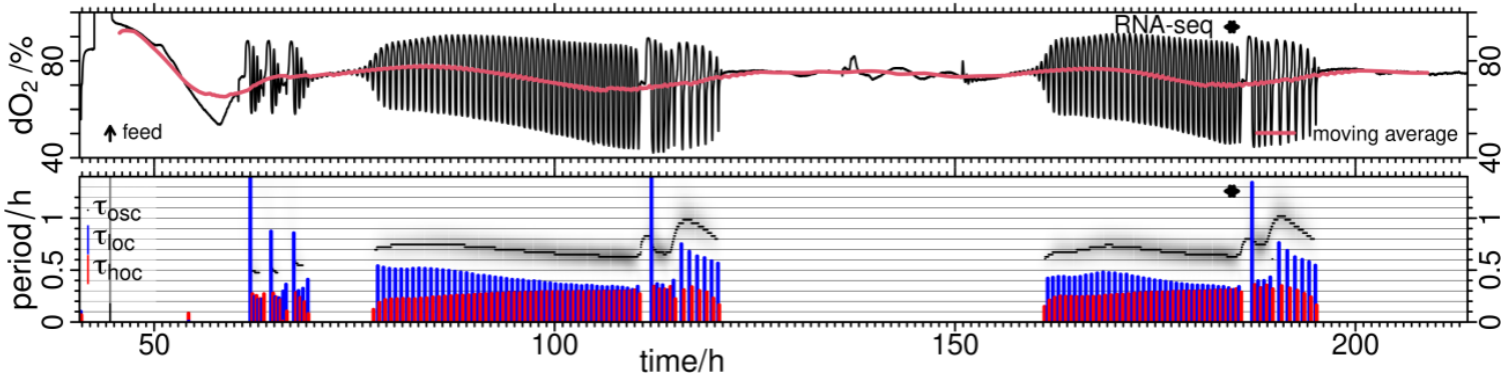
VII. Metabolism: feedbacks and the auto-catalytic cycles of life, and
VIII. The cell growth cycle as a cell-structural proofreading loop. **METABOLISM**

IX. Same, same in a cyanobacterium (circadian DNA supercoiling homeostasis).
X. Other eukaryotes: circadian and developmental clocks. **OTHER SPECIES**

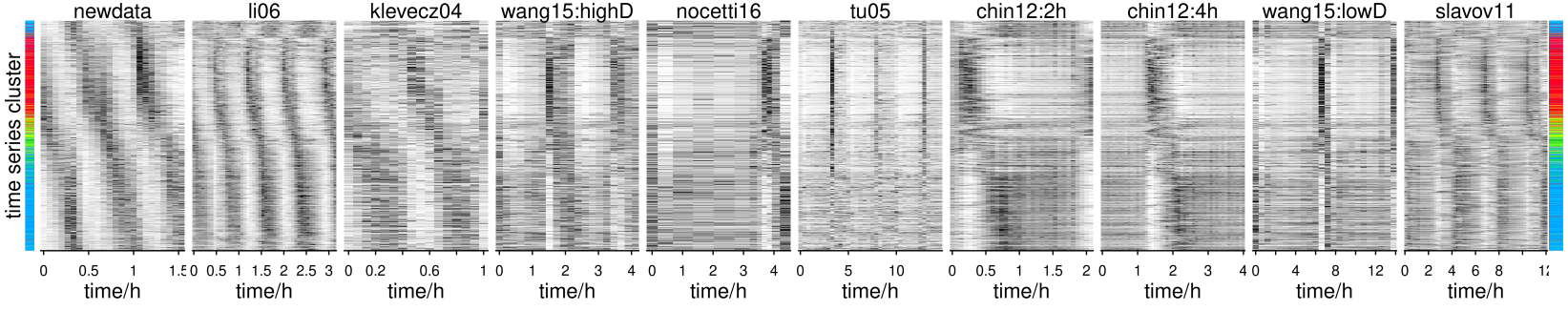
Discussion: *Do yeast cells dream of metabolic sheep?*

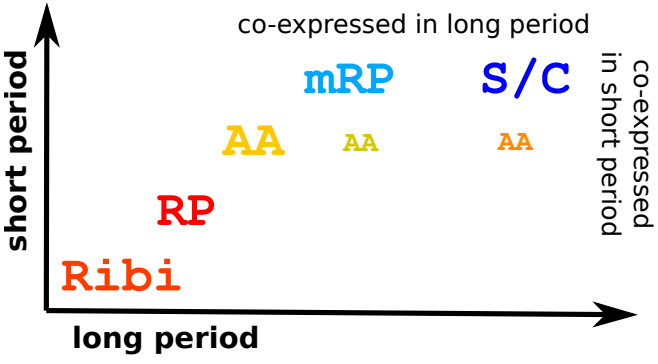
All slides, incl. references, at https://www.bioinf.uni-leipzig.de/~raim/lecture_series/

Respiratory (Metabolic) Oscillation in Budding Yeast



At high cell density: phases of High (HOC) and Low Oxygen Consumption (LOC).
here: distillery strain IFO 0233: exceptionally short periods, and **regular cycles or complex dynamics.**





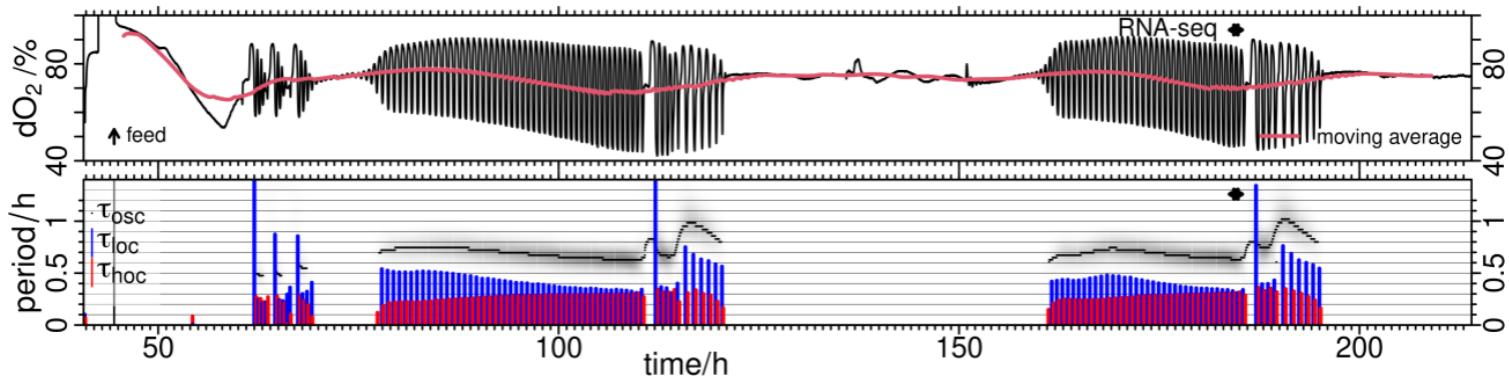
A conserved temporal transcription program:

Ribi → RP → AA → mRP → S/C

unspooled at periods τ_{osc} from 0.6 h to 120 h!

Lectures I&II: noncoding in LOC phase.

A General Pattern: Ribi/RP ⇔ mRP ⇔ S/C



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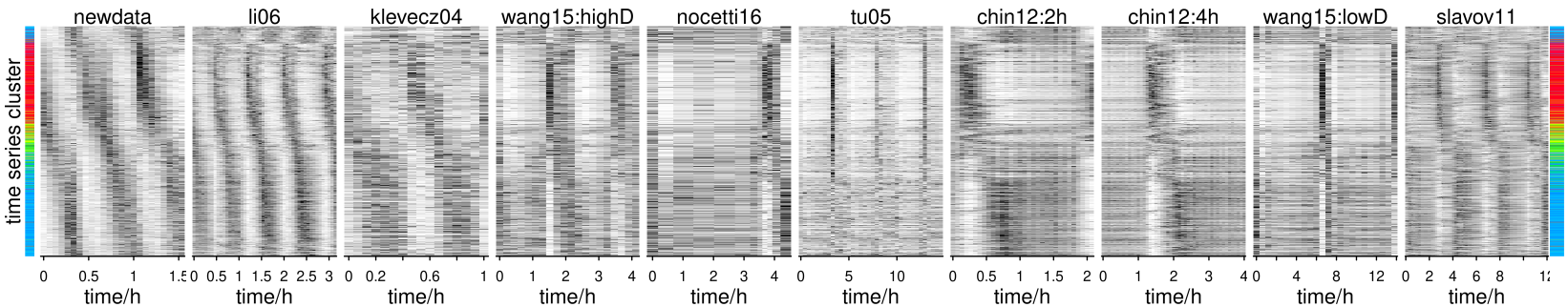
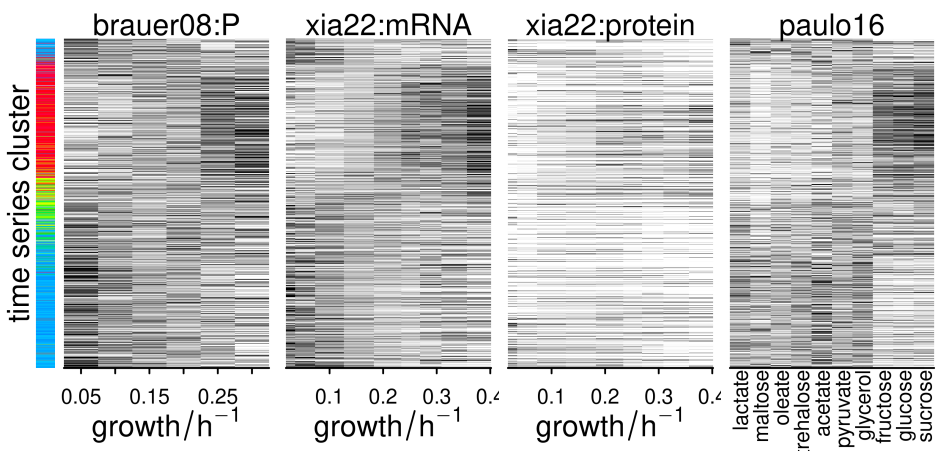
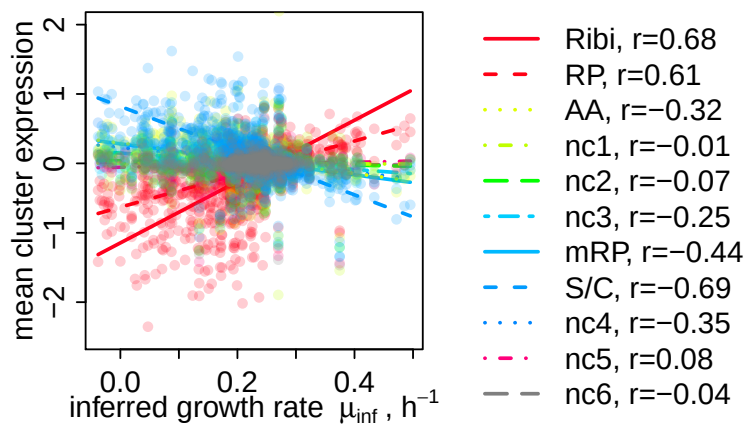
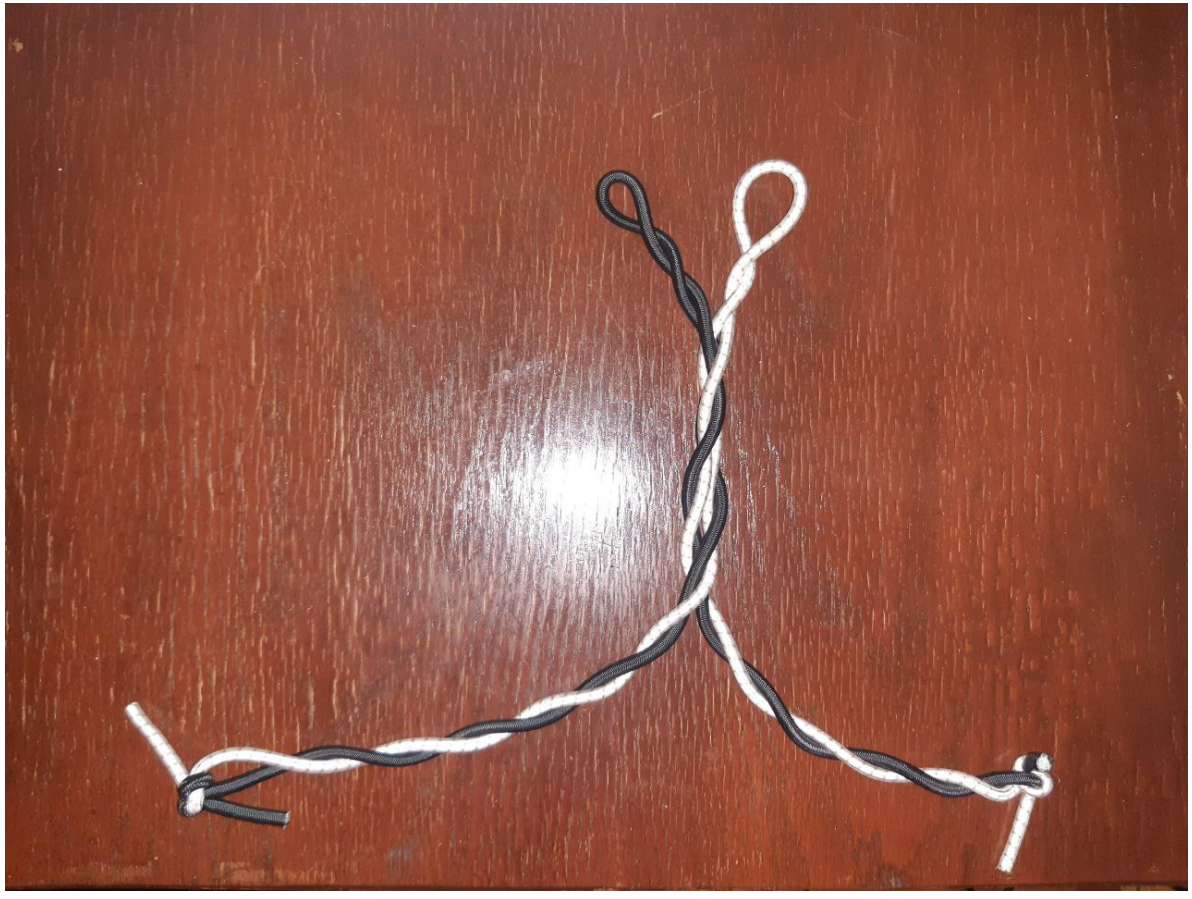






A Simple Mechanism for Global Gene Regulation?

Live: the rubber band model of DNA struture.



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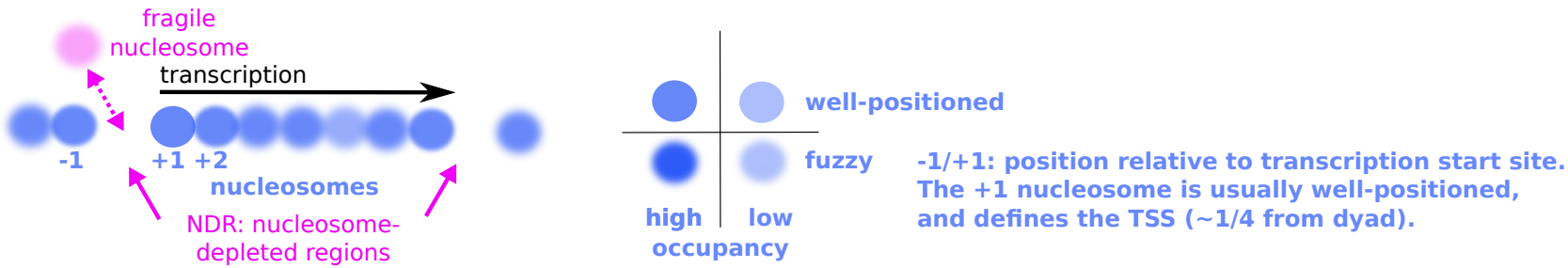
The Standard Nucleosome Configuration of Transcribed Units



Typical nucleosome configurations at transcribed genes, common to eukaryotes and archaea (and *chromatinized E. coli*).

Is there *a genomic code for nucleosome positioning*?

- The **contributions of DNA sequence, proteins and transcription** in establishing the local chromatin structure remain an **unsolved and controversial problem**, e.g., *pubpeer* (with figures) by Michael Eisen (2021)[1] on Segal et al. (2006).
- Translational positioning (e.g. Chereji et al. 2018): barrier model v. Rotational positioning (e.g. Cui et al. 2014): ~**10.5 bp period**.
- Rojec et al. (2019): *chromatinization of E. coli* with archaeal histones.

[1] https://pubpeer.com/publications/34904859EA5787B3927F952E0EED43

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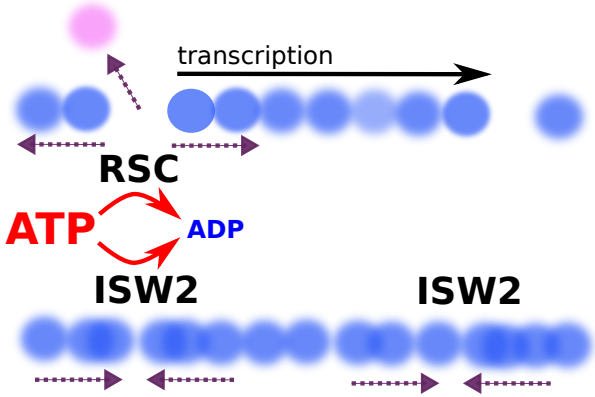
ATP-Dependent Nucleosome Remodelling



pushers: RSC, SWI/SNF

ATP-dependent nucleosome remodellers

pullers: ISW2, INO80

Conserved eukaryotic **motor proteins** that actively pull and wrap DNA, requiring a high [ATP]/[ADP] ratio:

- Kubik et al. (2019): **pushers** (RSC, SWI/SNF) $\Leftrightarrow$ **pullers** (ISW2, INO80): pushers open the promoter, pullers repress **or** define the TSS.
- Remodellers have **both genome-wide and specific functions**, where specificity is conferred by co-factors; e.g. lost (ISW2) in *Drosophila* (Donovan et al. 2021).
- *In vitro* reconstitution of *in vivo*-like nucleosome configurations requires the addition of **cell extract** and **ATP** (Zhang et al. 2011).
- **Nuclear ATP synthesis** required in breast cancer cells (Wright et al. 2016).
- ISW2 is specifically inhibited by ADP (Fitzgerald et al. 2004).

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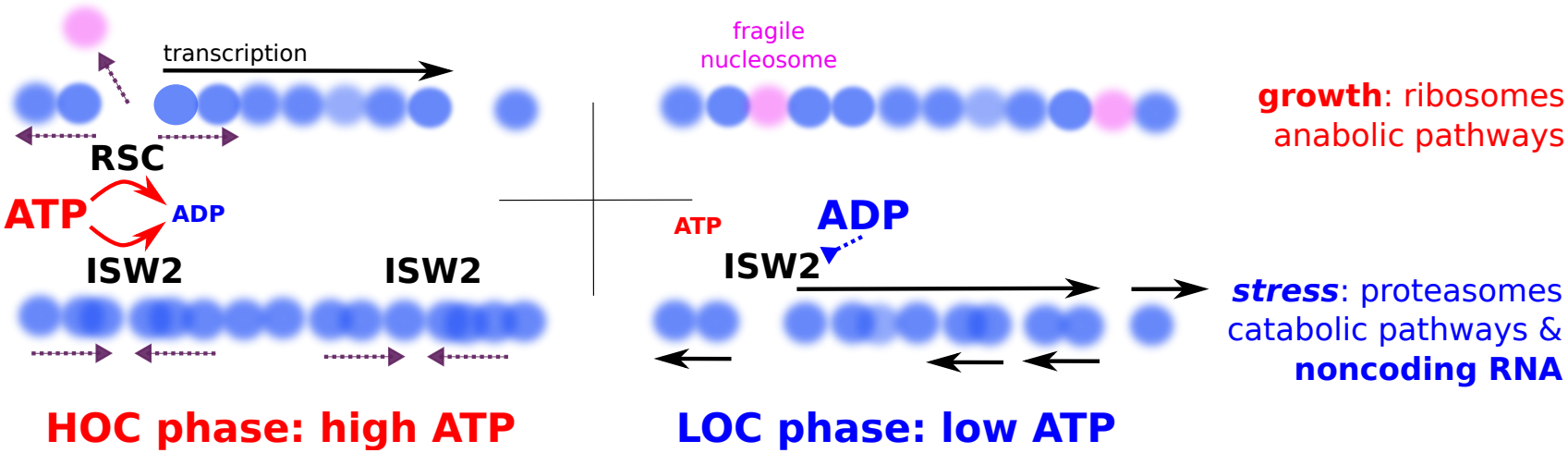
A (too) Simple Model



A 👋*pubmed conspiracy theory*👋 (Machné and Murray 2012; Amariei et al. 2014), based on . . .

- **"This makes a lot of sense"**:
  - Low ATP: stress and catabolic genes,
    High ATP: growth and anabolic genes.
- . . . and on **data integration**:
  - Nucleosome occupancy (MNase footprinting) ***in vivo***[2 a] **and** ***in vitro***[b],
  - RSC[c] and ISW2[d] binding sites/tracks (ChIP-seq), and
  - Overall nucleotide content (GC/AT, purines).

[2] a: Lee et al. (2007); b: Kaplan et al. (2009); c: Badis et al. (2008); d: Whitehouse et al. (2007).

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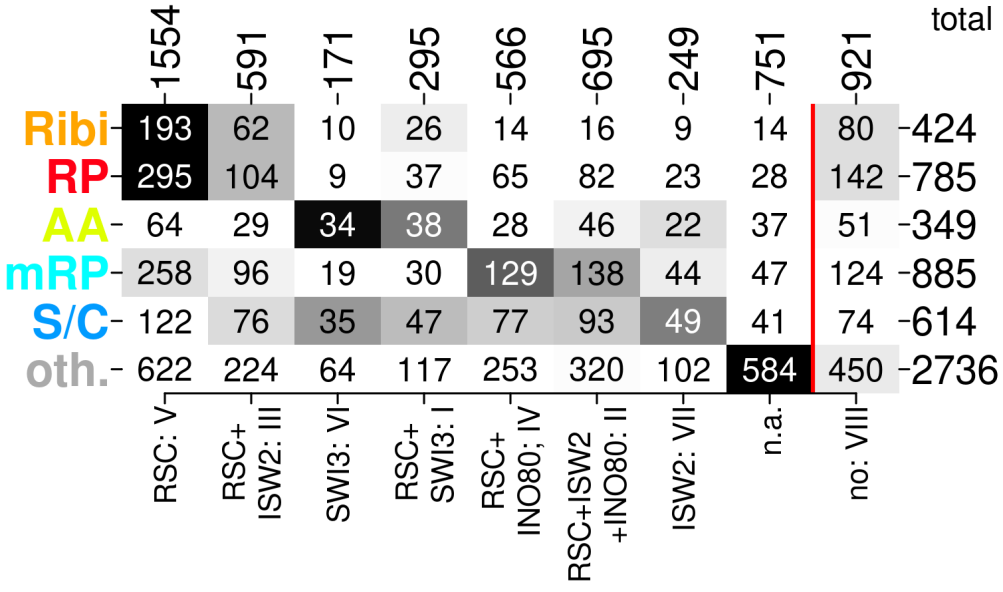
Extension of the Model



k-means clustering of promoters by Kubik et al. (2019), sorted along co-expression cohorts.

Novel data:

- Kubik et al. (2019): **pushers** (RSC, SWI/SNF) $\Leftrightarrow$ **pullers** (ISW2, INO80),
- Based on ChEC-seq (chromatin endogenous cleavage sequencing):
  $\Rightarrow$ inducible MNase fusion proteins, no ~~antibodies~~!
- **Extends our model to the four major groups**:
  - Ribi/RP: RSC; AA: SWI3,
  - mRP: INO80; S/C: ISW2.

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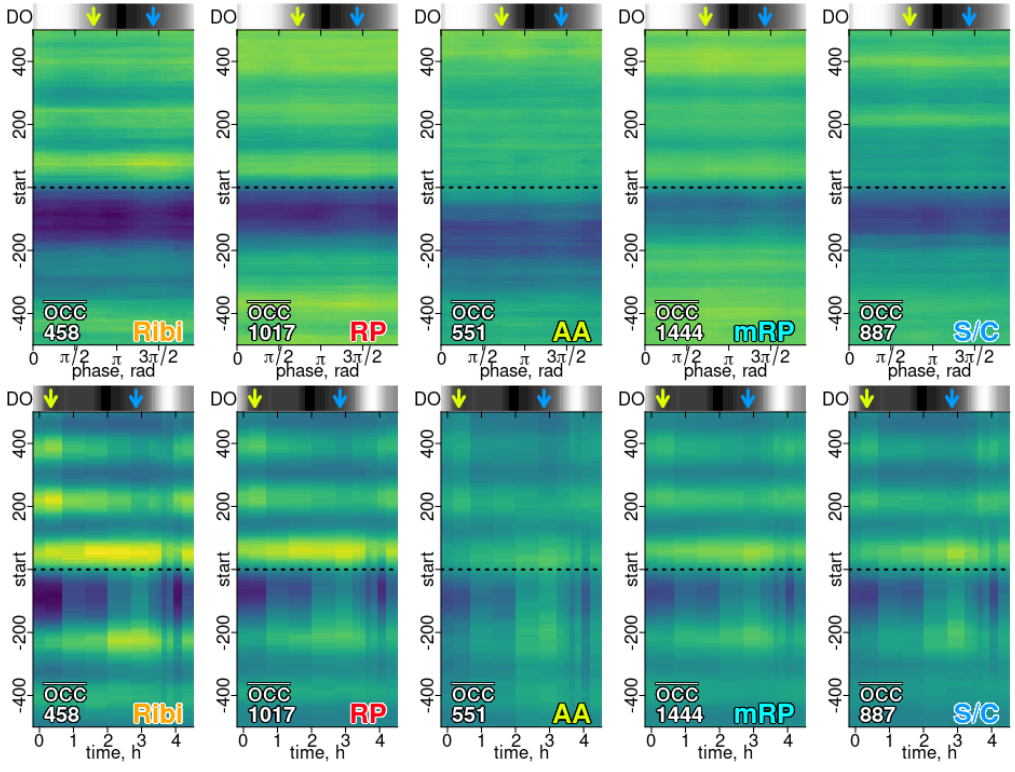
Chromatin Reset Points in `LOC`



Amariei et al. 2014:

- IFO 0233,
- Short period,
- MNase-tiling (⌀50 bp),
- Mean over 3 cycles.

Nocetti & Whitehouse 2016:

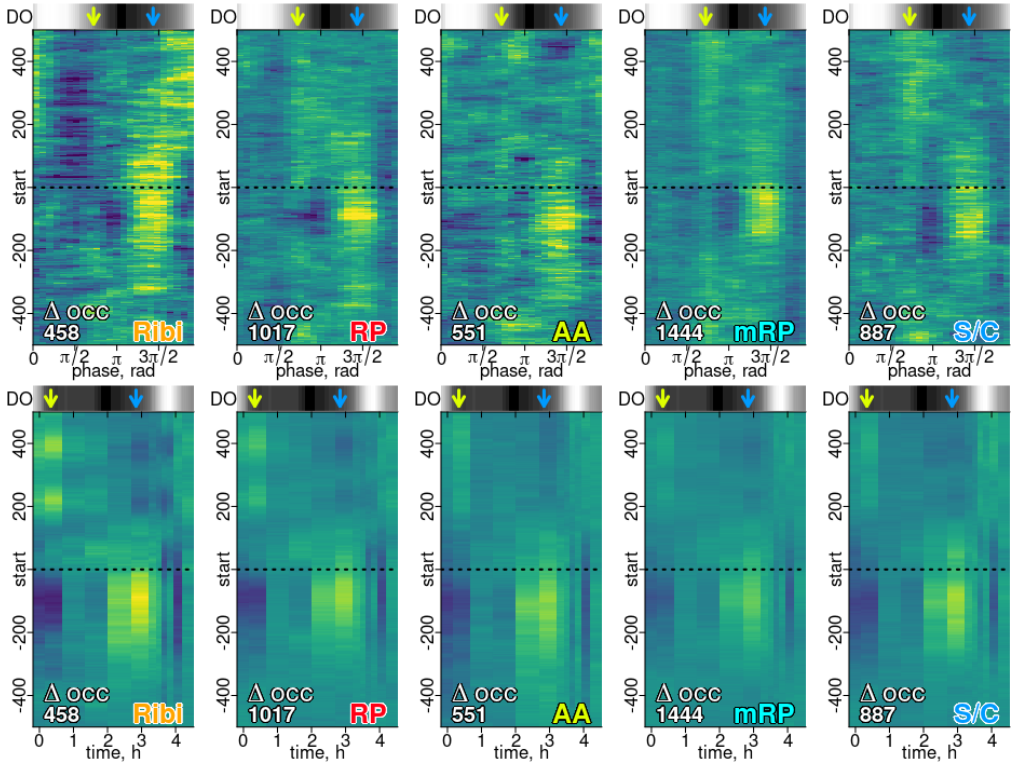
- CEN.PK122,
- Long period,
- MNase-seq,
- Only 1 cycle.

occ: DNA occupancy is the read-count of MNase digested, isolated chromatin, reflecting proteins bound to DNA; $\overline{\text{occ}}$: cohort means around the TSS (`start`).

The 5 cohorts show distinct DNA occupancy (protein-bound) patterns, reproducing the results by Machné and Murray (2012), based on data by Lee et al. (2007).

A Simple Mechanism for Global Gene Regulation?

Chromatin Reset Points in LOC



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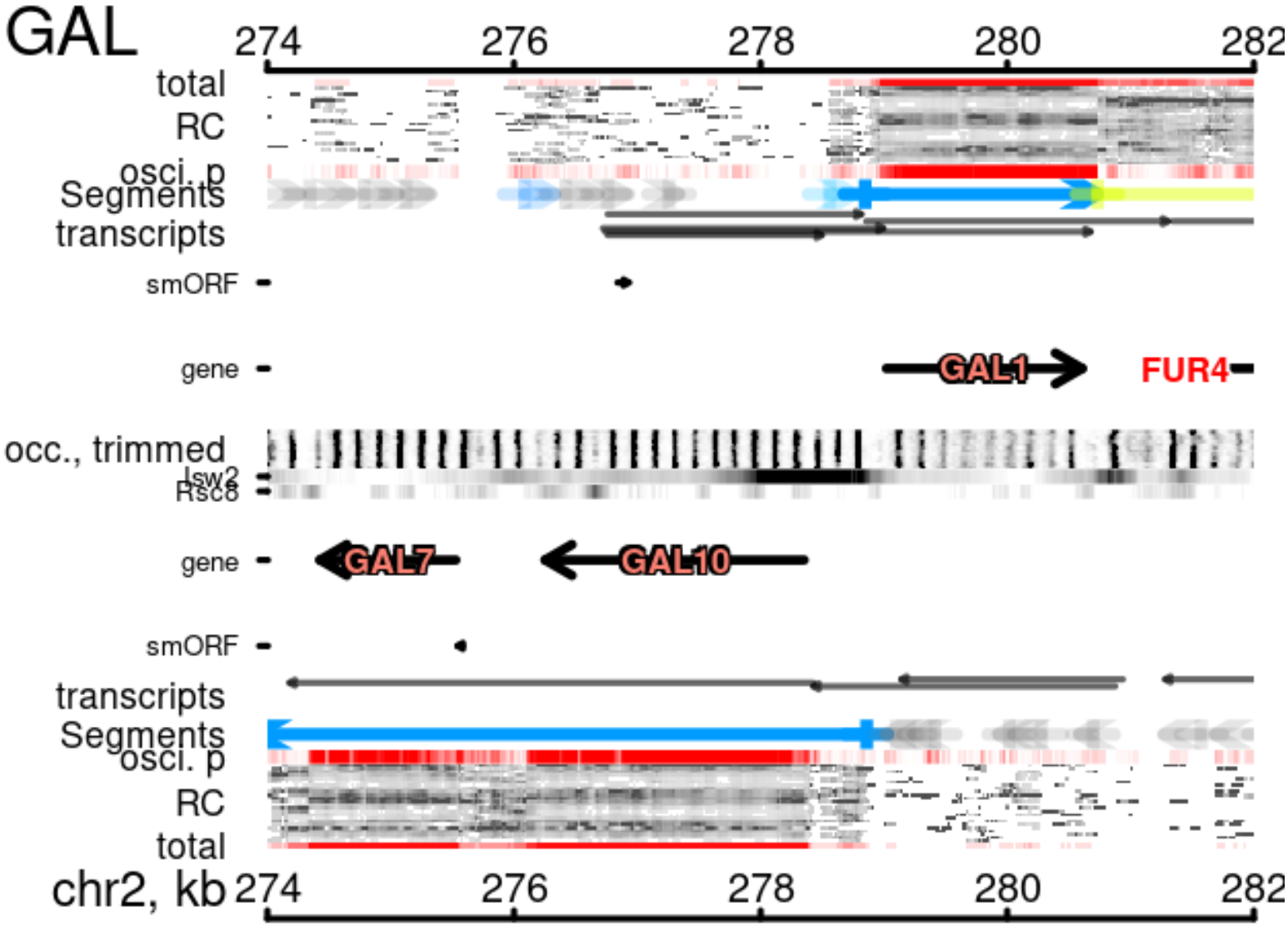
- Only 1 cycle.

$\Delta\text{occ}_i = \text{occ}_i - \frac{1}{n}\sum_{i=1}^{n} \text{occ}_i$: is the difference between occupancy at time point i and the temporal mean.

Chromatin reset points in early (gene bodies, yellow arrow) and late (promoters, blue arrow) LOC phase, **common to all cohorts**.

Subtle and Individual Signals at Promoters



Using the `genomeBrowser` data collection and plot tools;
see https://gitlab.com/raim/genomeBrowser, and `shiny` interface at http://yeast.bioinf.uni-leipzig.de/.

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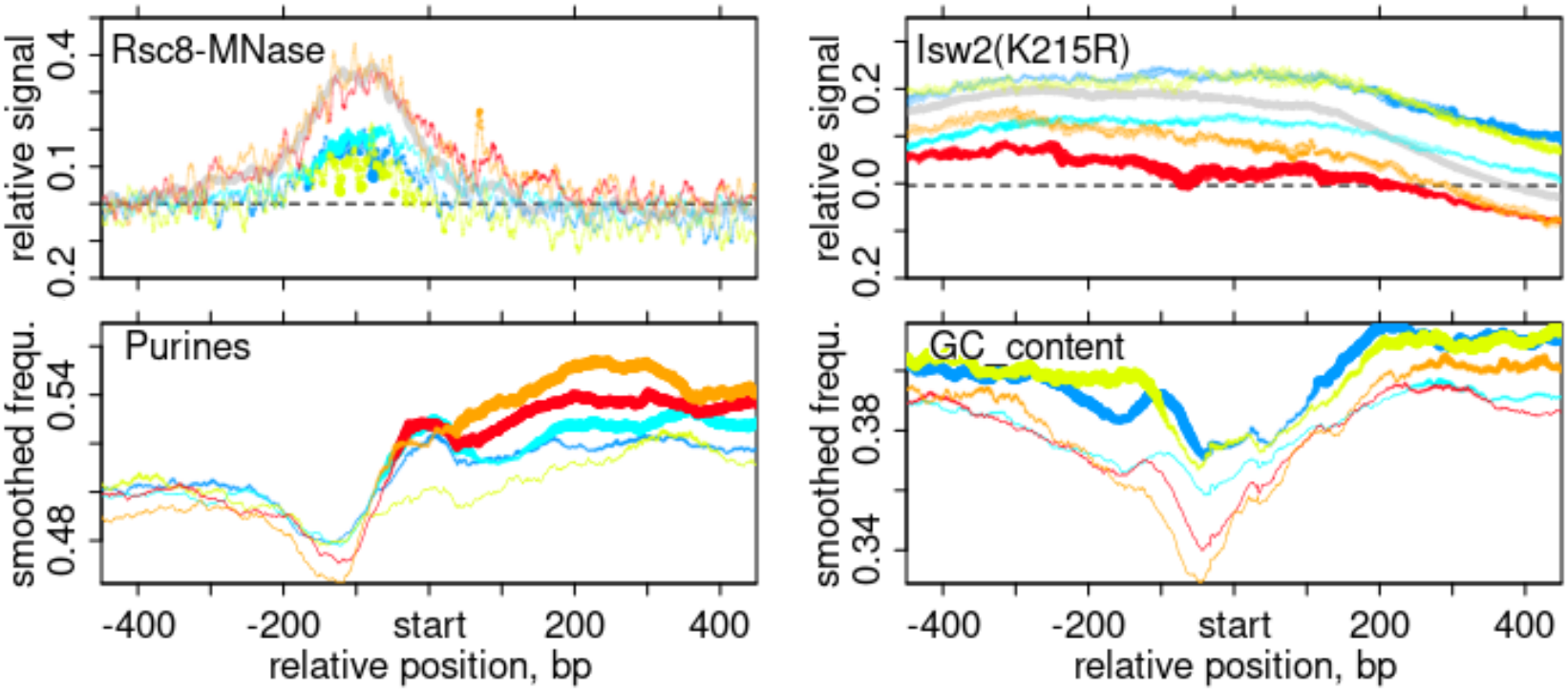
Promoter Structures

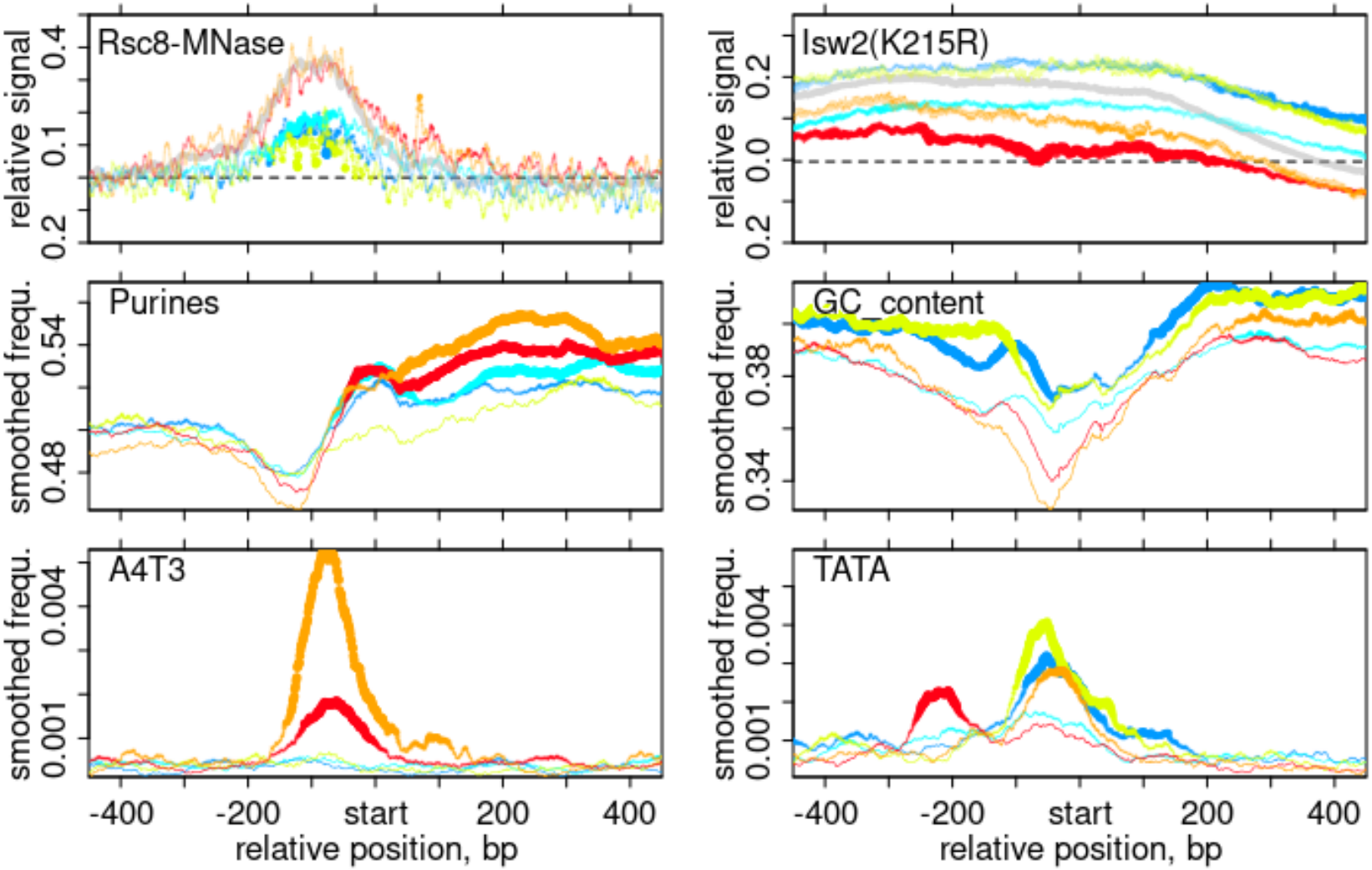


- Genes (segments) were aligned at transcription (segment) start sites and mean binding data or motif frequencies calculated at each relative position.
- Point sizes $\propto log(p)$ in local statistics tests, with all genes as the reference set:
  - t-test for binding data (5 bp moving average), or
  - cumul. hypergeom. distribution tests for sequence motifs (66 bp bins).

Data alignment was done with `segmenTools`'s `alignData`; statistics and plotting are not yet in the package.

A Simple Mechanism for Global Gene Regulation?

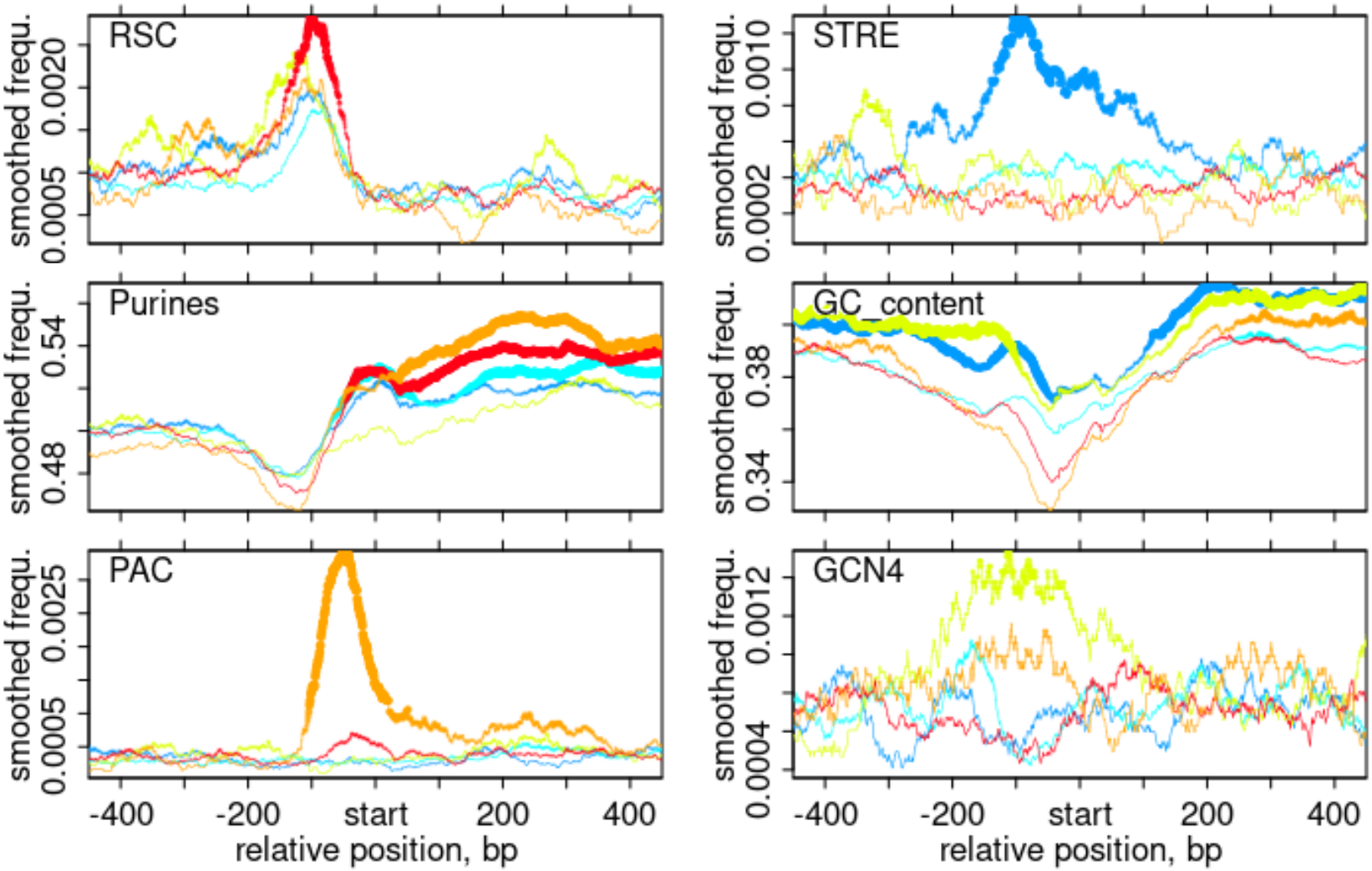
Promoter Structures



A4T3: `AAAATTT`, bent and stiff DNA, thought to NOT bind to nucleosomes;
TATA: `TATA[AT]A[AT][AG]` (Basehoar, Zanton, and Pugh 2004).

A Simple Mechanism for Global Gene Regulation?

Promoter Structures



RSC binding site: `CGCG` (Badis et al. 2008),
PAC motif: `GATGAG`.

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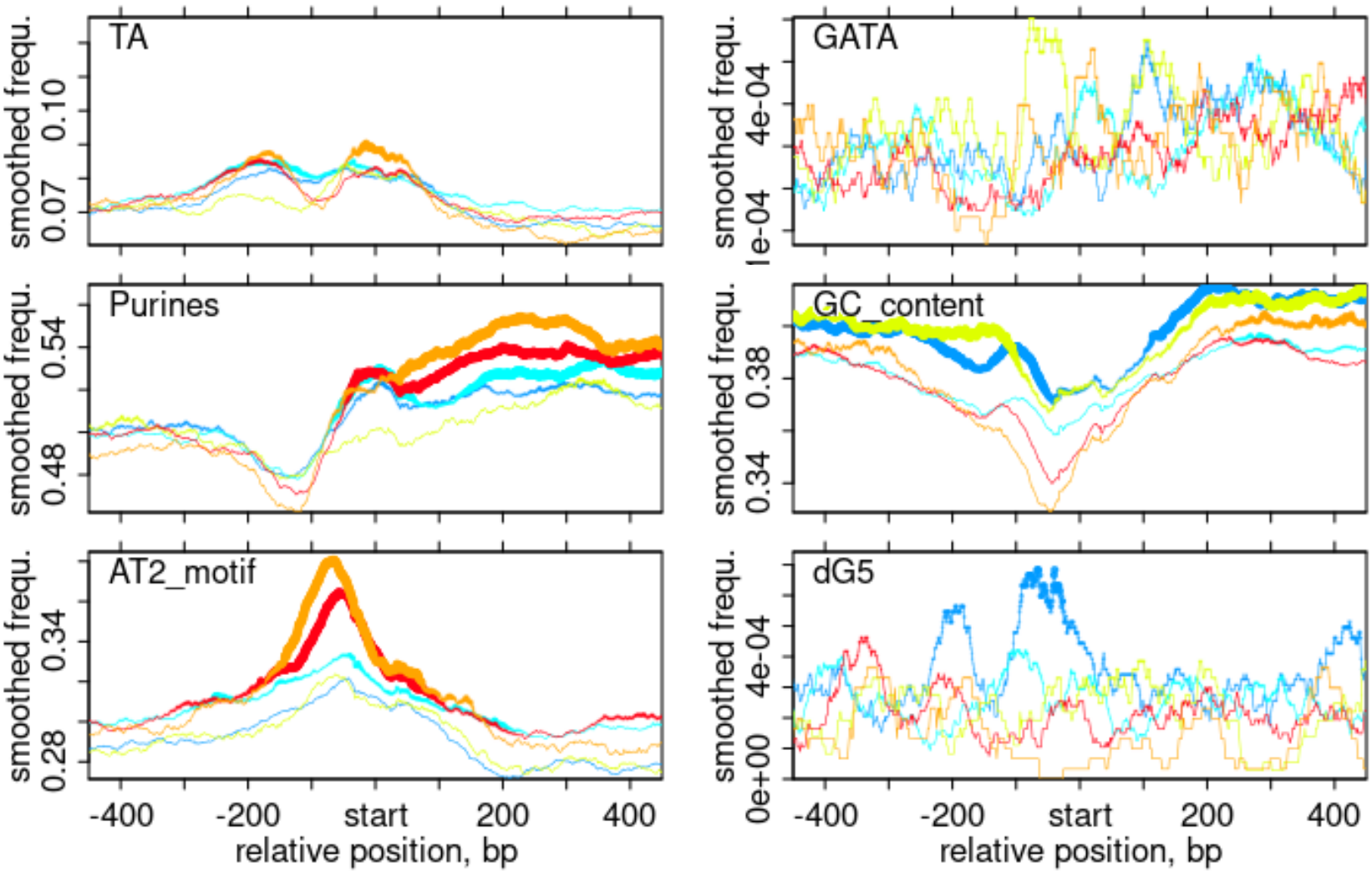
Promoter Structures



AT2 motif: ApA, TpT or ApT dinucleotide steps, **bent and stiff DNA**,
TA: TpA step, a bimodal **twist capacitor** (Reymer, Zakrzewska, and Lavery 2018).

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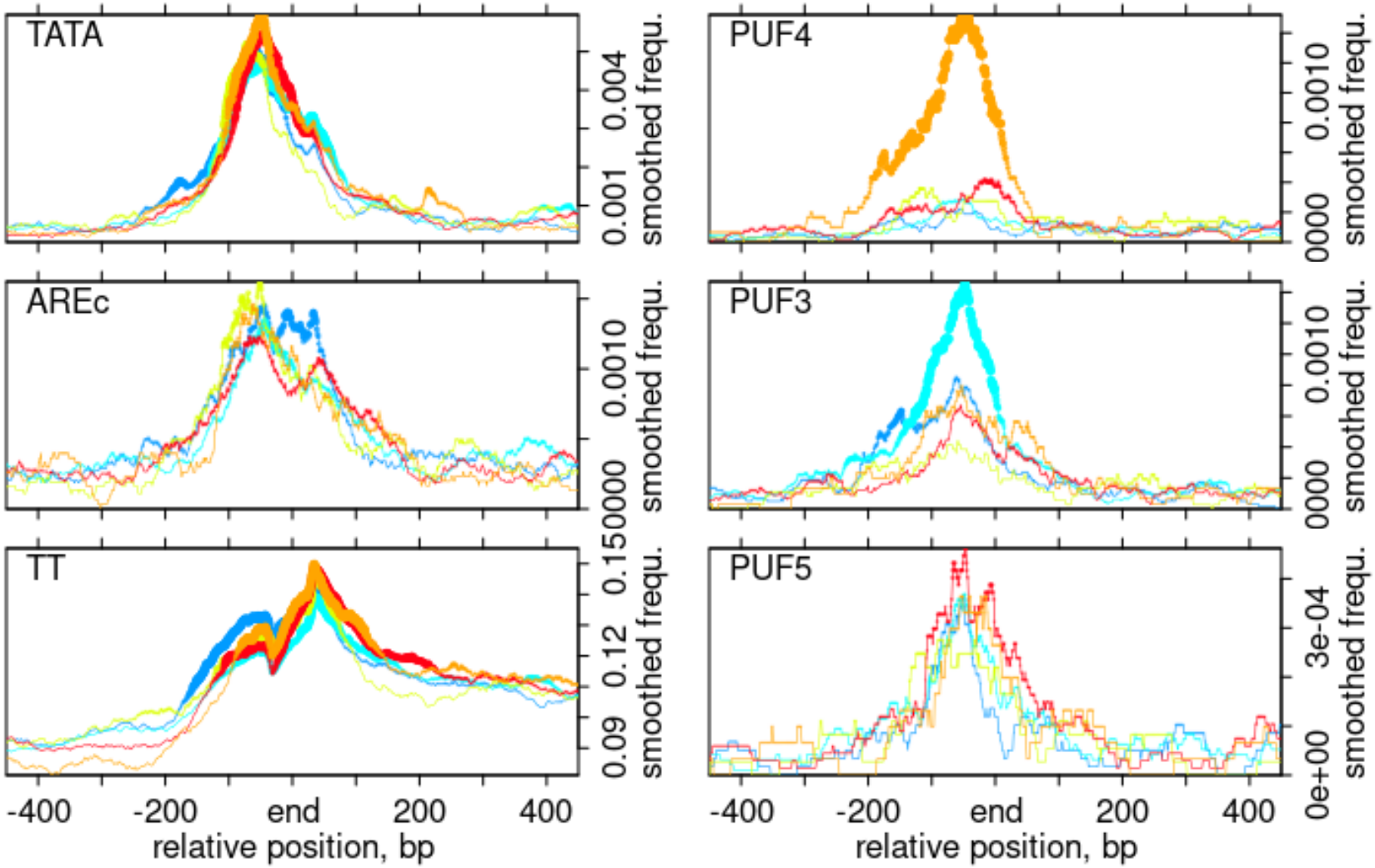
3'UTR Motifs



Pumilio-homology domain proteins (Puf3, Puf4, Puf5) are conserved in eukaryotes and mediate both mRNA localization (e.g. Puf5 to mitochondria) and degradation.

Same, same in a Cyanobacterium?

In eukaryotes and bacteria, the DNA is in a strained state, resulting from transcription, replication and ATP-dependent motor proteins (remodellers & topoisomerases). This strained state is required for expression of growth genes, and repression of stress genes. The torsional strain is channelled by DNA interacting enzymes, e.g., **into open bubble formation by RNA polymerases**.

Summary and Conclusion

- Summary of model, current state.
- Embedding into the growth cycle (LOC).

APPENDIX

Sequence- and ATP-Dependance of Remodellers

Sequence effects on rotational and translational positioning

- Segal et al. (2006): see long pubpeer comment (with figures) by Michael Eisen (2021)[3],
- Cui et al. (2014): rotational positioning model,
- Chereji et al. (2018): barrier model, but assuming promoters.

Subtle effects, difficult to see in genome-wide analysis

- A shift by +/- 10 bp (helical twist) can hide or expose TF binding motifs,
- A shift by +/- 5 bp can expose or bury TF binding motifs.

ATP-dependance

- Remodellers are **motor proteins**, unwrapping and pulling DNA from the nucleosome, one remodelling step can require >10 ATP.
- Zhang et al. (2011): *in vitro* reconstitution requires remodellers + ATP,
- *in vitro* RSC remodeling by 5 or 10 bp depended on ATP concentration,
- ISW2 is specifically inhibited by ADP.

[3] https://pubpeer.com/publications/34904859EA5787B3927F952E0EED43

Global Effects of ATP Depletion

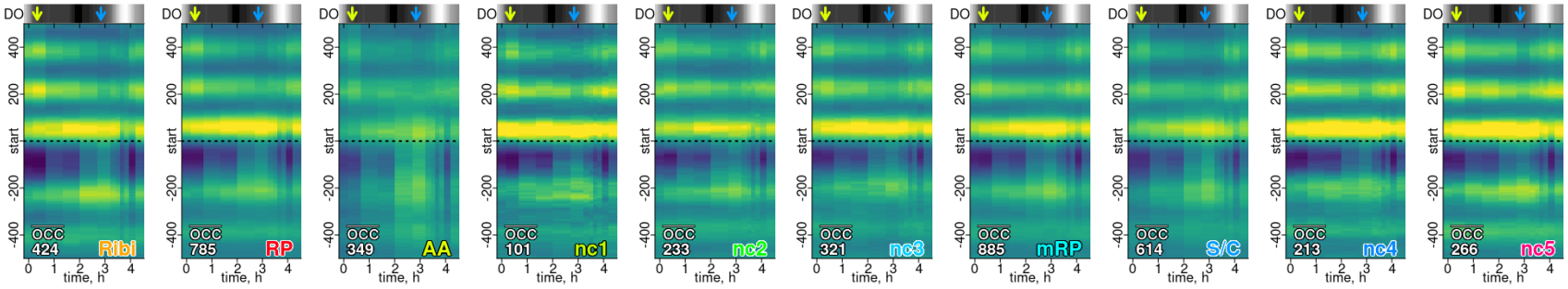
In eukaryotes and bacteria, the DNA is in a strained state, resulting from transcription, replication and ATP-dependent motor proteins (remodellers & topoisomerases). This strained state is required for expression of growth genes, and repression of stress genes. The torsional strain is channelled by DNA interacting enzymes, e.g., into open bubble formation by RNA polymerases.

Other Global Effects of ATP Depletion

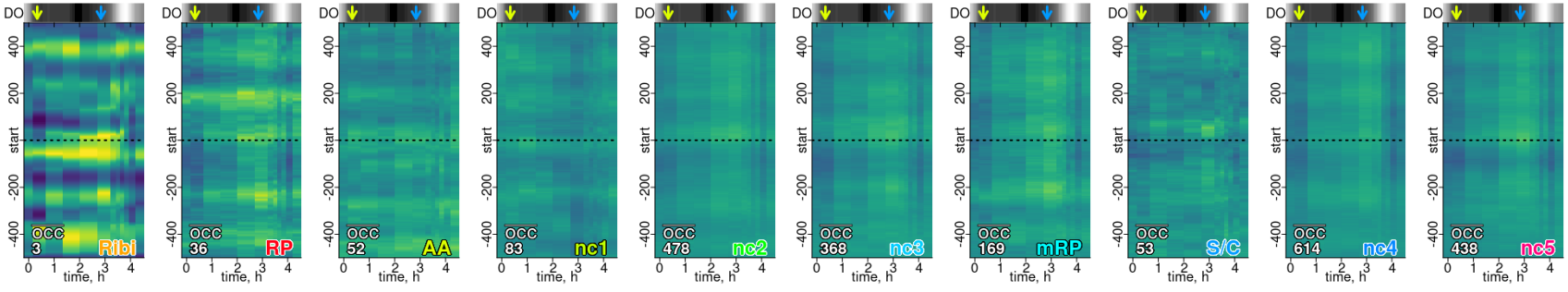
- RNA secondary structure formation (RNA helicases),
- Protein aggregation (solubility, chaperones, degradation),
- Change in ionic composition (ion pumps).

Chromatin Reset Points - Coding v Noncoding

Coding segments only:



Noncoding segments only:



Nocetti and Whitehouse (2016): long period, MNase-seq.

The few noncoding transcripts in HOC clusters show weaker and distinct nucleosome dynamics from their cluster's coding genes; with a fragile nucleosome appearing during LOC phase.

The many noncoding transcripts in LOC clusters show very weak average nucleosome profiles and dynamics.

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