Pervasive Transcription during the Low Energy Phase of Yeast Respiratory Oscillations.

Rainer Machné, Douglas B. Murray, Stephan H. Bernhart, Ilka M. Axmann, and Peter F. Stadler

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**

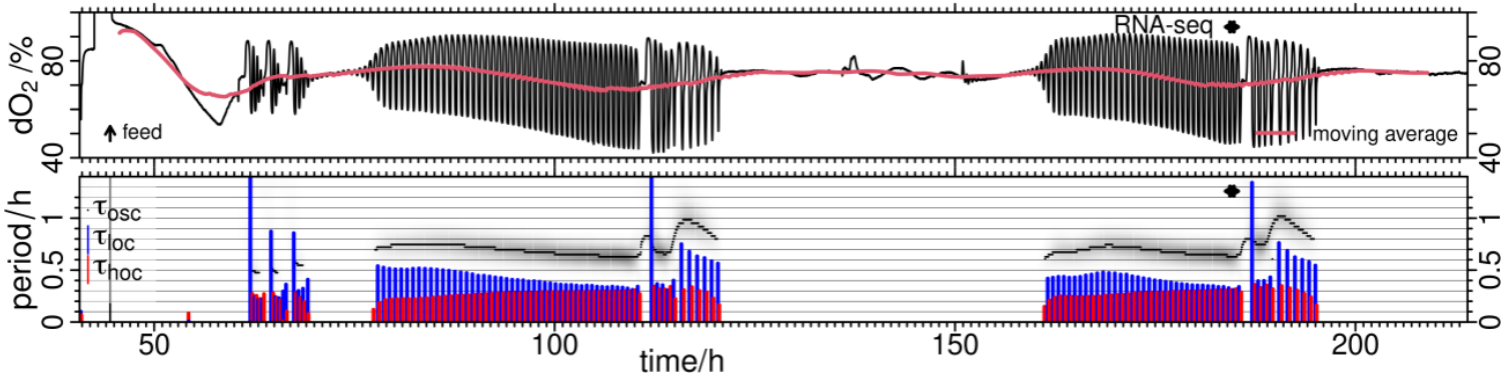
VII. Metabolism: feedbacks and the auto-catalytic cycles of life, and
VIII. The cell growth cycle as a cell-structural proofreading loop. **METABOLISM**

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?*

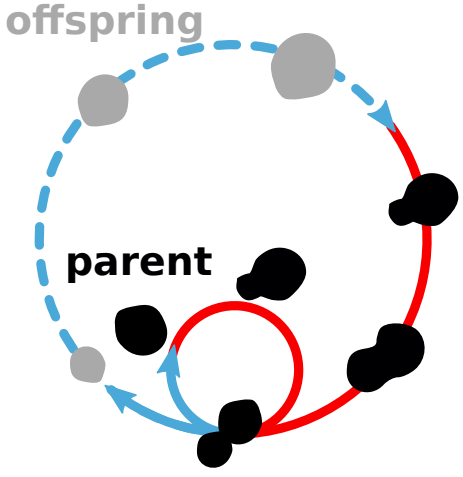
All slides, incl. references, at https://www.bioinf.uni-leipzig.de/~raim/lecture_series/

Respiratory (Metabolic) Oscillation in Budding Yeast



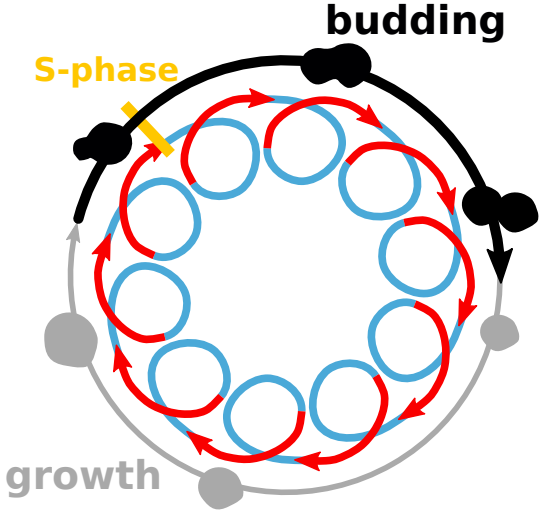
At high cell density: phases of High (HOC) and Low Oxygen Consumption (LOC).
here: distillery strain IFO 0233: exceptionally short periods, and **regular cycles or complex dynamics**.

Long period (v.Meyenburg, 1969):



$\tau_{osc} < \tau_{doubling}$

Short Period, IFO 0233 (Kuriyama, 1992):



$\tau_{osc} \ll \tau_{doubling}$

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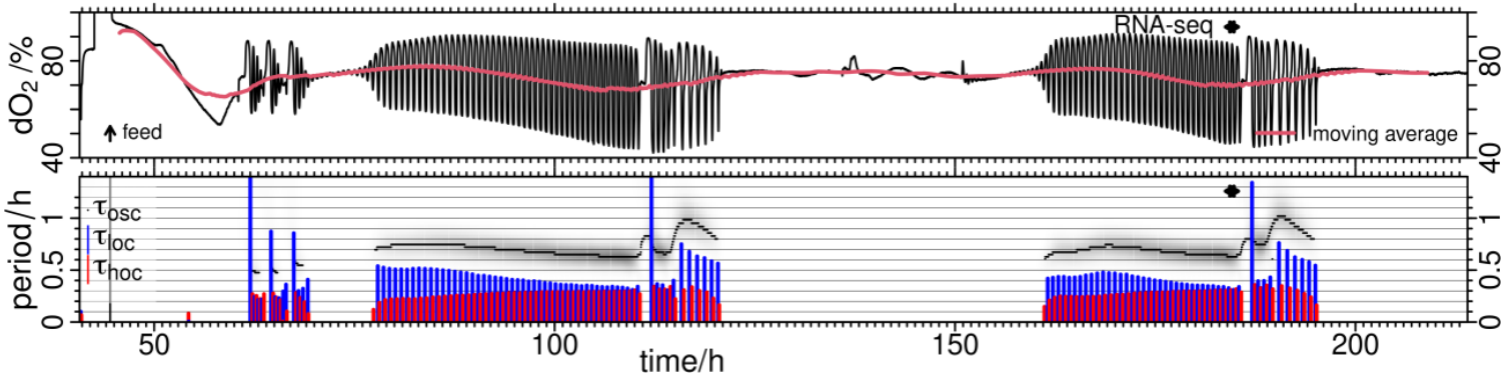

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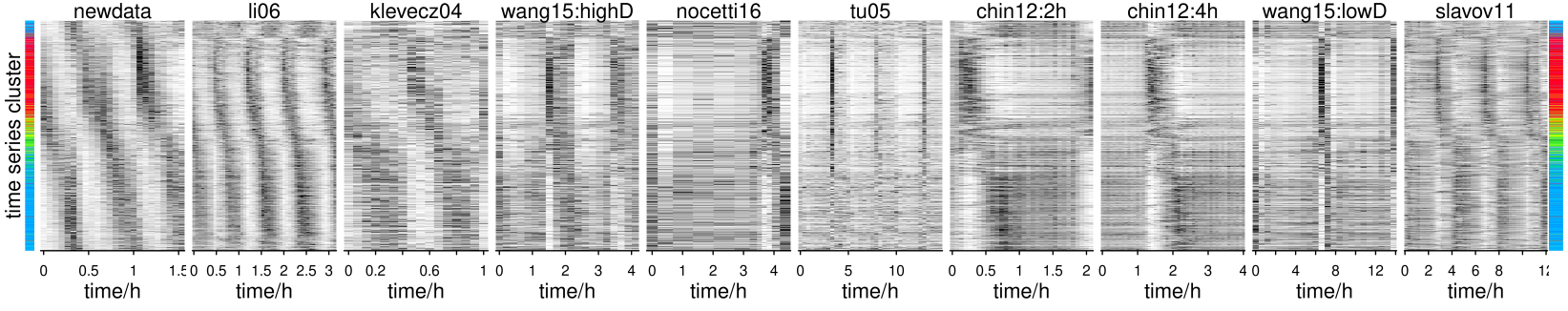
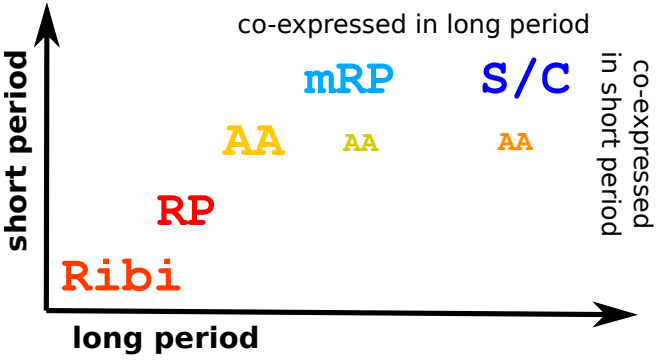


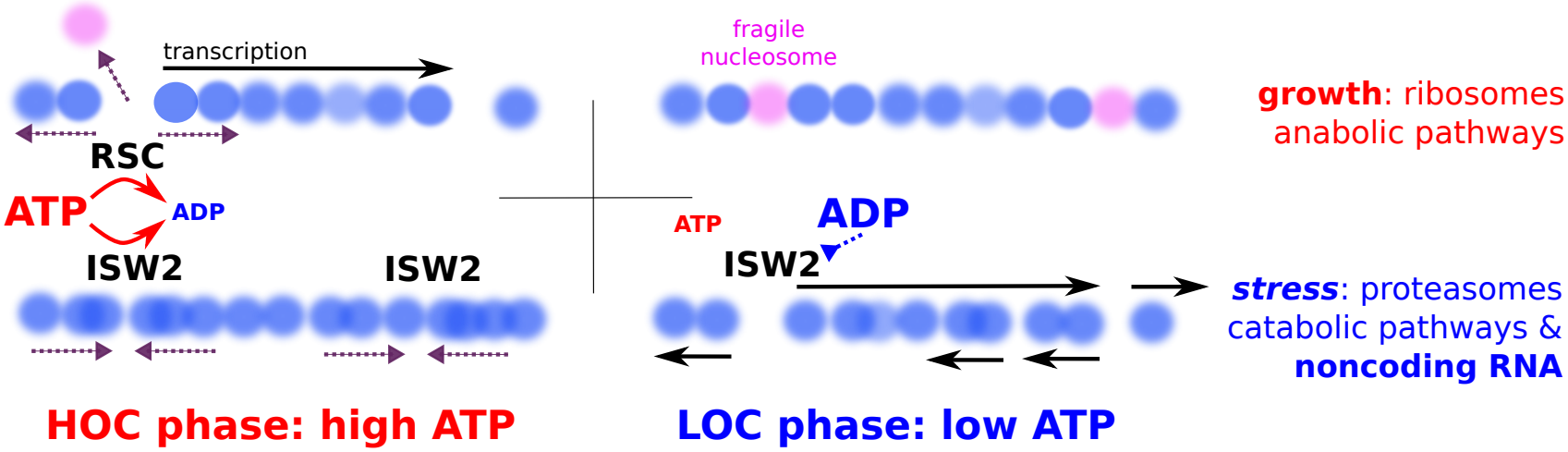


A conserved temporal transcription program:

Ribi → RP → AA → mRP → S/C

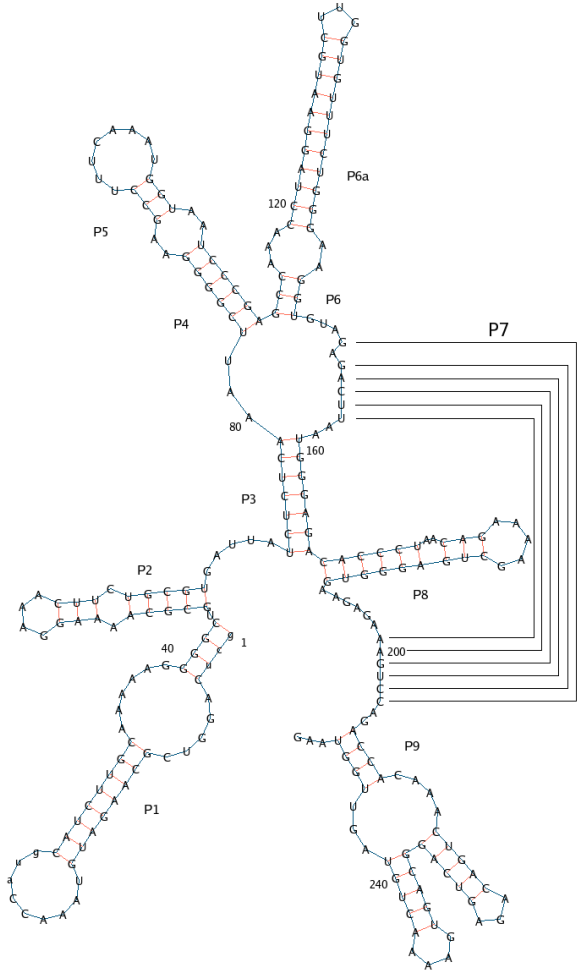
unspooled at periods τ_{osc} from 0.6 h to 120 h!

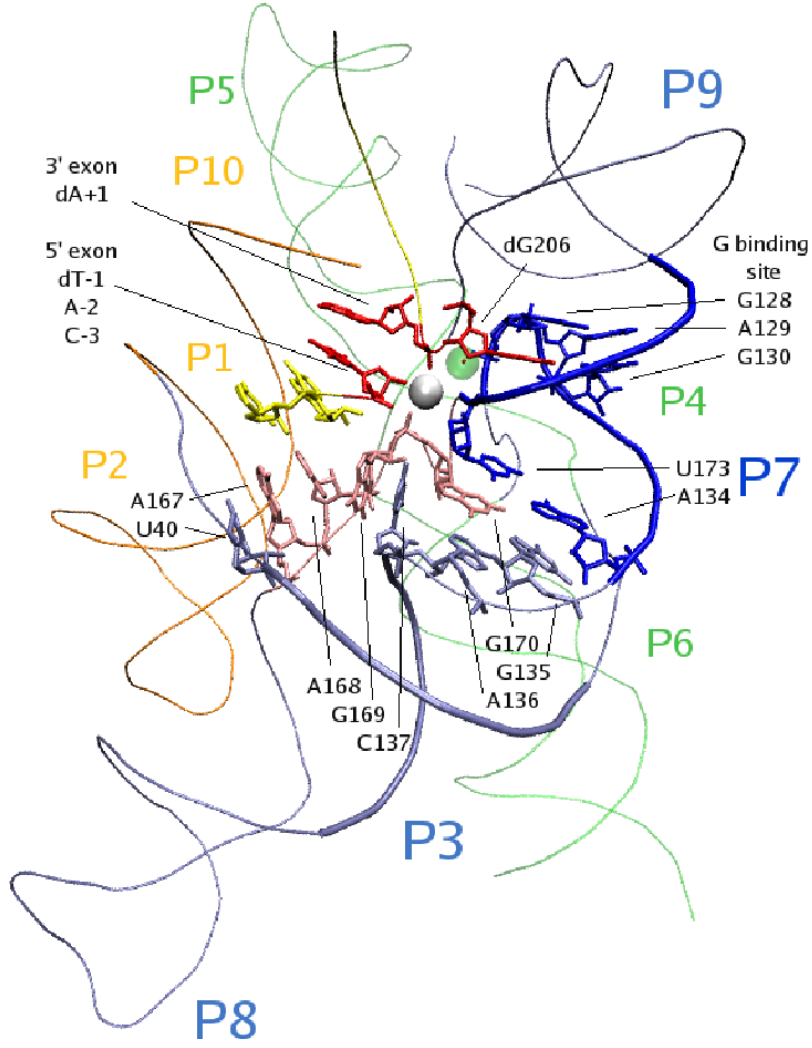
Lecture III - DNA as a metabolic sensor:



Machné and Murray (2012), Amariei et al. (2014)

Prediction: pervasive **noncoding transcription** during the low energy states of the cell, e.g. *stress* or LOC phase.





Group I self-splicing intron in the fMet-tRNA of *Synechocystis* sp. PCC 6803,
Splicing, and thereby **protein translation**, may be light- and/or redox-dependent,
P2 loop encodes a **homing endonuclease** (not shown) specific for intron-less fMet-tRNA.

$\Rightarrow$ mobile *regulatory* elements, **Lectures II and IV**.

Noncoding Transcription: RNA and Evolution

Noncoding transcription: produces RNAs that do not code for a protein.

- **Ribozymes**: RNA may[1]/can[2] catalyze reactions (Nobel 1989), commonly with a bivalent cationic co-factor (Mg^{2+}, Fe^{2+}):
  - Translation (rRNA), splicing (snRNA).
- **Ancient RNA world**, at the origin of life (OoL):
  - RNA as both enzyme and genetic material, *cf.* Eigen's *hypercycle* and *quasispecies*[3],
  - An **axiomatic model of the genotype-phenotype map**[4],
  - Current OoL discussion: RNA-first or metabolism-first[5],
  - Until the *Great Oxidation Event* (GOE)[6]: $[Fe^{2+}] \gg [Mg^{2+}]$?
- **Extant RNA world**, *junk DNA* or *function*:
  - Increase of noncoding genomic fraction with developmental complexity[7]?
  - Easily **evolvable** small RNA regulators.

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[1]: Woese (1967), Crick (1968), Orgel (1968); [2]: Cech (1990), Altman (1984); [3]: Eigen and Schuster (1977); [4]: Schuster et al. (1994), Stadler et al. (2001); [5]: Preiner et al. (2020); [6]: Hsiao et al. (2013), Okafor et al. (2017); [7]: Mattick (2001), Mattick (2023)

Noncoding Transcription: Pervasive?

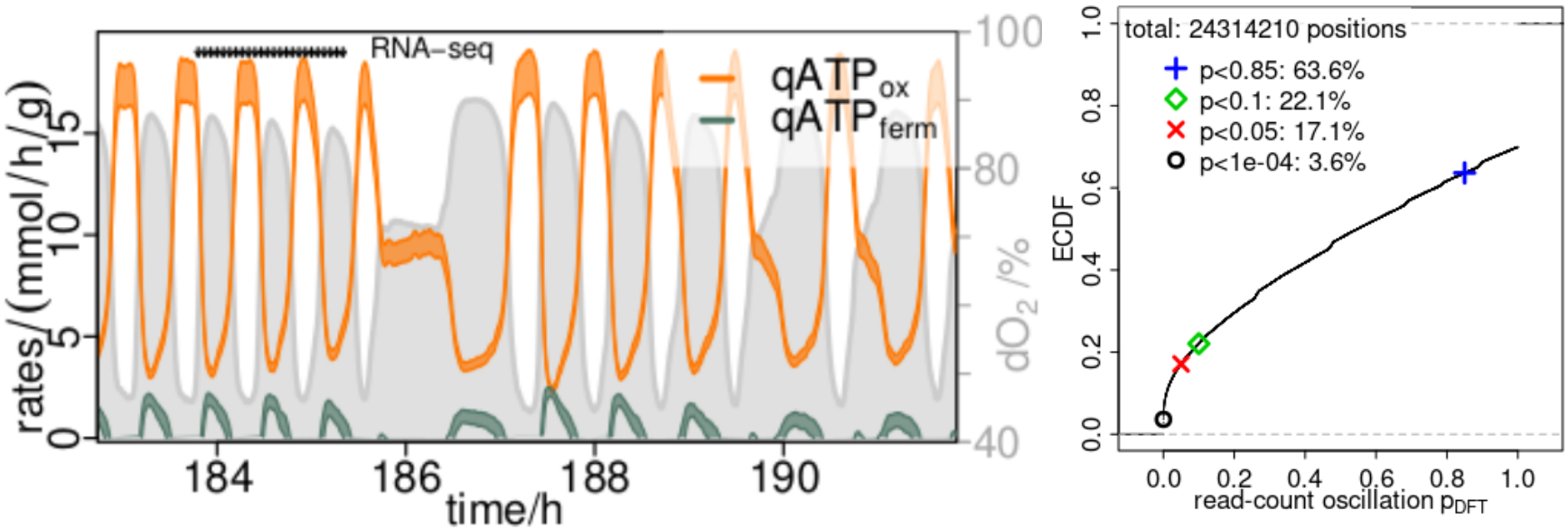
Noncoding transcription: produces RNAs that do not code for a protein.

- Many roles are now well understood:
  - catalytic: rRNA, snRNA, RNase P,
  - regulatory: miRNA, Xist, chemo- and thermosensors,
  - immunity: siRNA, sgRNA.
- But early tiling-array and sequencing data indicated **persavive transcription** (∼genome-wide) beyond the known cases:
  - lncRNAs: regulatory upstream and antisense transcripts,
  - circRNAs as miRNA sponges,
  - scaffolds for RNA binding protein (RBP)-containing droplets,
  - . . . **but no general picture exists**.
- Can also be **detrimental**: R-loops, replication fork barriers.
  - local genome instability and **evolvability**?

Prediction: pervasive **noncoding transcription** during the low energy states of the cell, e.g. *stress* or `LOC` phase.

Similarity-Based Segmentation of an RNAseq Time Series

We (i) sampled for a poly(A)-RNA-seq time series in high temporal resolution:



. . . **confirming that noncoding transcripts are enriched in `LOC` phase**, where qATP and ATP reach their minima (Lecture VI):

- 76% of the genome covered by reads,
- 17% of the genome showed periodic ($p < 0.05$) abundance.
-

The Discrete Fourier Transform (DFT) of the time series yields phase and amplitude, and a permutation test provides a p-value for each genome position.

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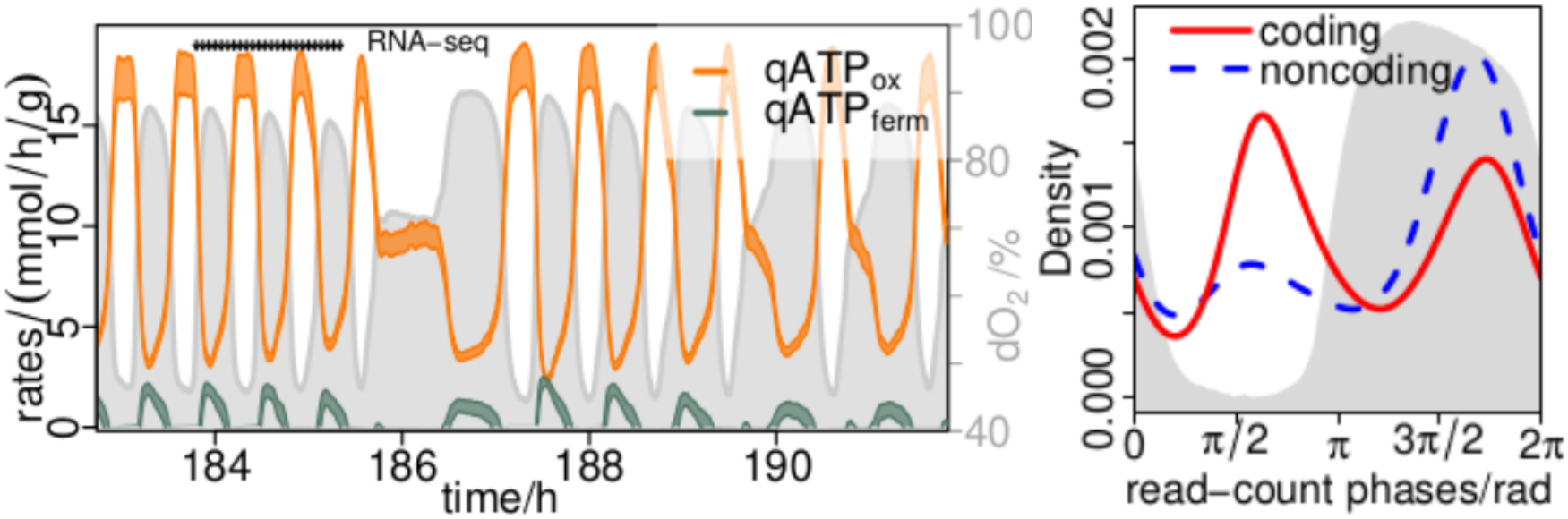

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- Noncoding transcription mostly peaks in `LOC` phase.

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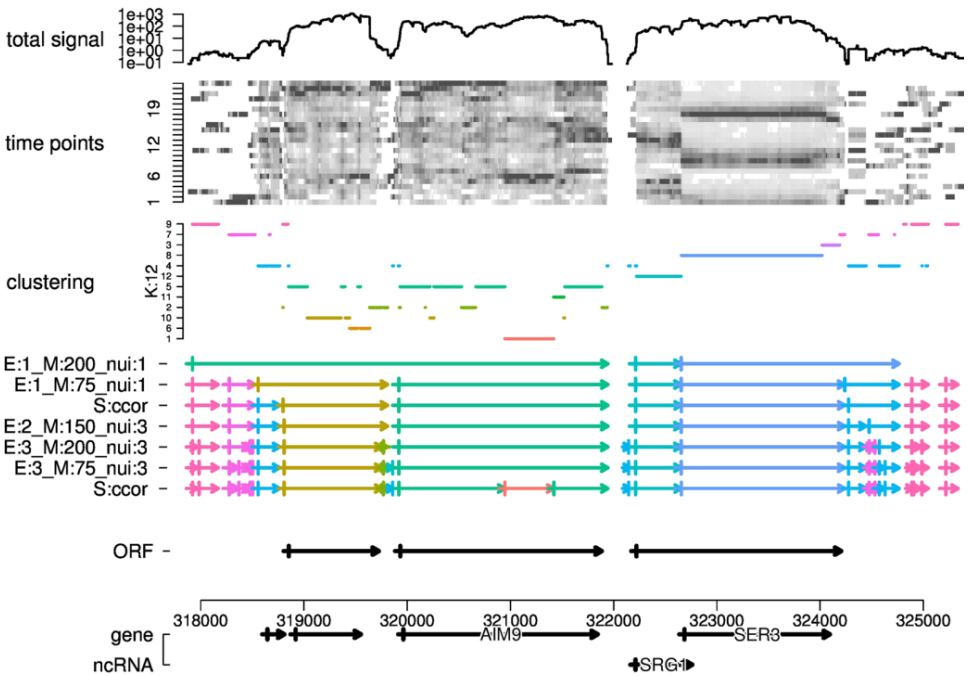
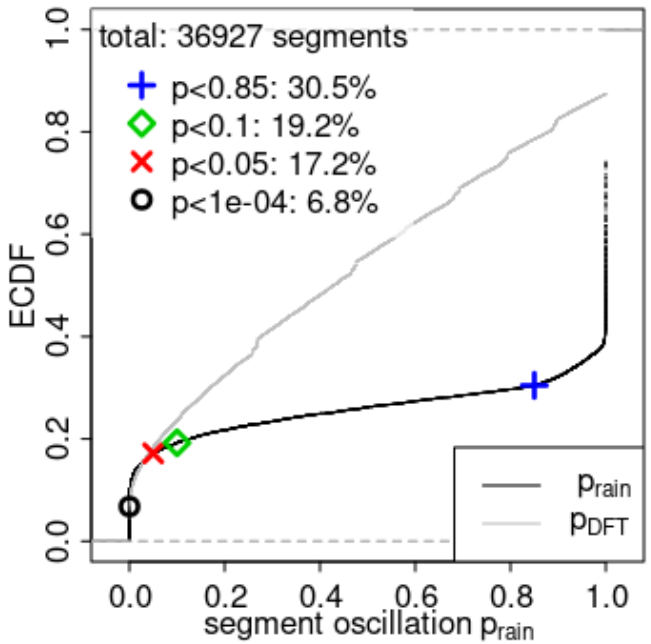
Similarity-Based Segmentation of an RNAseq Time Series

We (i) sampled for a poly(A)-RNA-seq time series in high temporal resolution, and (ii) developed a novel algorithm (`segmenTier`) to find co-expressed segments, **independent of the genome annotation (unbiased)**:





- Similarity-based segmentation by a dynamic programming algorithm,
- *Note*: segments $\neq$ transcripts: `segmenTier` does not resolve overlaps,
- $\approx$ 37k segments, $\approx$ 26k short and noisy, $\approx$ 11k for clustering.

Segmentation algorithms available at CRAN: R packages `segmenTier`, `dpseg` and `CONSSEG` (Machné, Murray, and Stadler 2017; Saker et al. 2021). p-values by the `rain` package (Thaben and Westermark 2014).

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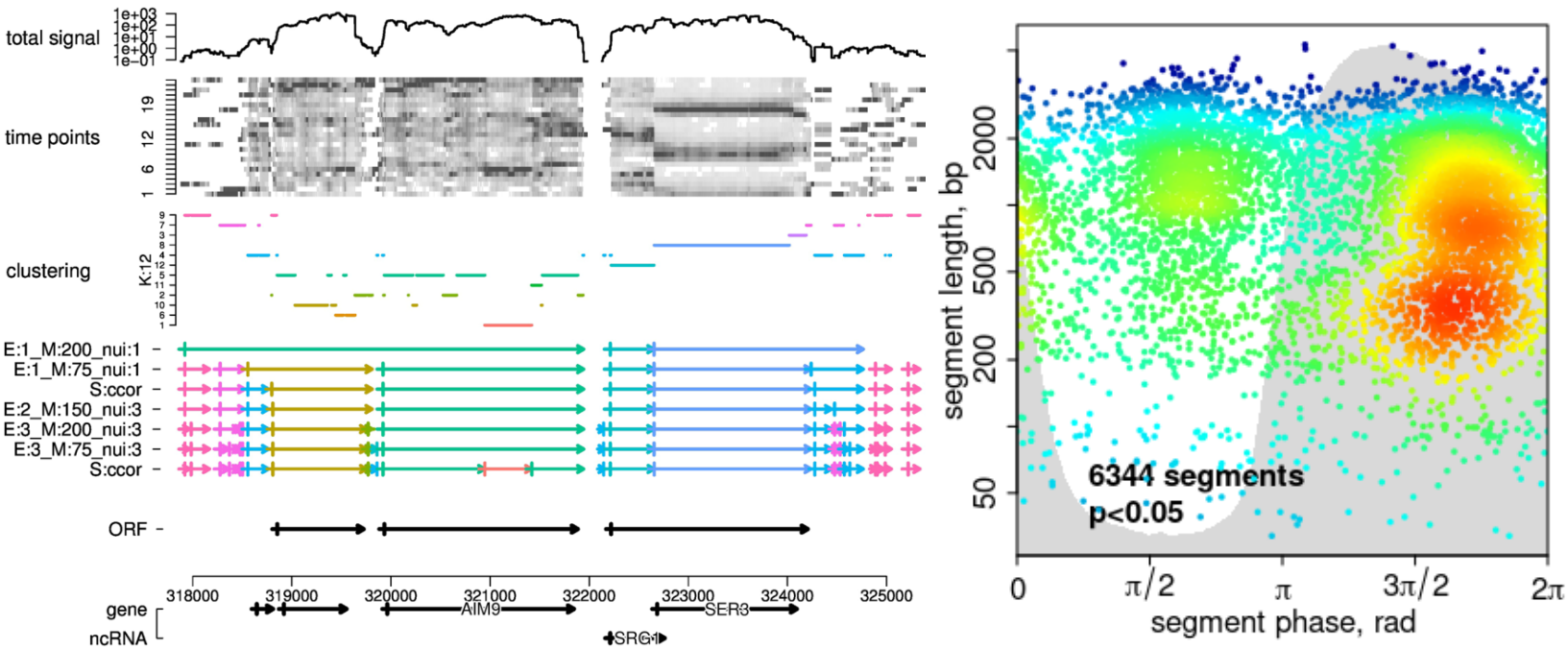

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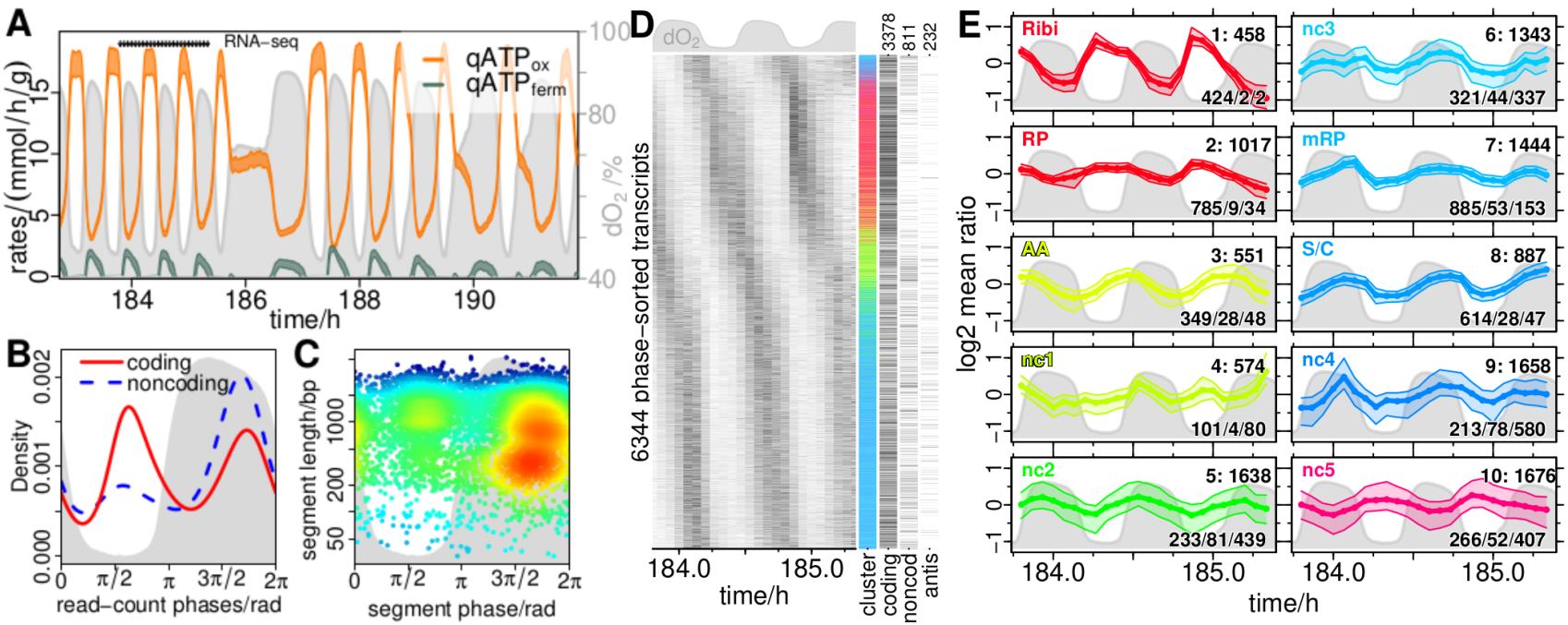
Similarity-Based Segmentation of an RNAseq Time Series

We (i) sampled for a poly(A)-RNA-seq time series in high temporal resolution, and (ii) developed a novel algorithm (`segmenTier`) to find co-expressed segments, **independent of the genome annotation (unbiased)**, and (iii) clustered temporally co-expressed segments:

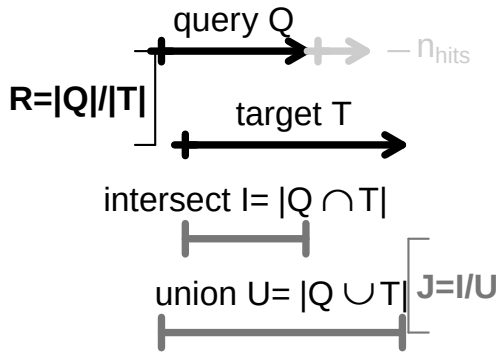


- Similarity-based segmentation by a dynamic programming algorithm,
- *Note*: segments $\neq$ transcripts: `segmenTier` does not resolve overlaps,
- Noncoding transcription mostly peaks in LOC phase.

Model-based clustering of the DFT of time series data (Machné and Murray 2012; Lehmann et al. 2013; Behle et al. 2022); using `segmenTools`' `clusterTimeseries2` function.

Noncoding Transcripts in LOC phase

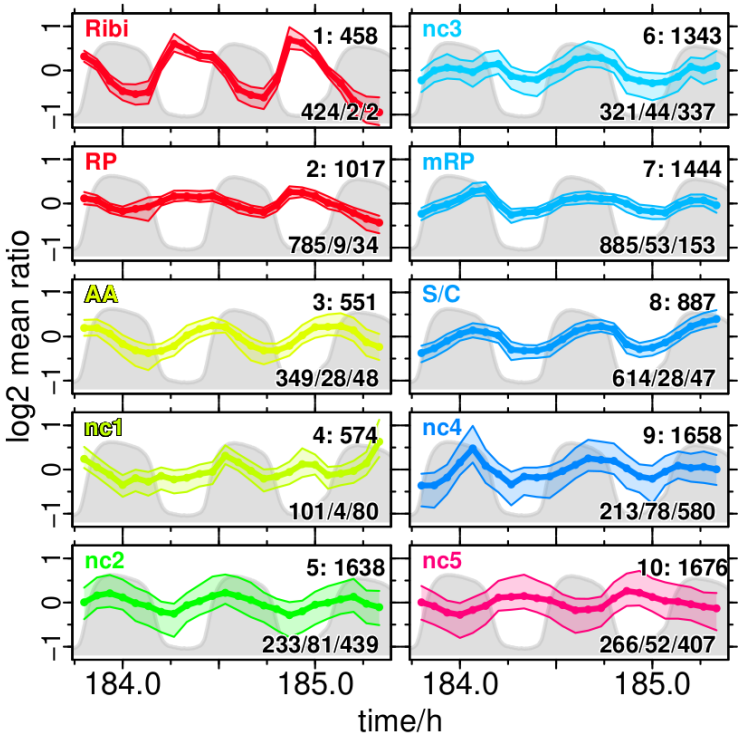
Where are those transcript candidates in the genome?

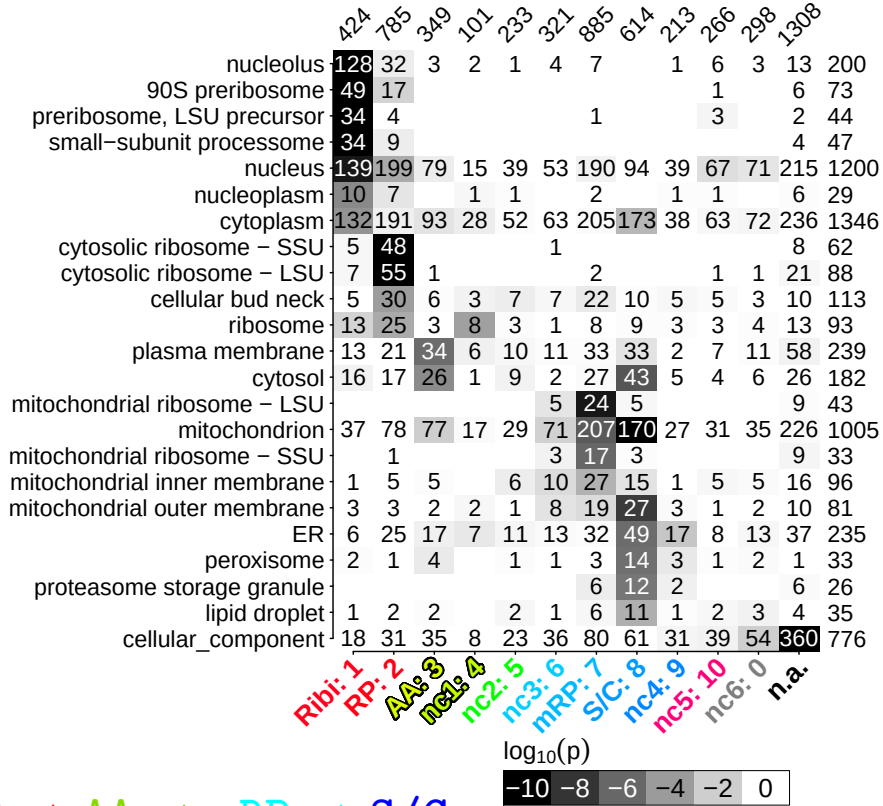


- Local J and length ratio R between individual overlapping pairs to annotate individual segments, and
- Global Jaccard index J between segment classes, and $n = 10000$ permutations of the query set (our segments): enrichments evaluated as $p = \frac{1}{n} \sum_{i=1}^{n} [J_{\text{random,i}} \geq J_{\text{real}}]$.

Segment analysis tools of the `segmenTools` R package (github.com/raim/segmenTools), with statistics partially based on `BEDtools` (Quinlan and Hall 2010).

Segment Annotation: Known Temporal Program

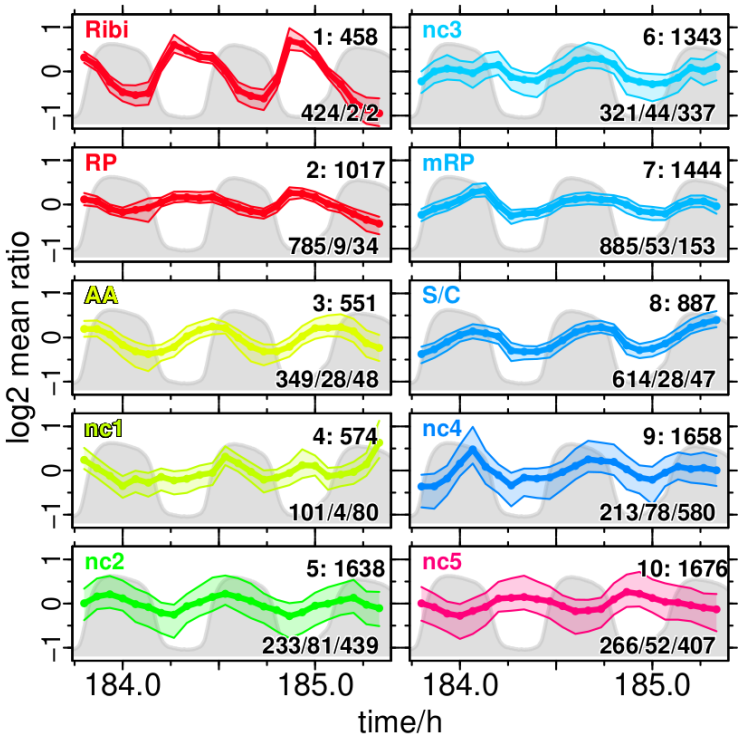


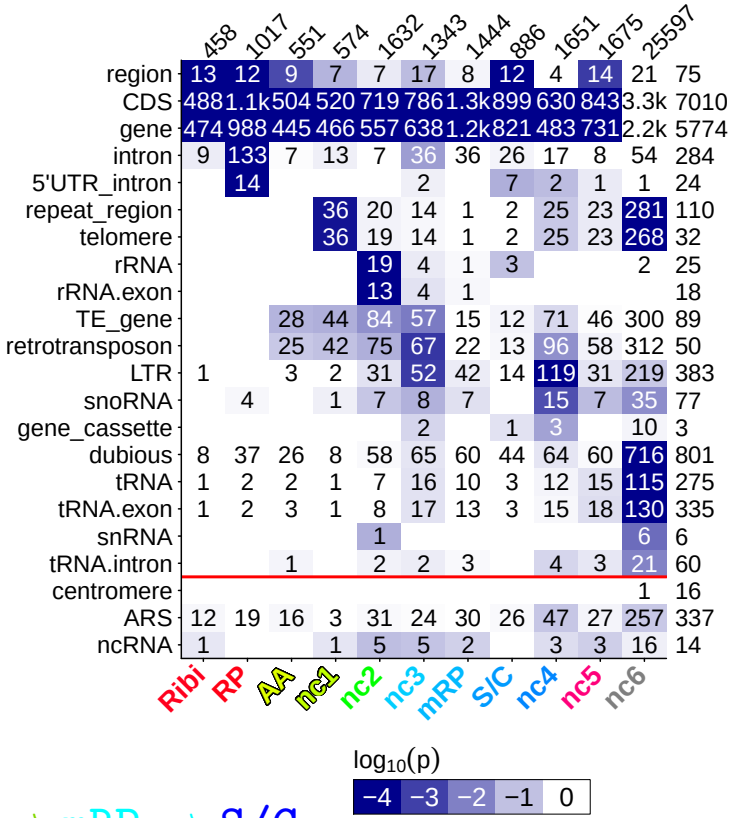


- Five coding clusters: Ribi → RP → AA → mRP → S/C, reflect the **known temporal program** (Machné et al. 2012).

Segments with $J_{ORF} > 0.5$ were assigned to a coding gene; cumulative hypergeometric distribution tests for segment clusters v. GO annotation were calculated; GO categories with signifcant hits were sorted along the temporal program; using `segmenTools`' `clusterAnnotation`, `sortOverlaps` and `plotOverlaps` functions.

Segment Annotation: Noncoding Clusters

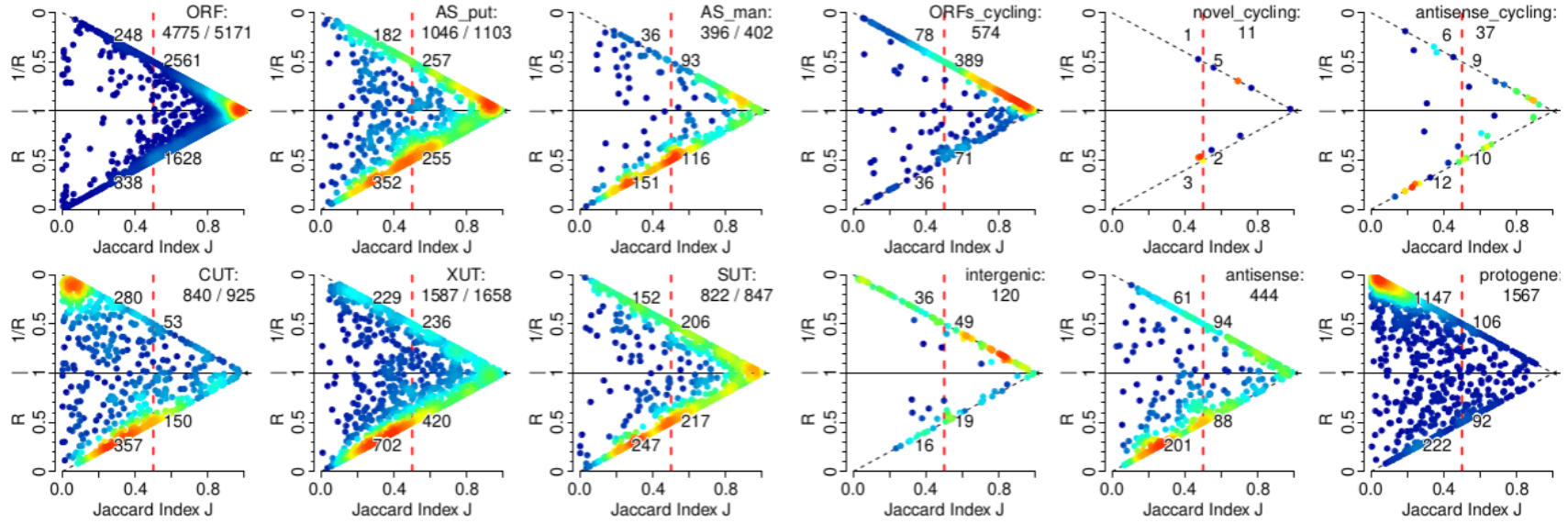


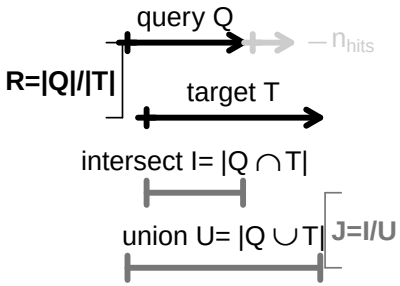


- Five coding clusters: Ribi → RP → AA → mRP → S/C,
- Five noncoding clusters:
  - nc1 shows a mixed profile, enriched at telomeres,
  - nc2 in early LOC phase, enriched at rRNA,
  - nc3-nc4 span LOC phase, enriched at snoRNA, **transposons**,
  - nc5 is associated with HOC phase,
  - +nc6: pre-filtered short and noisy segments.

Global Jaccard index (J) between segment cluster and genome annotation type; queries permutated $n = 10000$ times and J_r calculated; p-values: fraction of permutations where $J_{\text{random}} > J_{\text{real}}$; script `segmentOverlaps.R`.

Overlaps with Published Transcript Sets





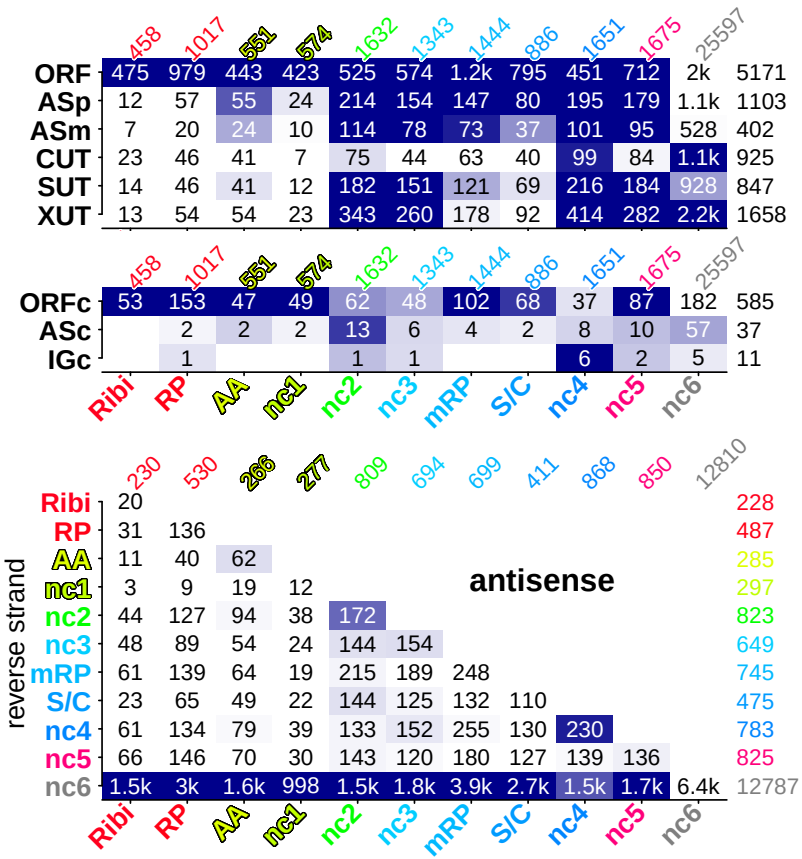
Query Q: our segments,
Target T: published transcripts,
$R = J = 1$ for $Q = T$ (identical segments).

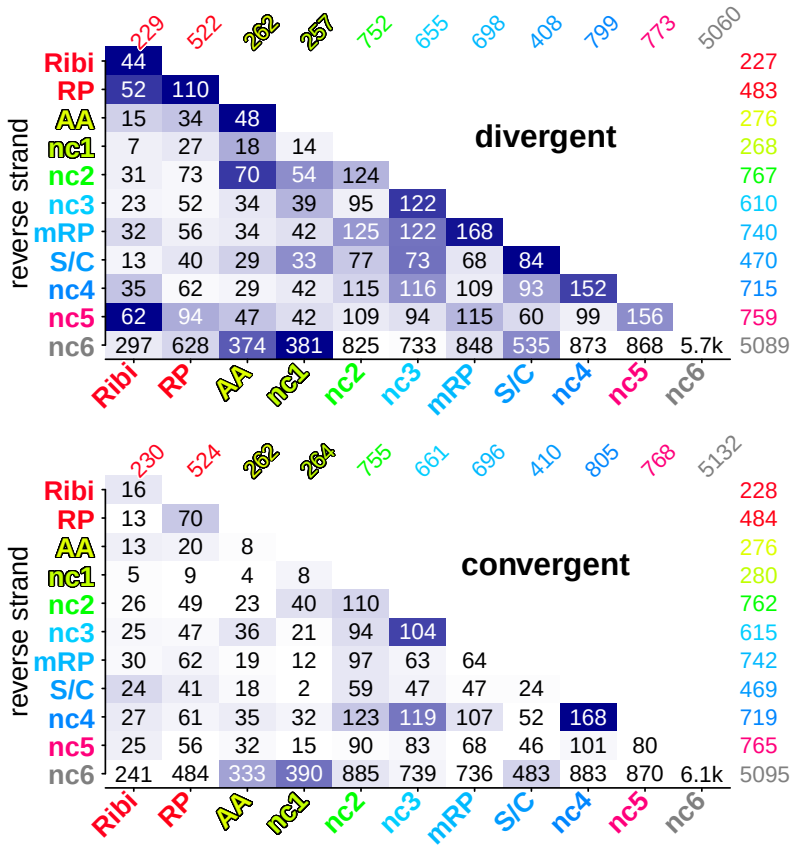
$R < 1$: Q **segments shorter than** T,
$\frac{1}{R} < 1$: Q **segments longer than** T.

J-R plots of the Jaccard indices J v the length ratios R of individual overlapping pairs $[Q_i, T_j]$ indicate how well our segments correspond to existing genomic interval sets.
We find **only few full-length matches** with previous noncoding sets:
condition-specific noncoding transcription landscape.

Using `segmenTools`' `segmentAnnotate` and `jrplot` functions.

Noncoding transcripts in LOC phase

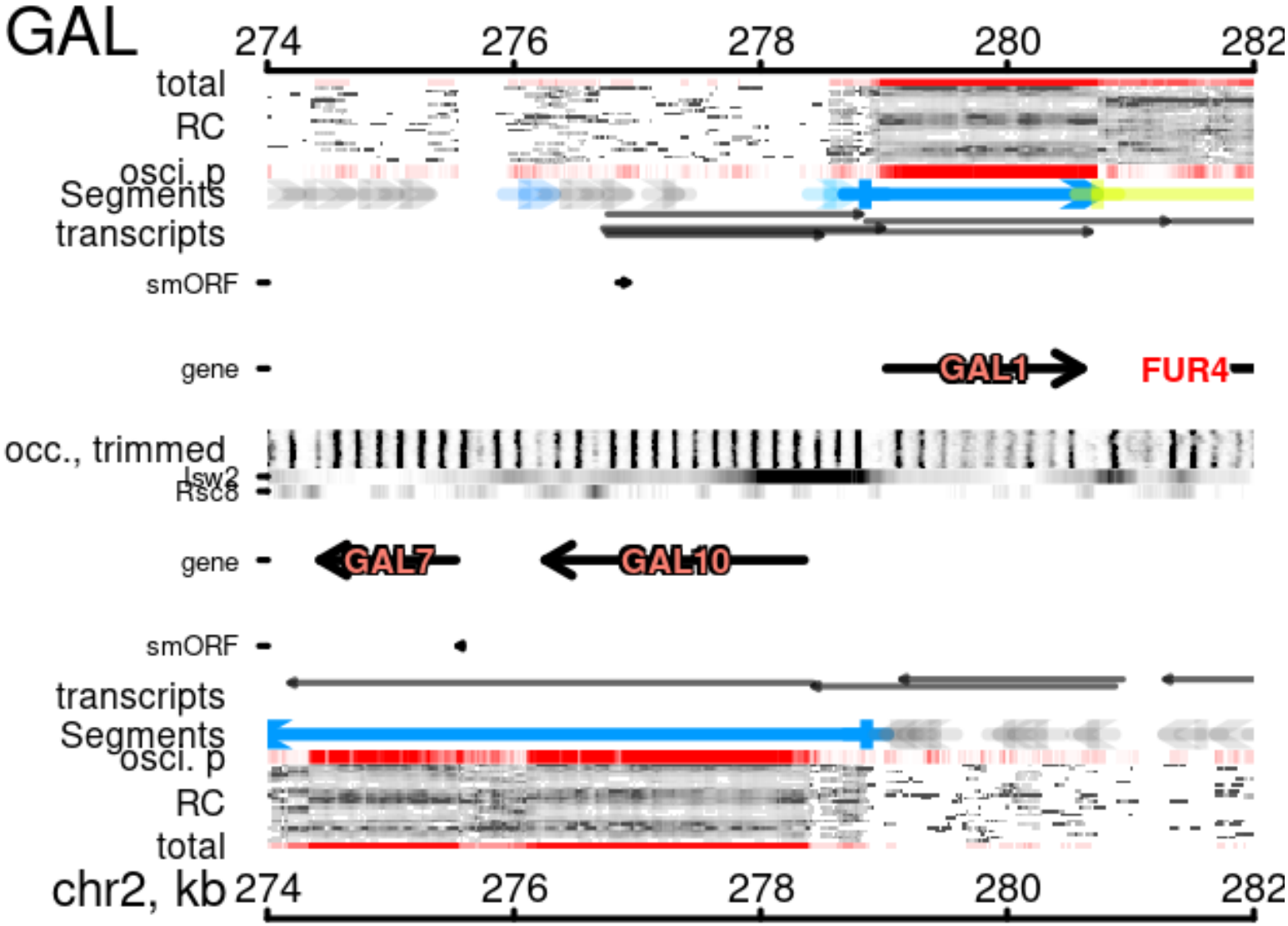




- LOC phase-enrichment of previously described noncoding transcripts (Yassour et al. 2010; Xu et al. 2009; van Dijk et al. 2011; Granovskaia et al. 2010) ,
- Antisense show **no systematic phase-relation** to their sense trancripts,
- Divergent promoters from the same phase and cluster (diagonal),
- **Clusters nc2–nc4 often converge on themselves and on each other.**

Using `segmenTools`' coordinate mapping functions to search antisense; 1 kb upstream of segment start site (divergent); and 1 kb downstream of segment end (convergent); via the R script `segmentOverlaps.R`.

Antisense Loci in Genomic Context



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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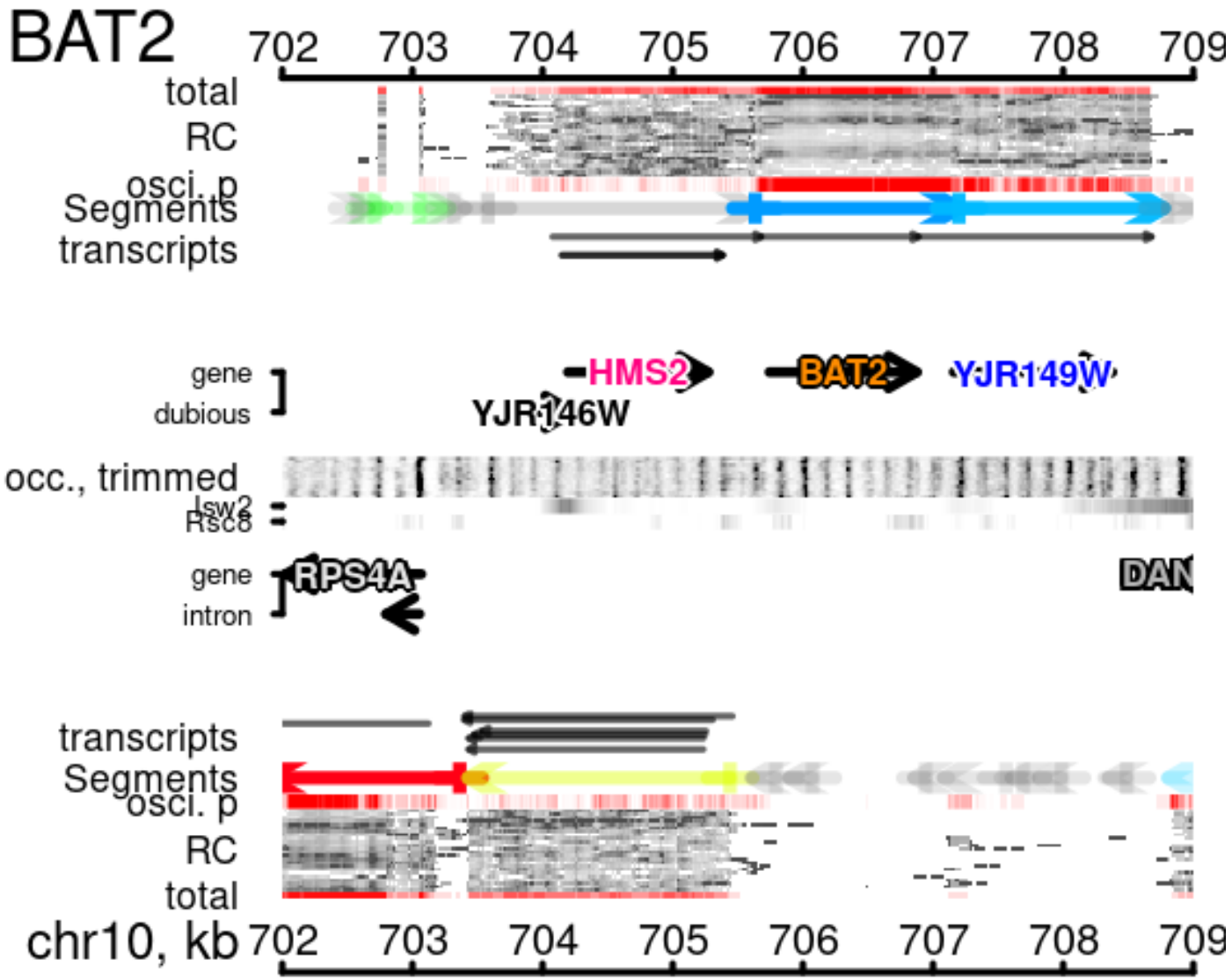

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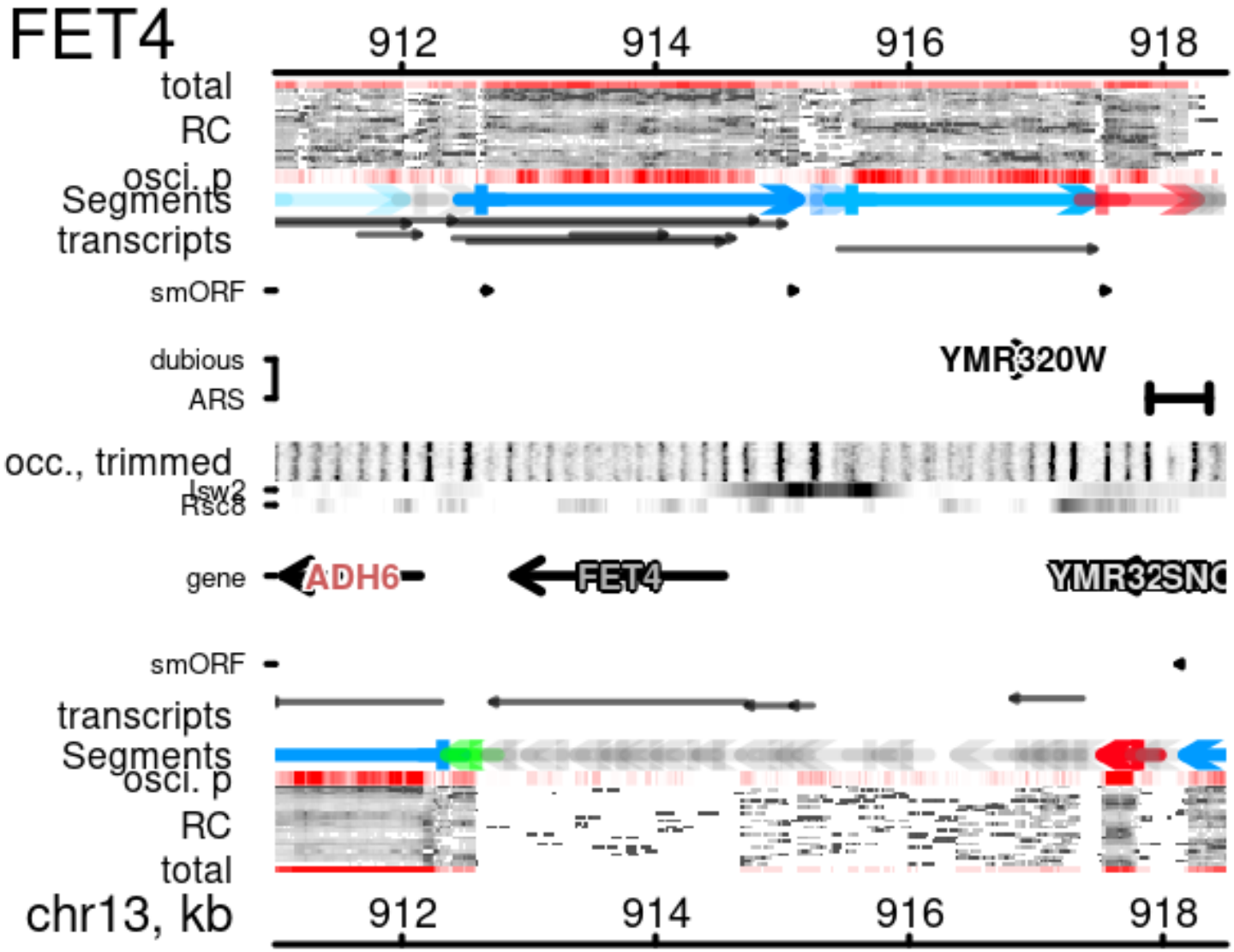

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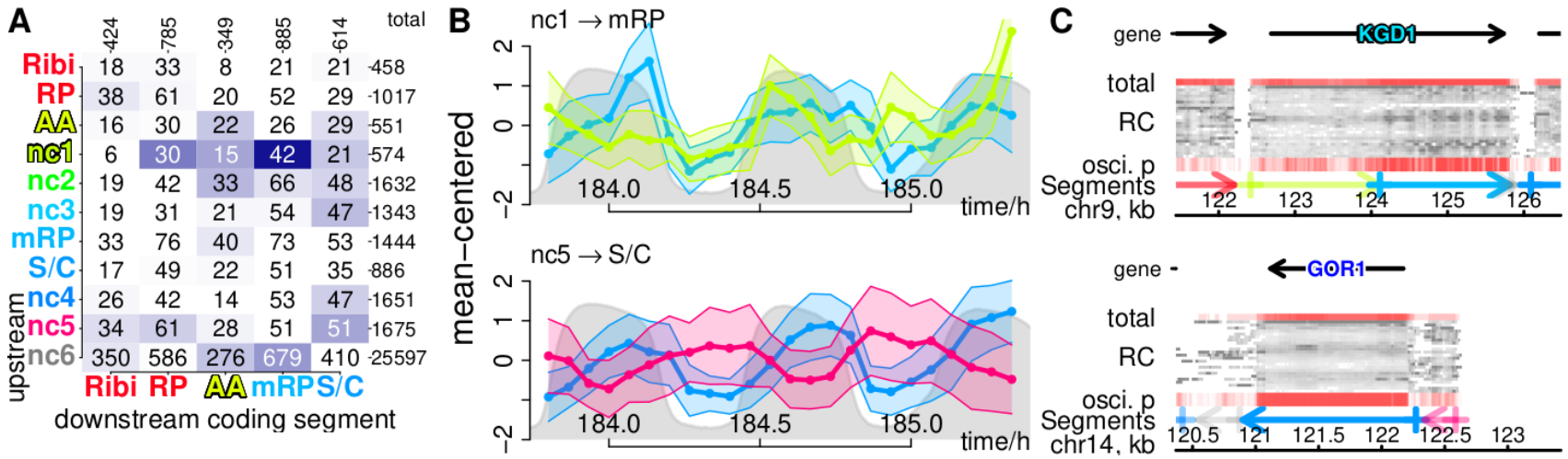
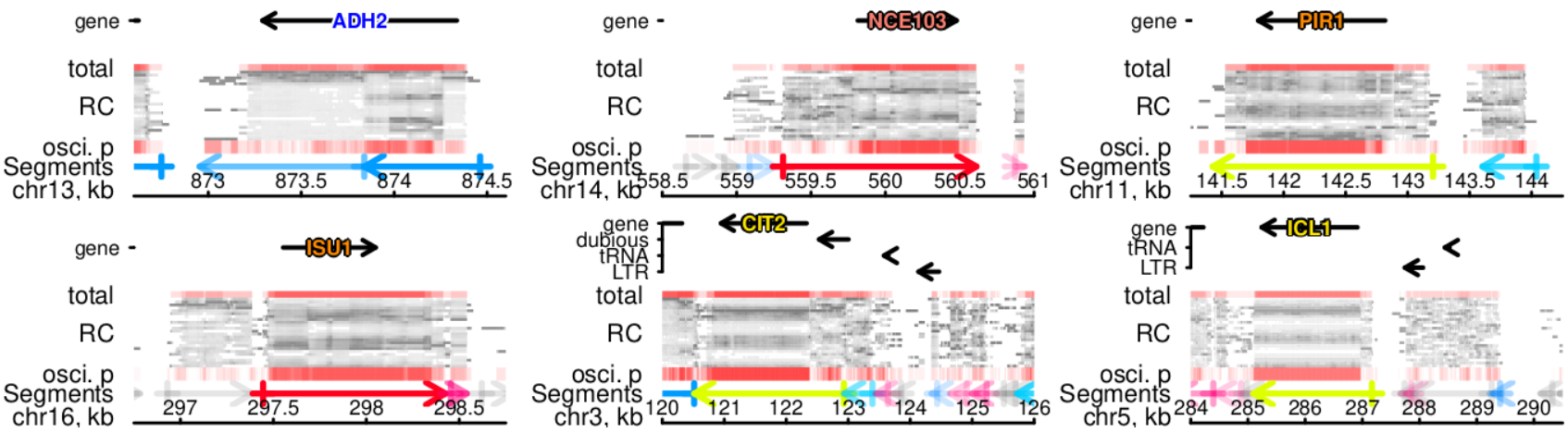
Promoter-Associated Transcripts (PARs)



PAR transcription can e.g. remodel promoter nucleosomes of downstream target genes, e.g. SRG1/SER3 (Martens, Laprade, and Winston 2004).

- Clusters `nc1` (`mixed`) and `nc5` (~`HOC`) show **weak** enrichments upstream of `mRP` and `S/C` clusters,
- The downstream target genes are typical for their cluster.

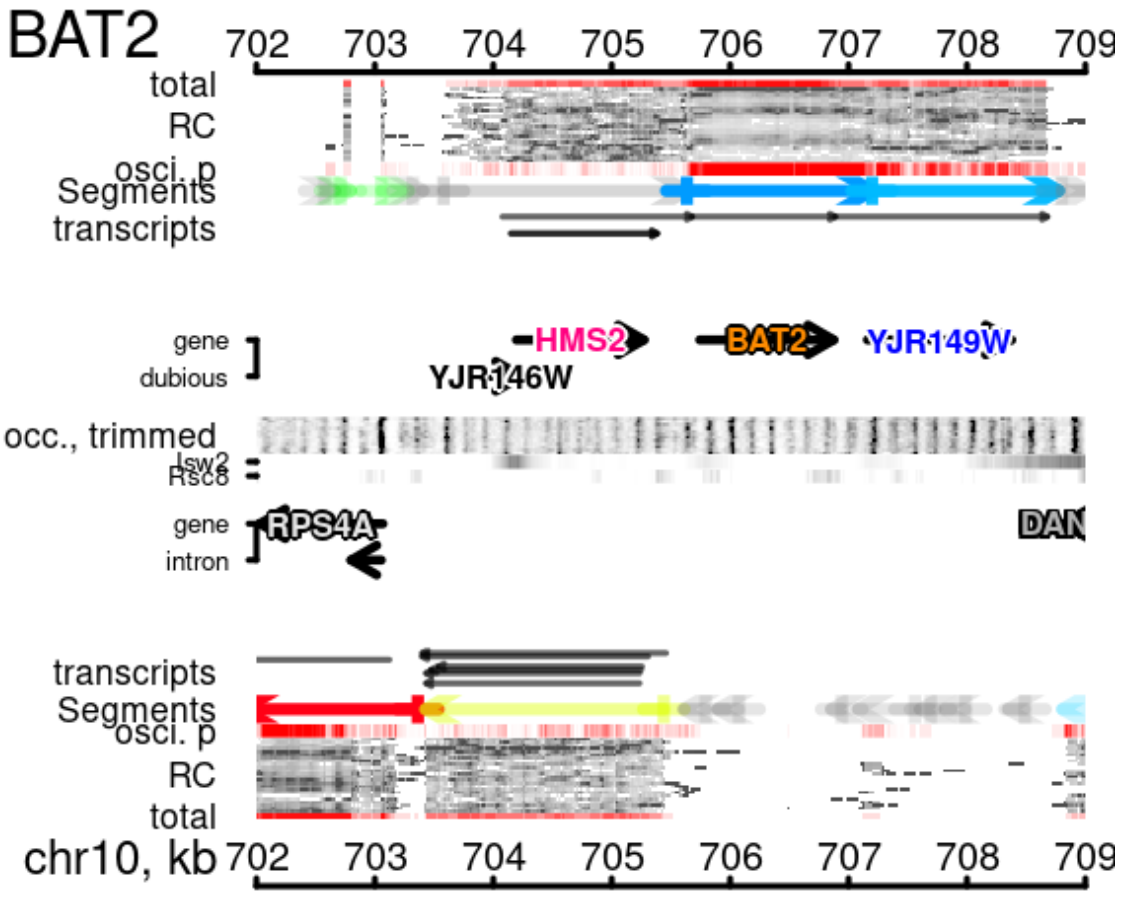
Promoter-Associated Transcripts (PARs)



False negatives: PARs not segmented or not enriched, e.g.

- *Often seen* ($p =$?) in cluster `Ribi` and `AA` genes encoding for **short-lived** enzymes in carbon sequestration and the autocatalytic glyoxylate cycle (**Lecture VII: feedbacks**).
- **Note** the tRNAs and long-terminal-repeats (LTR): LTR flank LTR retrotransposons and often remain after transposon loss (Solo-LTR).

Further Observations



- Small transcripts flanking introns in antisense !?
  - related to spliRNAs (Taft et al. 2010) ?
- 5' $\rightarrow$ 3' and 3' $\rightarrow$ 5' gradients:
  - dpseg algorithm!
  - different RNA degradation pathways?

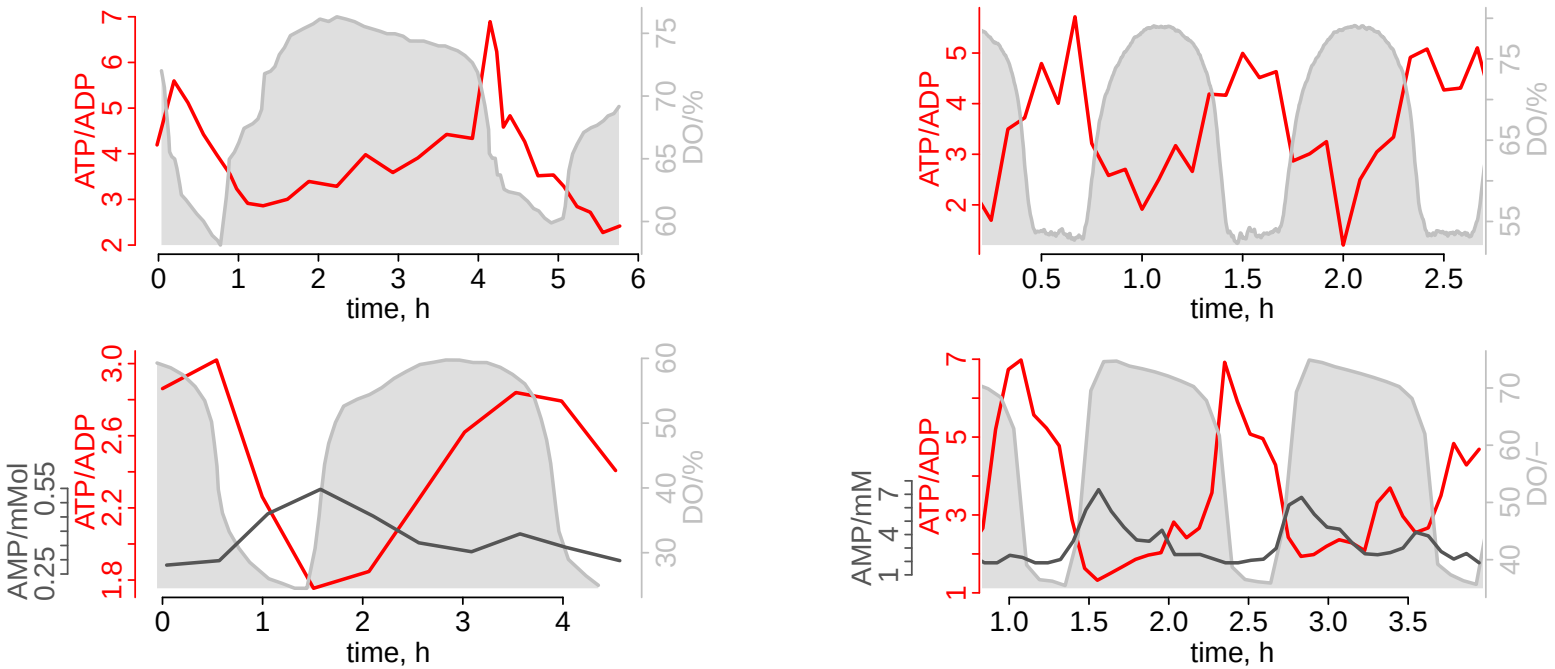
Noncoding Transcription - Summary

- Unprecedented temporal and spatial resolution of transcriptional dynamics on the yeast genome, but it provides **more questions than answers**.
- **Pervasive noncoding transcription is strongly biased but not exclusive to `LOC` phase** . . . **in our conditions**.
- **Weak overlap** with previously described sets of noncoding transcripts: highly condition-specific, strain-specific or even cell-specific.

- Highly individual transcription patterns around coding genes (antisense, promoter-associated), **several candidates for further investigation**, e.g. carbon sequestration and the glyoxylate cycle (Lecture VII).

Noncoding Transcription - Outlook 👋

- **Possible roles of noncoding transcription during LOC phase and/or at low ATP?**



- HOC phase ends with a pulse of amino acid synthesis and protein translation (Murray, Beckmann, and Kitano 2007; O' Neill et al. 2020),
- Protein degradation peaks in LOC phase (O' Neill et al. 2020),
- Low pH (Satroutdinov, Kuriyama, and Kobayashi 1992; O' Neill et al. 2020) changes protein net charges; K^+ is high (O' Neill et al. 2020),
- Increased resistance to heat shocks (Uno et al. 2002; Slavov et al. 2012).

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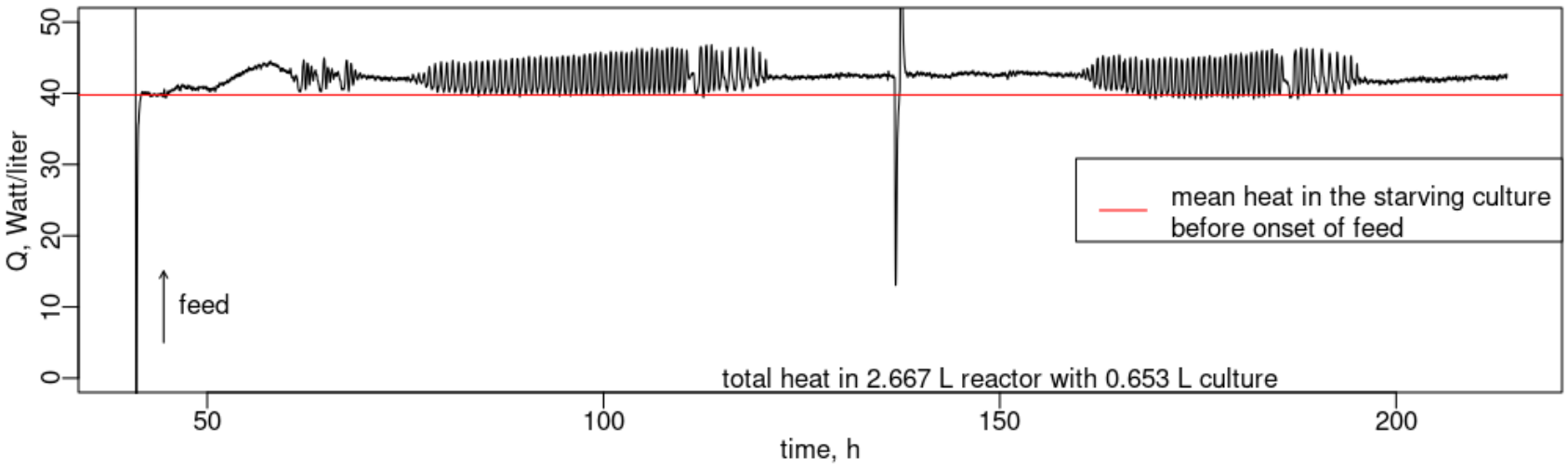
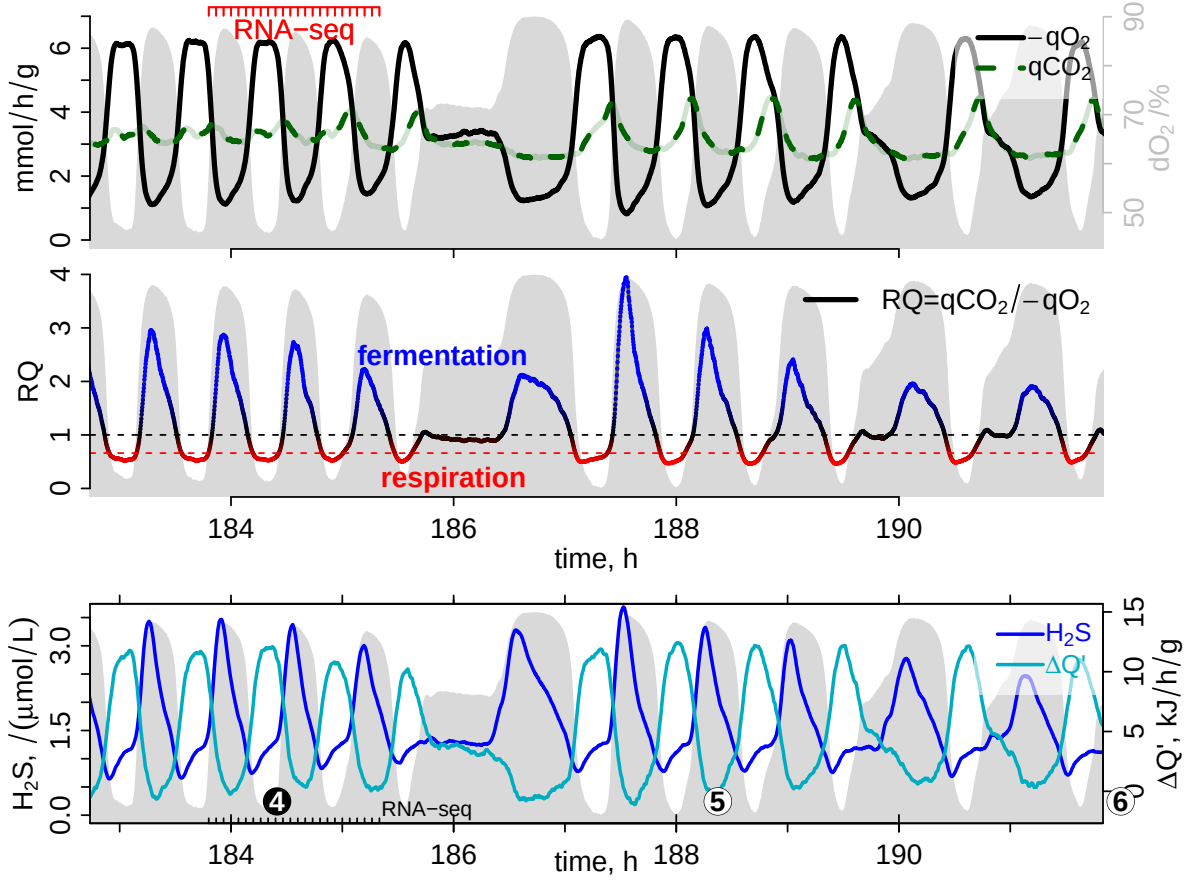


Calorimetric estimation of heat production from temperature control, via Fourier's law: $Q'_{\text{tot}}(t) = \frac{\mathrm{d}Q_{\text{tot}}(t)}{\mathrm{d}t} = \frac{kA}{\Delta x}\,(T_{\text{culture}} - T_{\text{waterbath}})$.

Noncoding Transcription - Outlook 👋

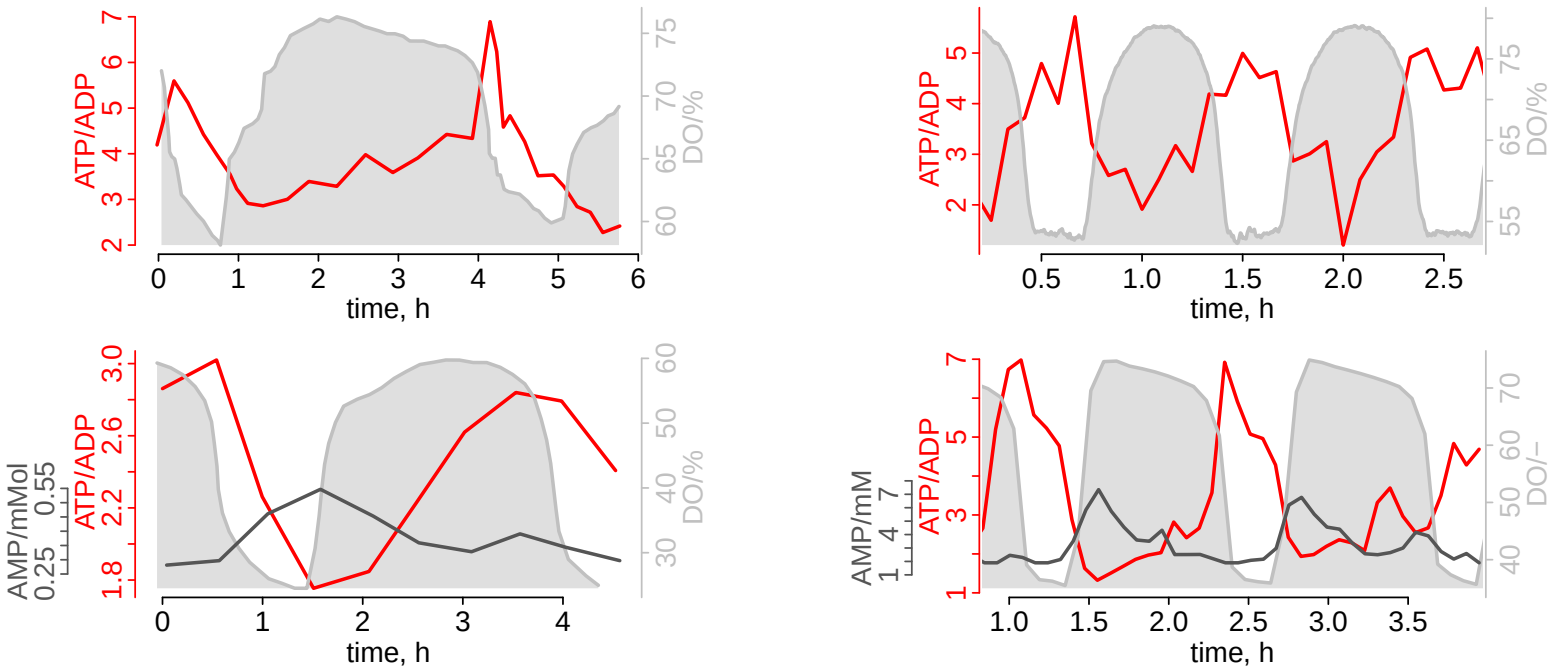
- **Possible roles of noncoding transcription during LOC phase and/or at low ATP?**



... where cells literally cool down to starvation level.

Noncoding Transcription - Outlook

- **Possible roles of noncoding transcription during LOC phase and/or at low ATP?**

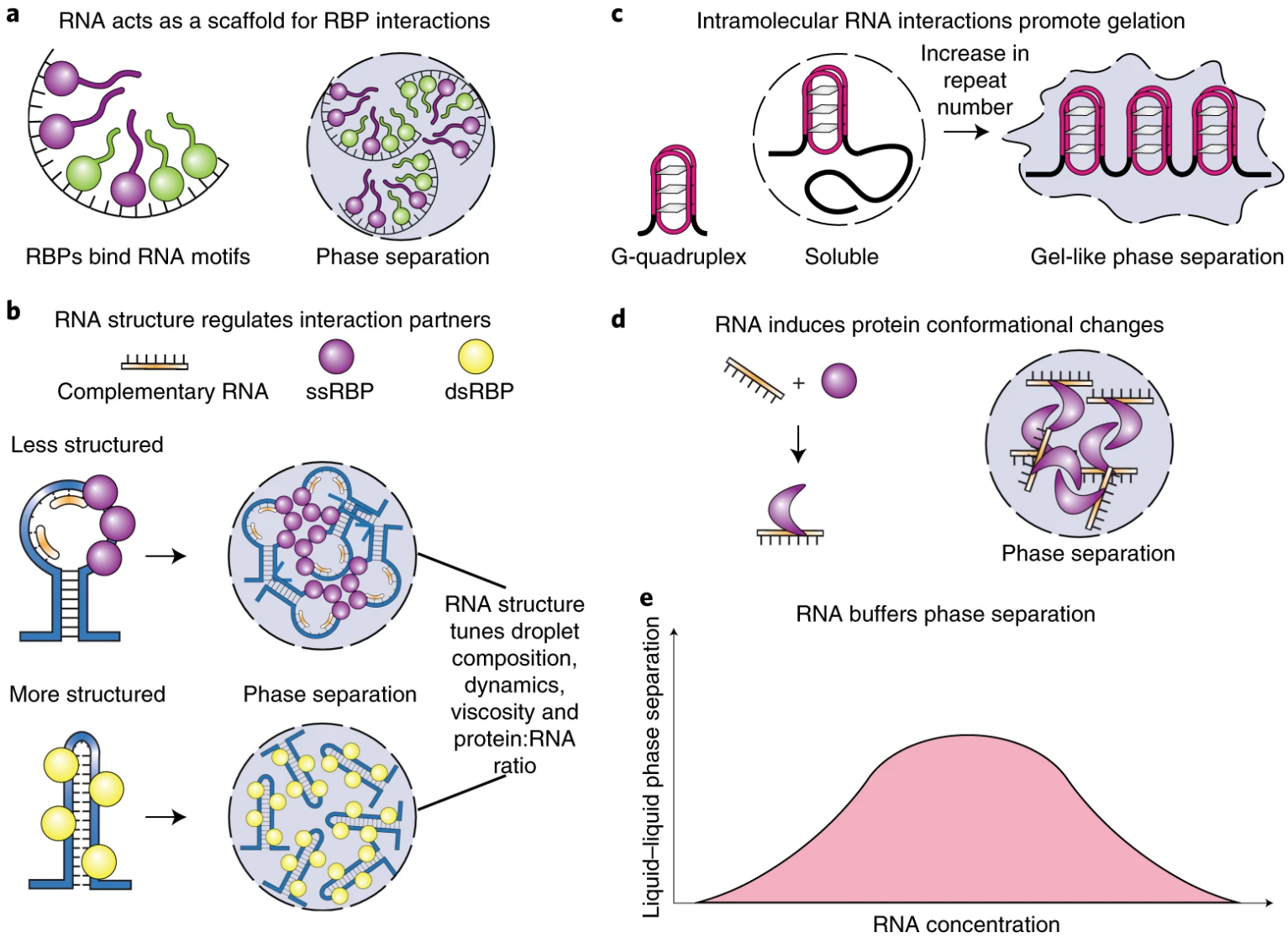


- RNA helicases require ATP: RNA more structured?
- ATP keeps proteins soluble, chaperones require ATP: protein aggregation?

cf. the **Association-Induction hypothesis** by Gilbert Ling?

Pollack (2001): Cells, gels and the engines of life.

Noncoding Transcription - Outlook 👋



Wiedner and Giudice (2021); also see Mattick (2023)

Noncoding Transcription - Outlook 👋

- **Possible roles of noncoding transcription during LOC phase and/or at low ATP** in . . .
  - . . . **structural proteome homeostasis:**
  - Scaffolds for RBP phase separation (Wiedner and Giudice 2021),
  - Inhibition of protein aggregation (Maharana et al. 2018).

Global Changes in LOC Phase:

- HOC phase ends with a pulse of amino acid synthesis and protein translation (Murray, Beckmann, and Kitano 2007; O' Neill et al. 2020),
- Protein degradation peaks in LOC phase (O' Neill et al. 2020),
- ATP is low in LOC phase (Meyenburg 1969; Machné and Murray 2012),
- Low pH (Satroutdinov, Kuriyama, and Kobayashi 1992; O' Neill et al. 2020) changes protein net charges; K^+ is high (O' Neill et al. 2020),
- Increased resistance to heat shocks (Uno et al. 2002; Slavov et al. 2012).

👋 **Self-assembly and repair, cell-structural proofreading, in LOC phase?** 👋

Noncoding Transcription - Outlook 👋

- **Possible roles of noncoding transcription during LOC phase and/or at low ATP** in ...
  - ... **structural proteome homeostasis.**
  - ... **structural genome homeostasis and evolution:**
  - Next: transcription at LTR Retrotransposons.

Next Lectures

II. Transcription at LTR Retrotransposons.
III. DNA as a metabolic sensor:
ATP-dependent nucleosome remodellers
and chromatin reset points in LOC phase,
IV. Chromosomal domains and genome evolution (IFO 0233),
V. Protein homeostasis by pulse width modulation of gene expression.

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