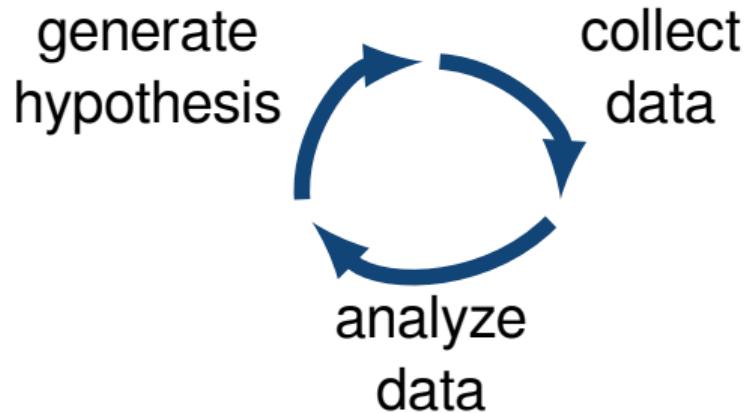


Genomic analyses of transcription elongation factors and intragenic transcription

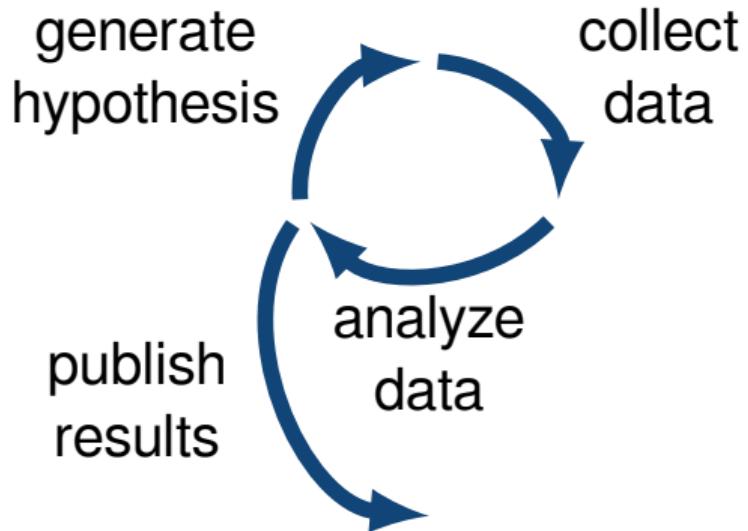
James Chuang

June 19, 2019

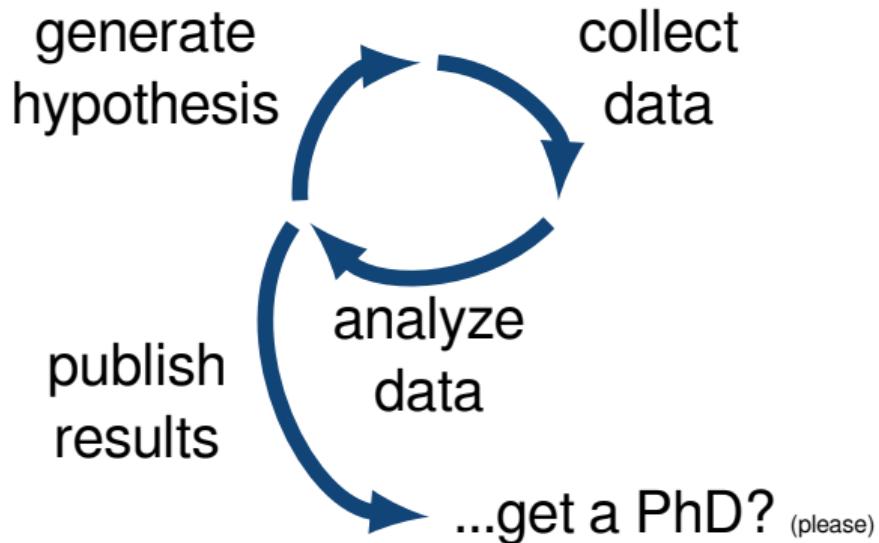
The scientific process

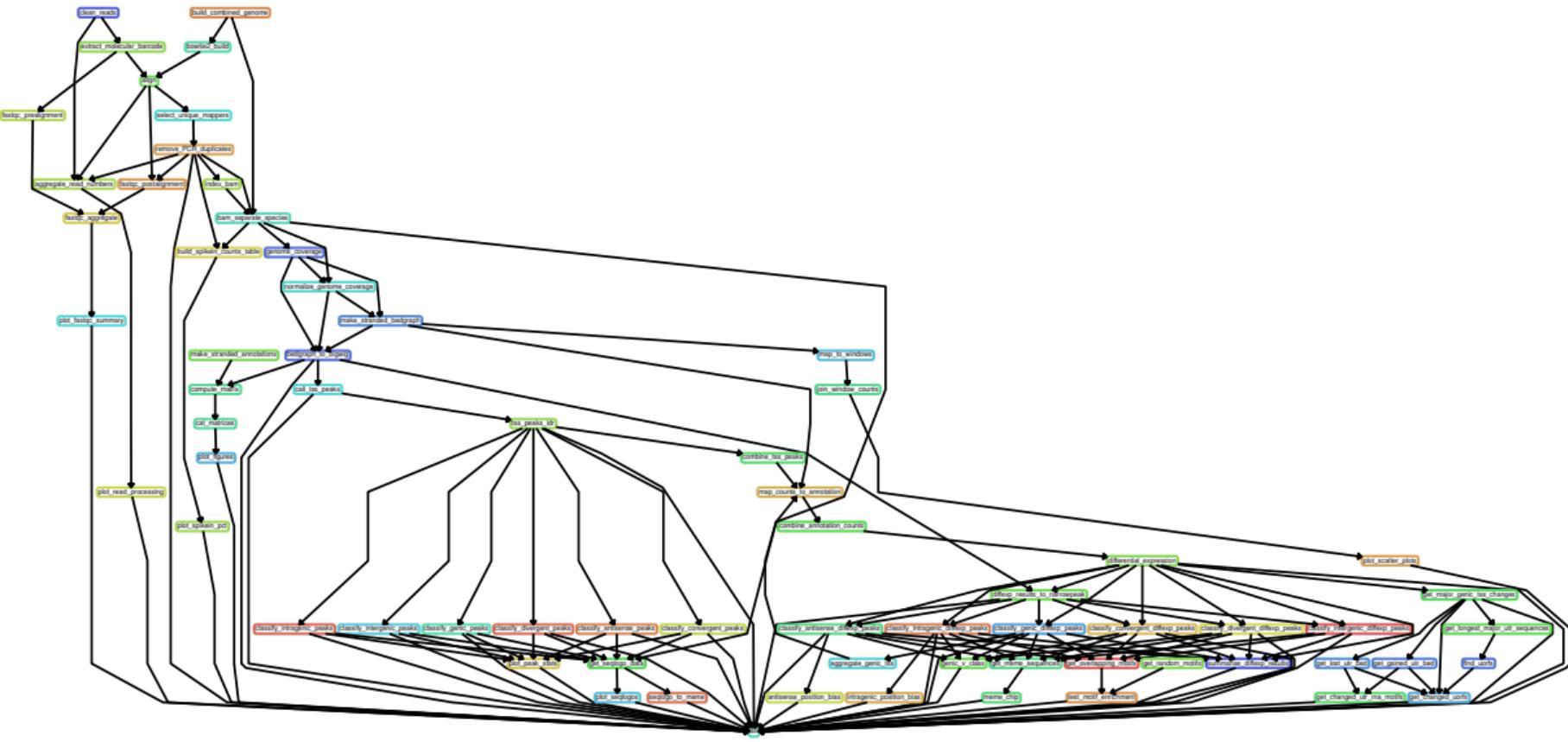


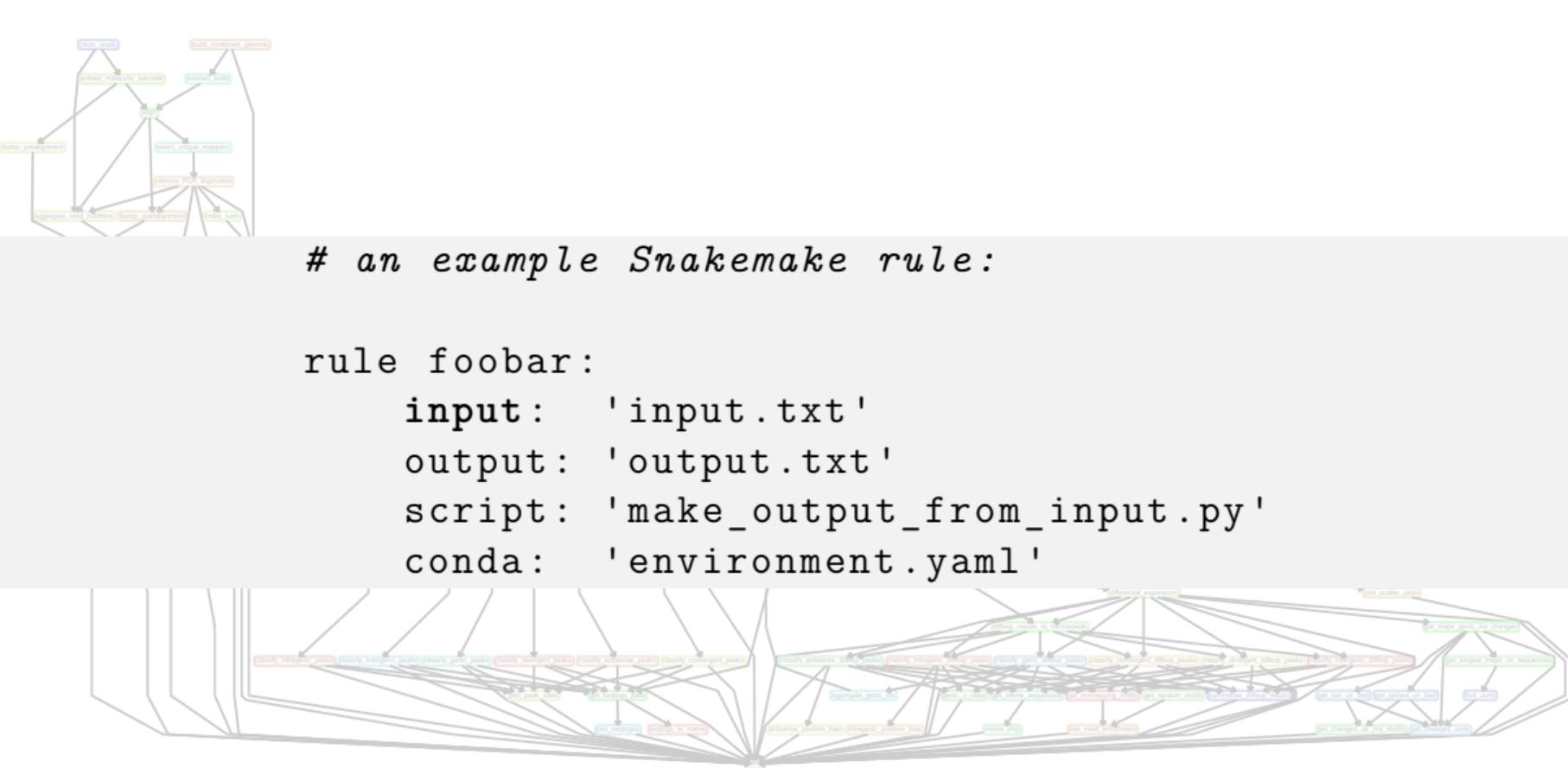
The scientific process



The scientific process







Köster and Rahmann (2012). *Bioinformatics*



September 7, 2018

Journal article

Open Access

Spt6 is required for the fidelity of promoter selection

Doris, Stephen M; Chuang, James; Viktorovskaya, Olga; Murawska, Magdalena; Spatt, Dan; Churchman, L. Stirling; Winston, Fred

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Data collector(s)

Spatt, Dan

Data manager(s)

Chuang, James

Other(s)

Churchman, L. Stirling

Researcher(s)

Doris, Stephen M; Viktorovskaya, Olga; Murawska, Magdalena

All data analyses supporting our publication "Spt6 is required for the fidelity of promoter selection". Reproduce the figures of the paper starting from raw data, as well as thousands of figures and analyses that aren't shown.

See README.md for instructions.

For updated versions of the pipelines used, see our [github page](#).

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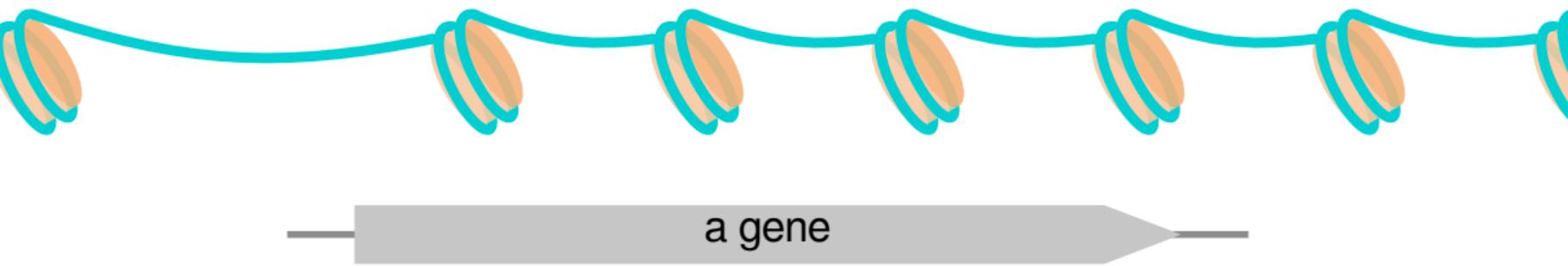
Keyword(s):

Spt6 transcription chromatin genomics
intragenic transcription TSS-seq ChIP-nexus NET-seq
MNase-seq

Published in:

Molecular Cell.

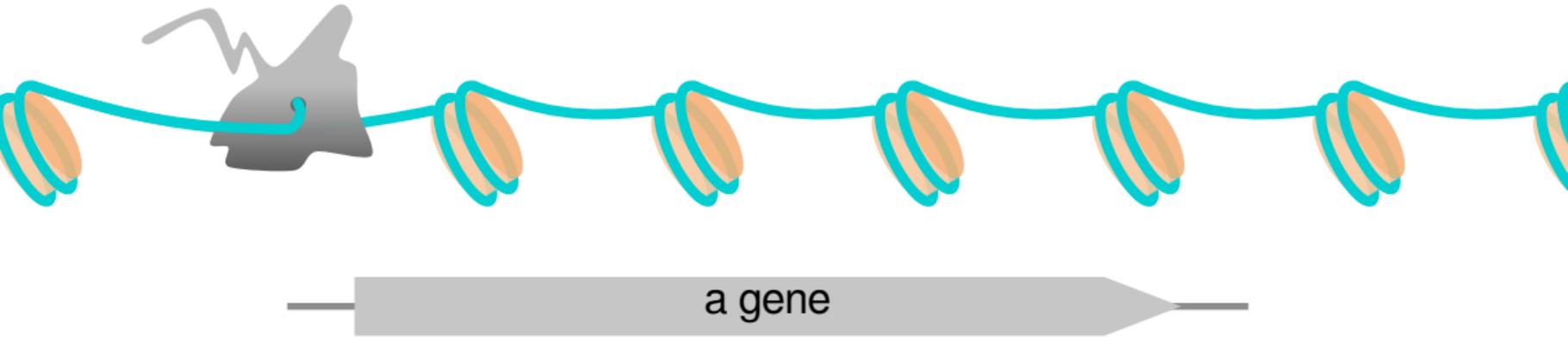
An introduction to transcription



An introduction to transcription

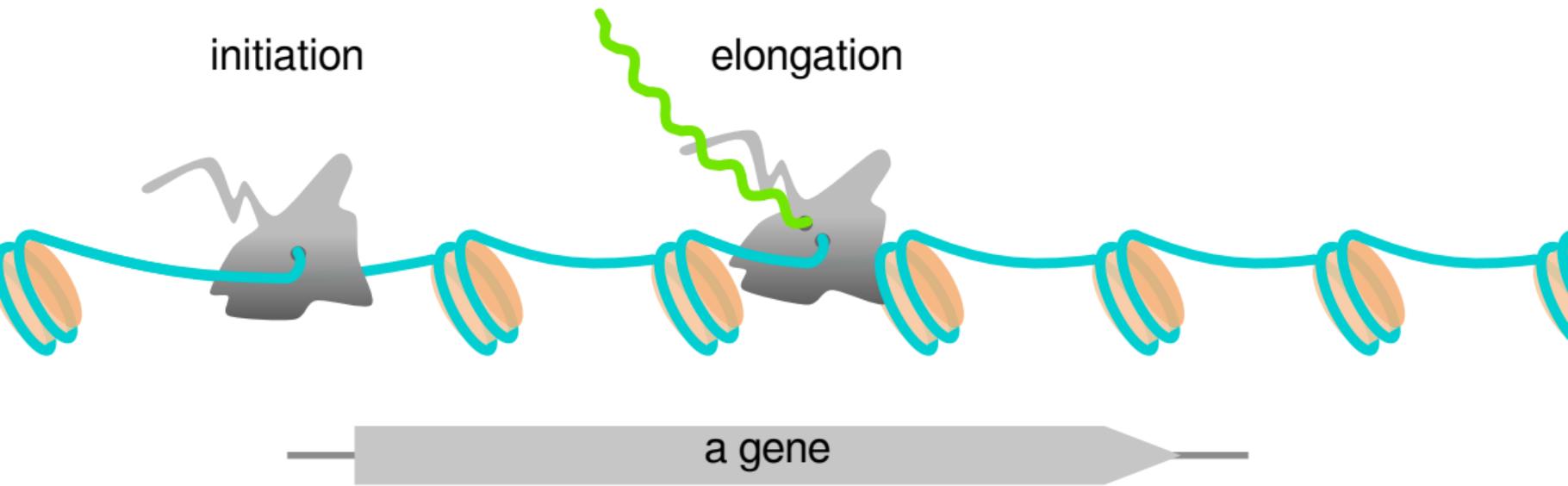
RNA polymerase II

initiation



An introduction to transcription

RNA polymerase II



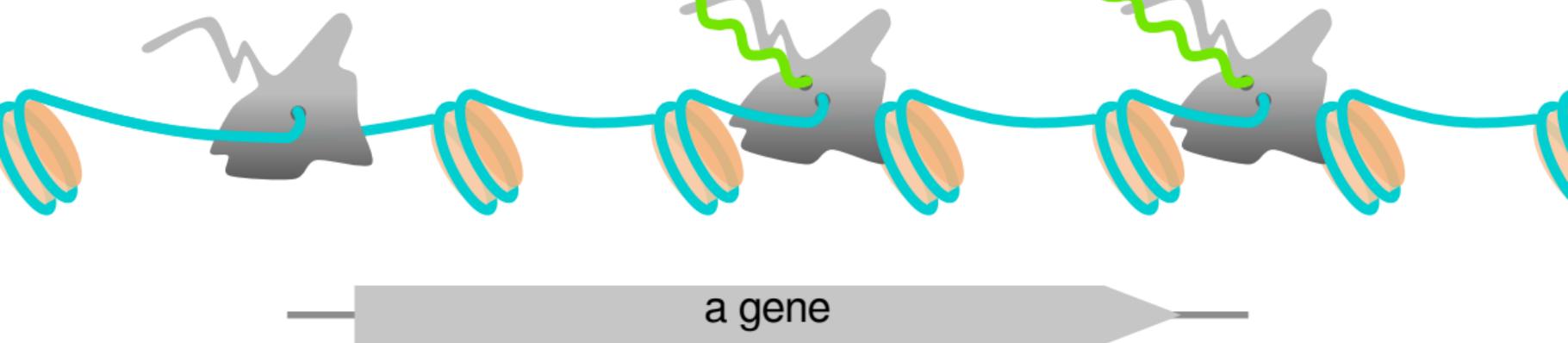
An introduction to transcription

RNA polymerase II

initiation

elongation

termination



An introduction to transcription

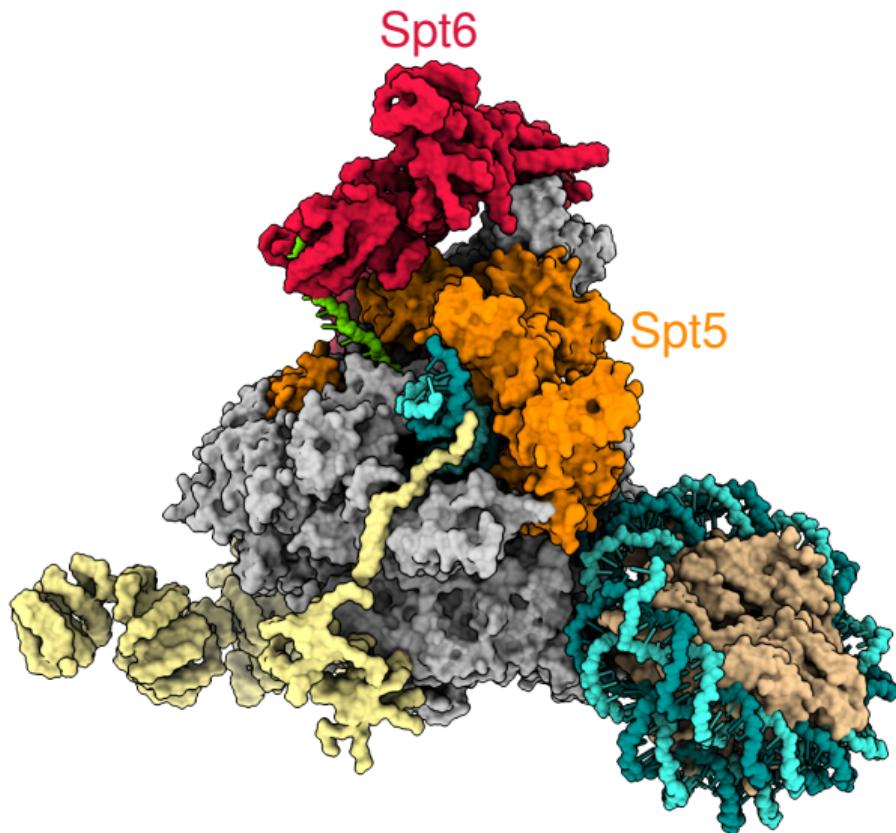
RNA polymerase II

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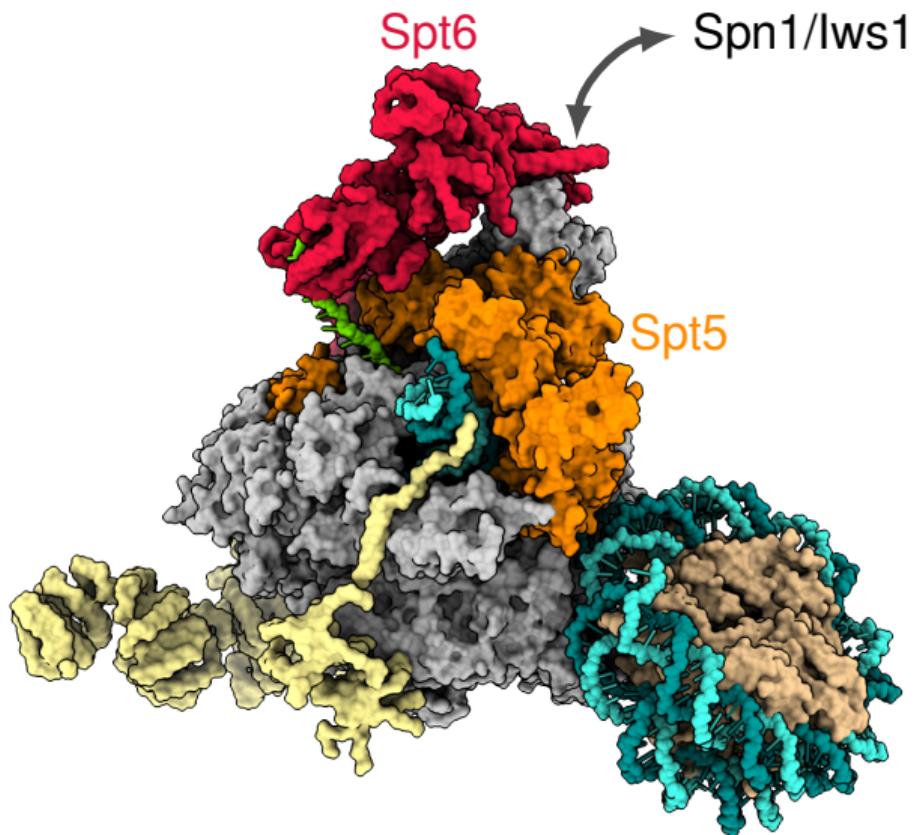
elongation

termination

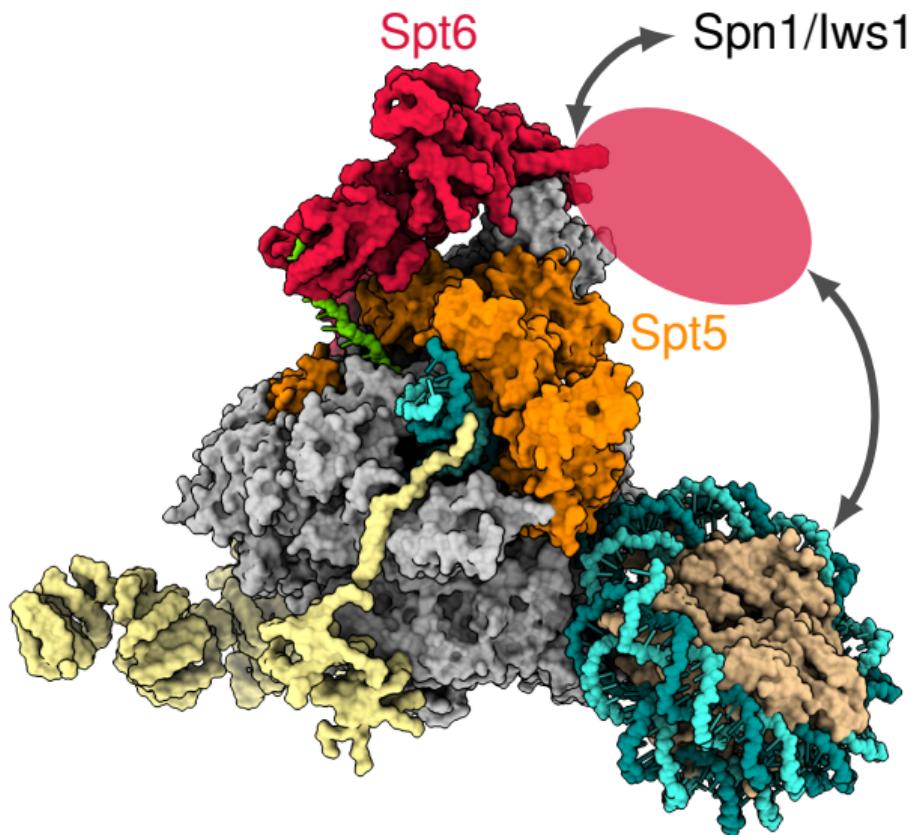




Vos *et al.* (2018). *Nature*
Farnung *et al.* (2018). *Nat. Commun.*



Vos *et al.* (2018). *Nature*
Farnung *et al.* (2018). *Nat. Commun.*



Vos *et al.* (2018). *Nature*
Farnung *et al.* (2018). *Nat. Commun.*

Spt6 project collaborators

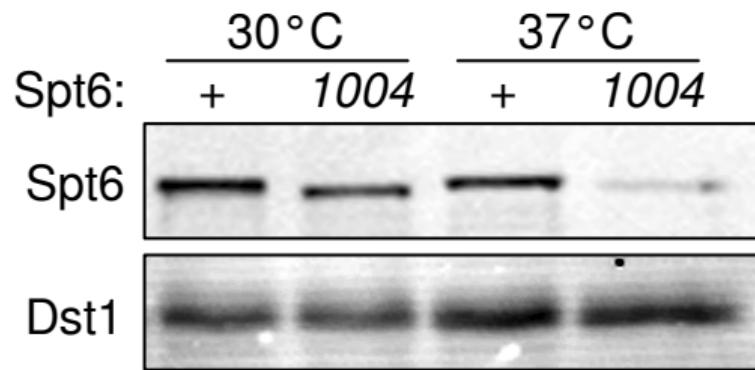
Steve Doris TSS-seq and ChIP-nexus

Olga Viktorovskaya MNase-seq

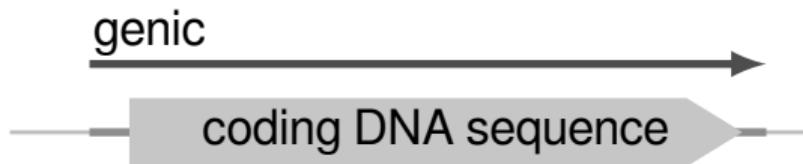
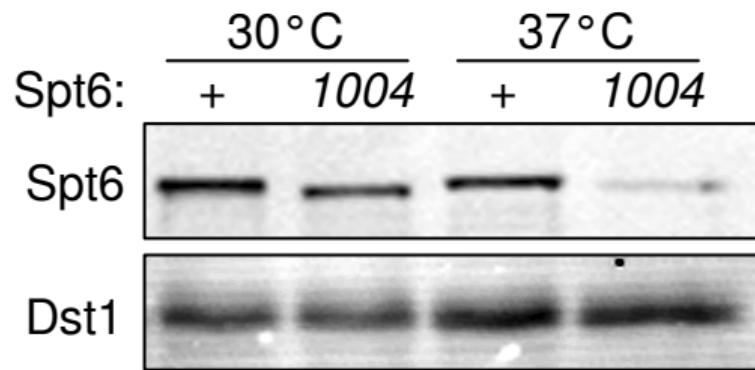
Magdalena Murawska NET-seq

Dan Spatt Northern, Western, and ChIP experiments

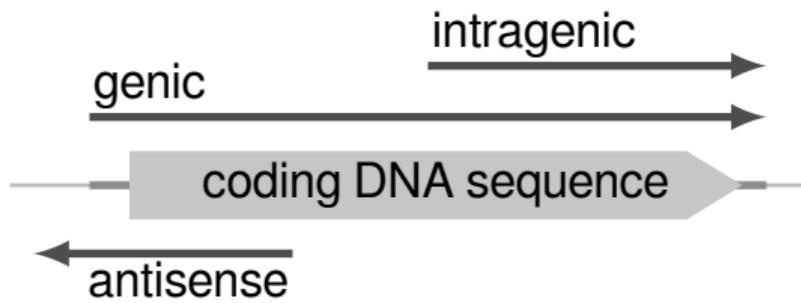
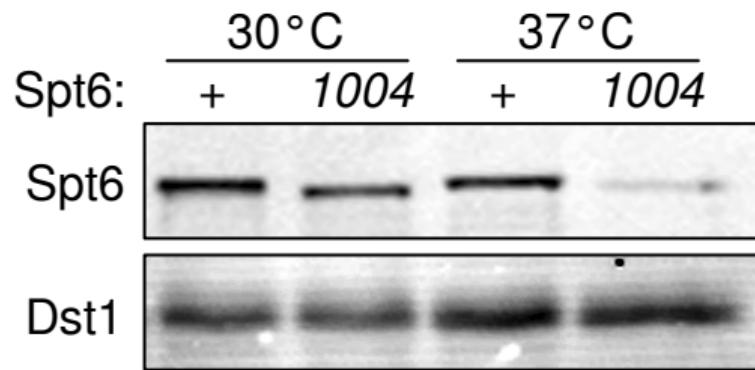
The *spt6-1004* mutant expresses **intragenic transcripts**

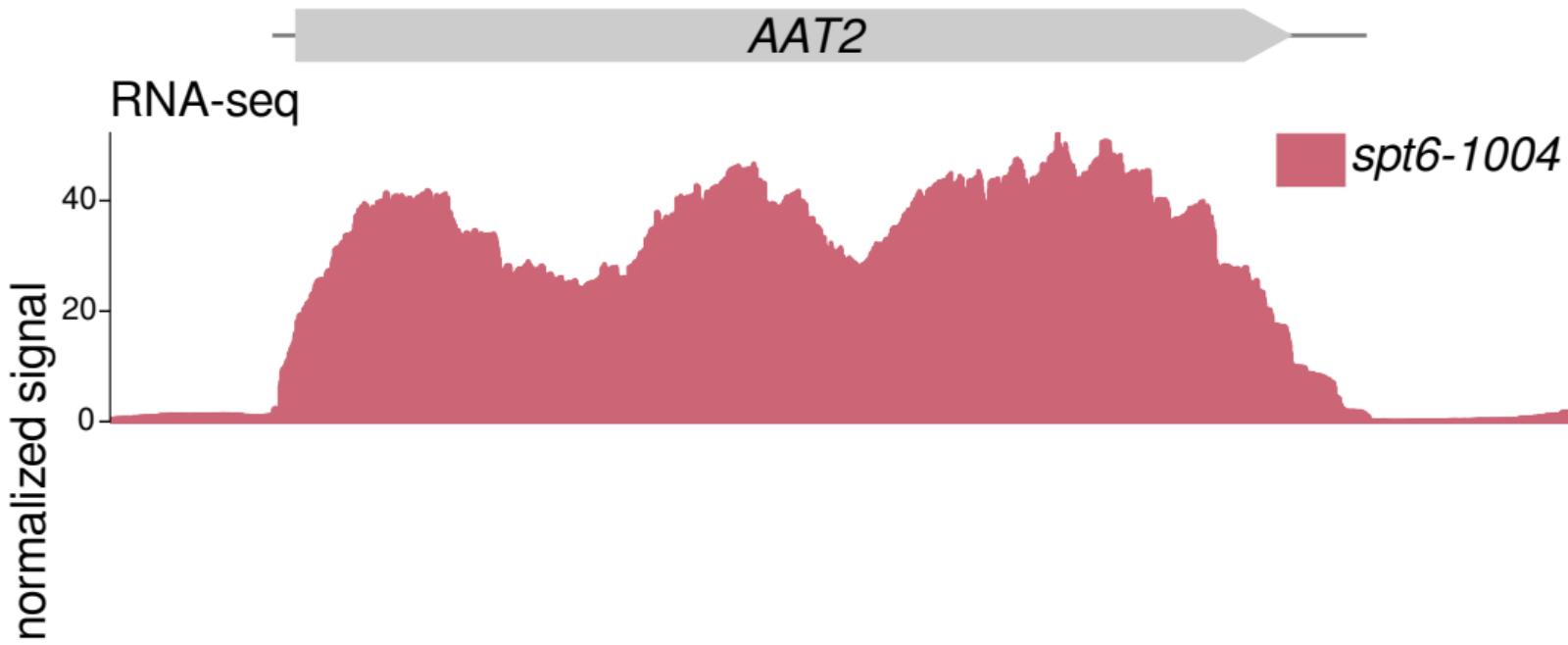


The *spt6-1004* mutant expresses intragenic transcripts

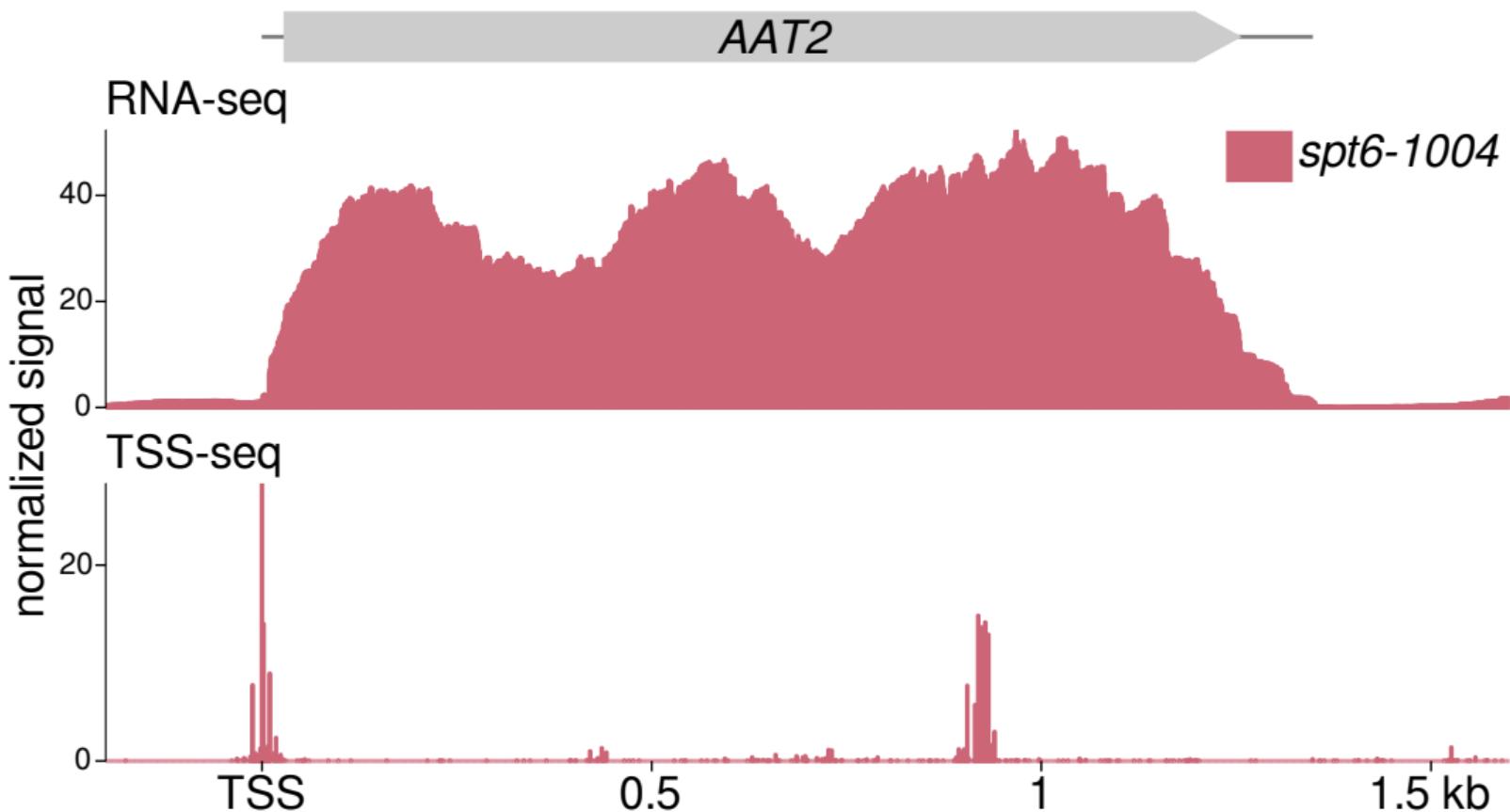


The *spt6-1004* mutant expresses intragenic transcripts

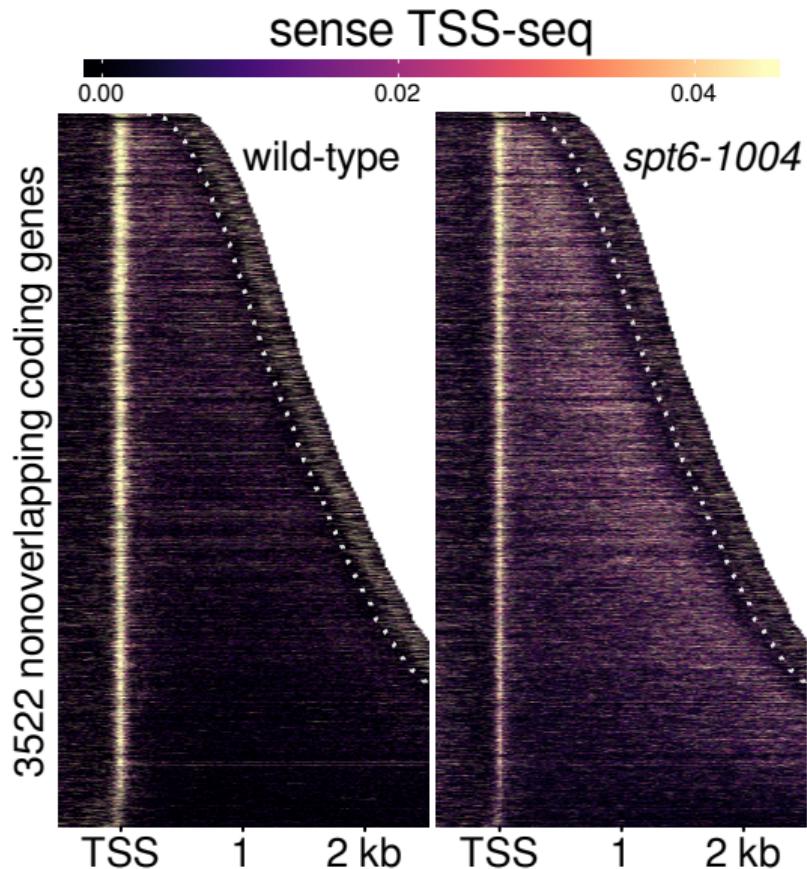


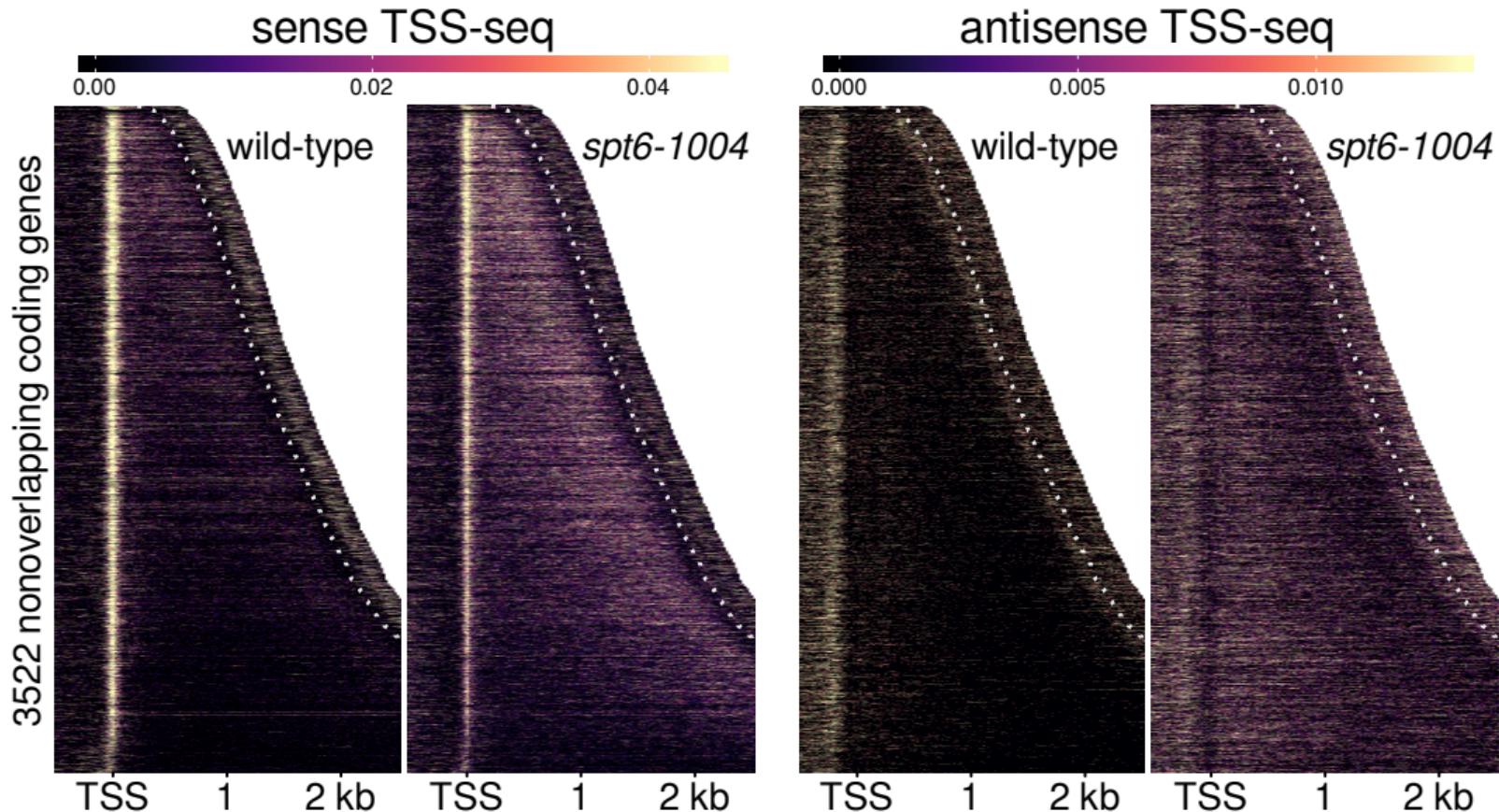


RNA-seq: Uwimana *et al.* (2017). *Nucleic Acids Res.*

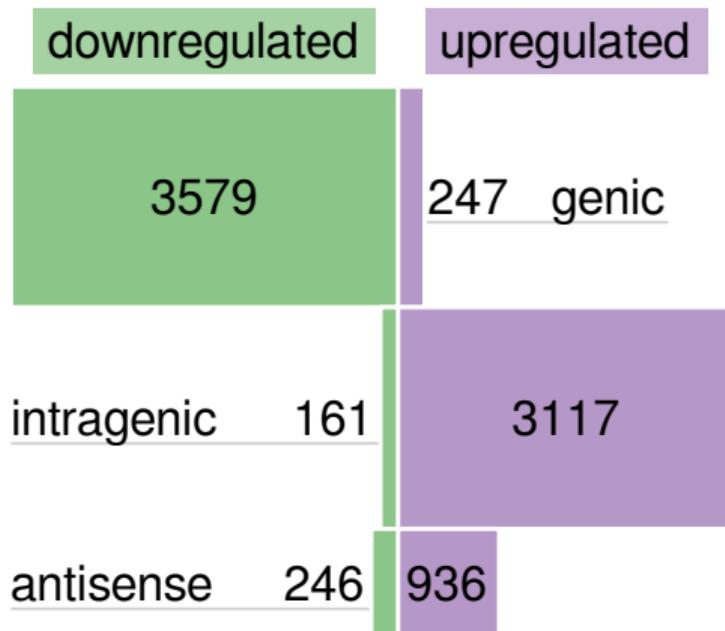


RNA-seq: Uwimana *et al.* (2017). *Nucleic Acids Res.*

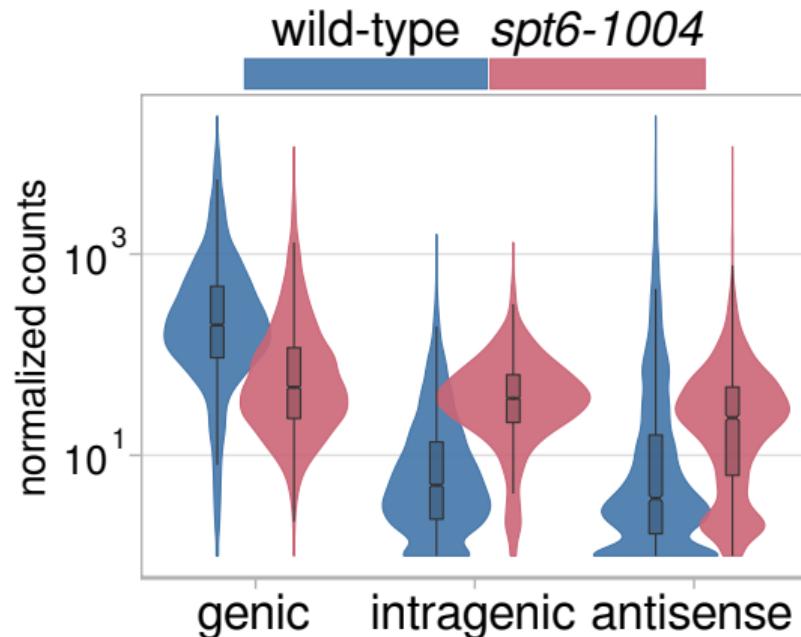
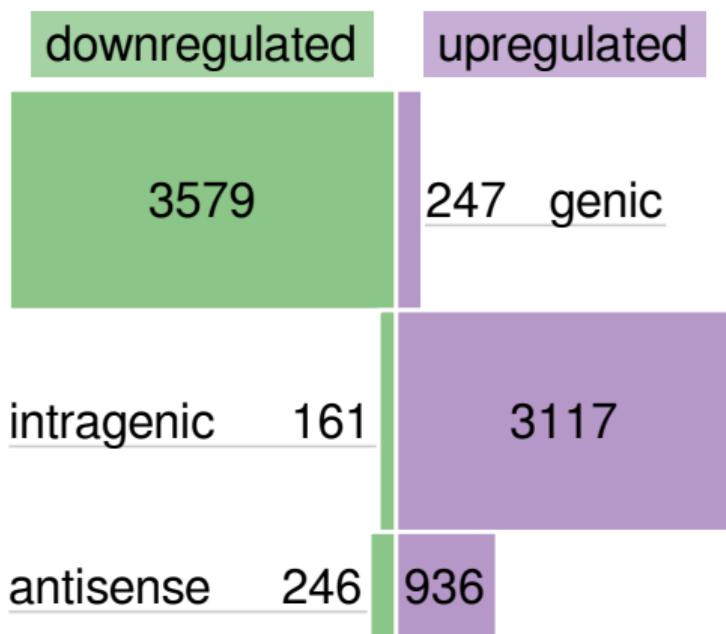




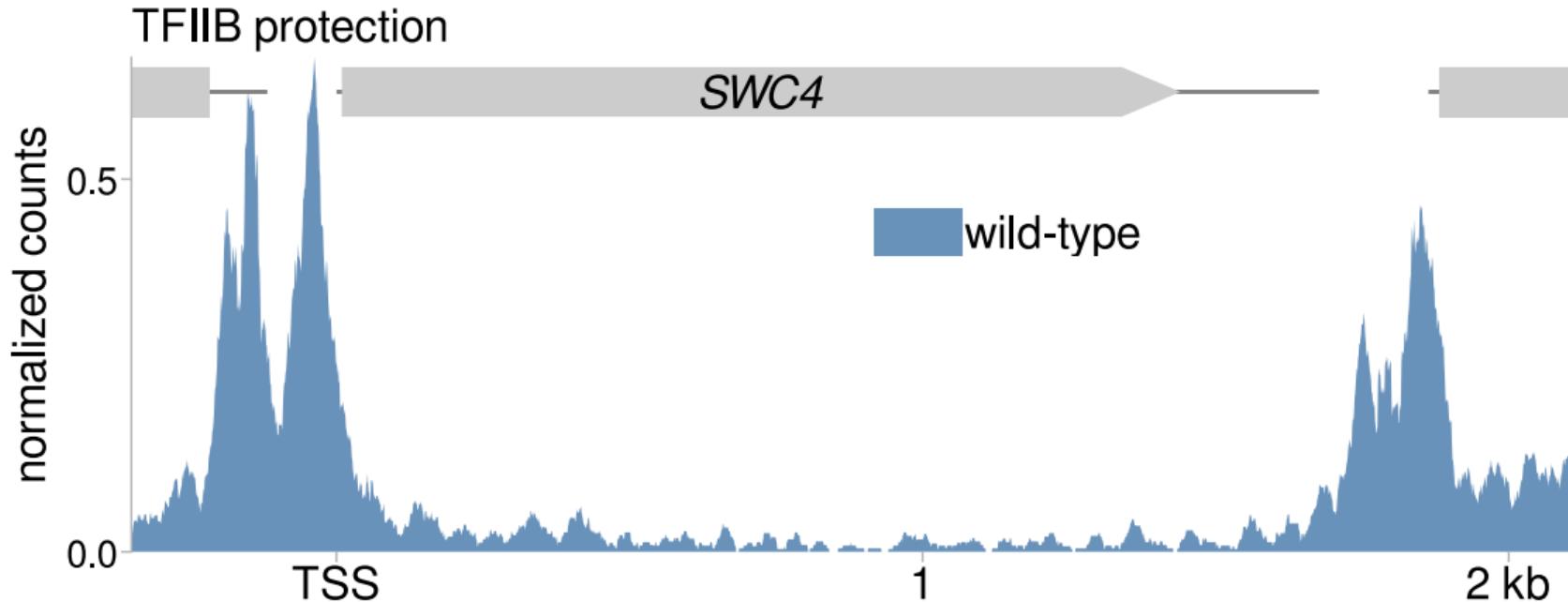
Genic transcript abundances are reduced in *spt6-1004*



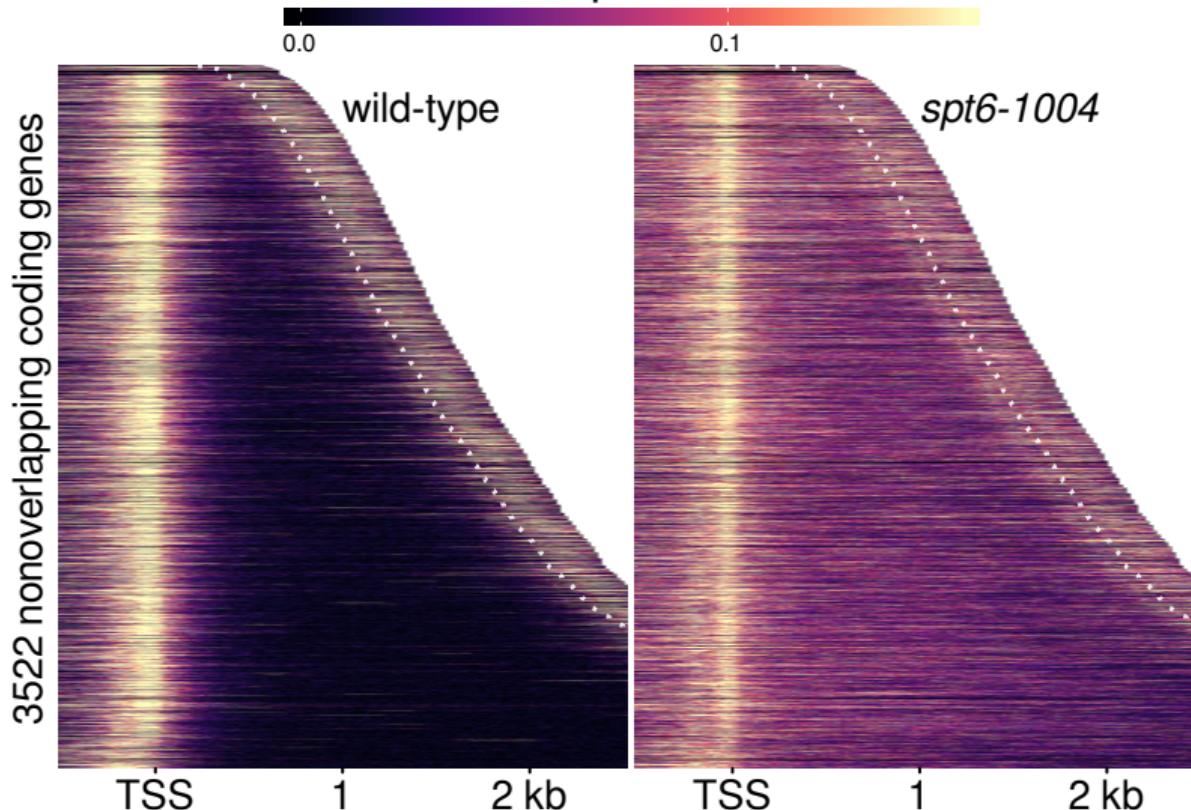
Genic transcript abundances are reduced in *spt6-1004*



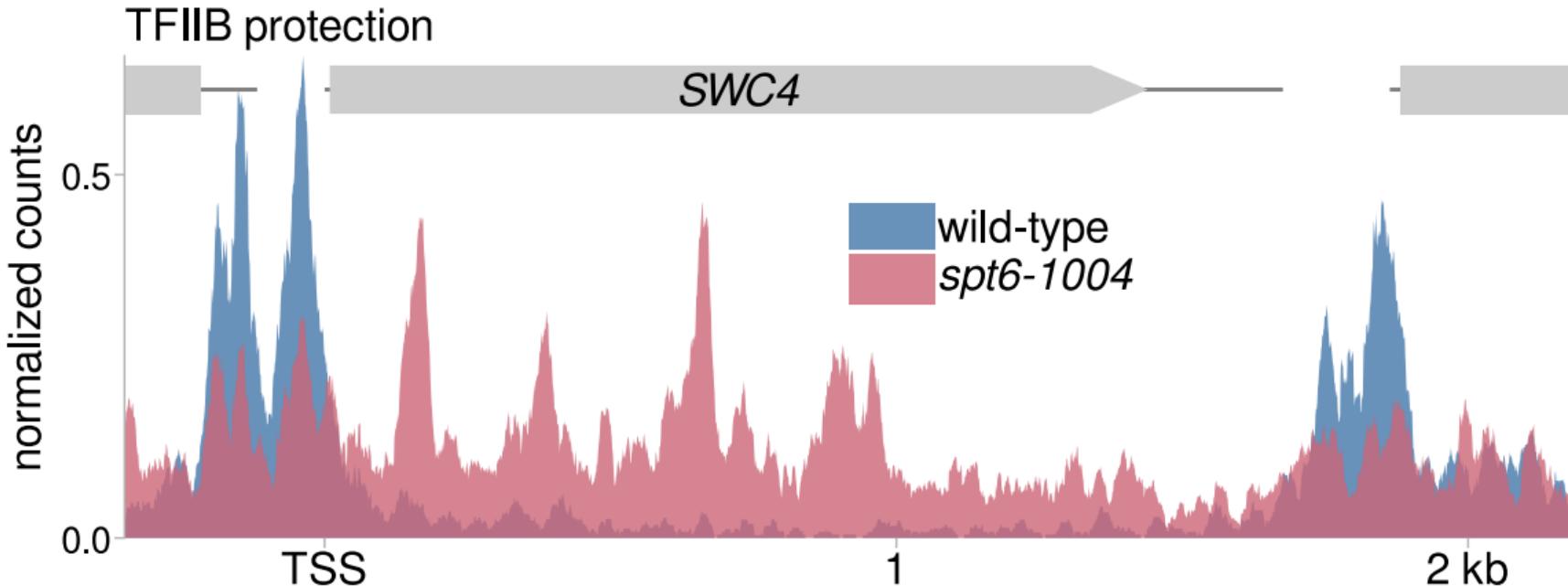
TFIIB ChIP-nexus measures transcription initiation



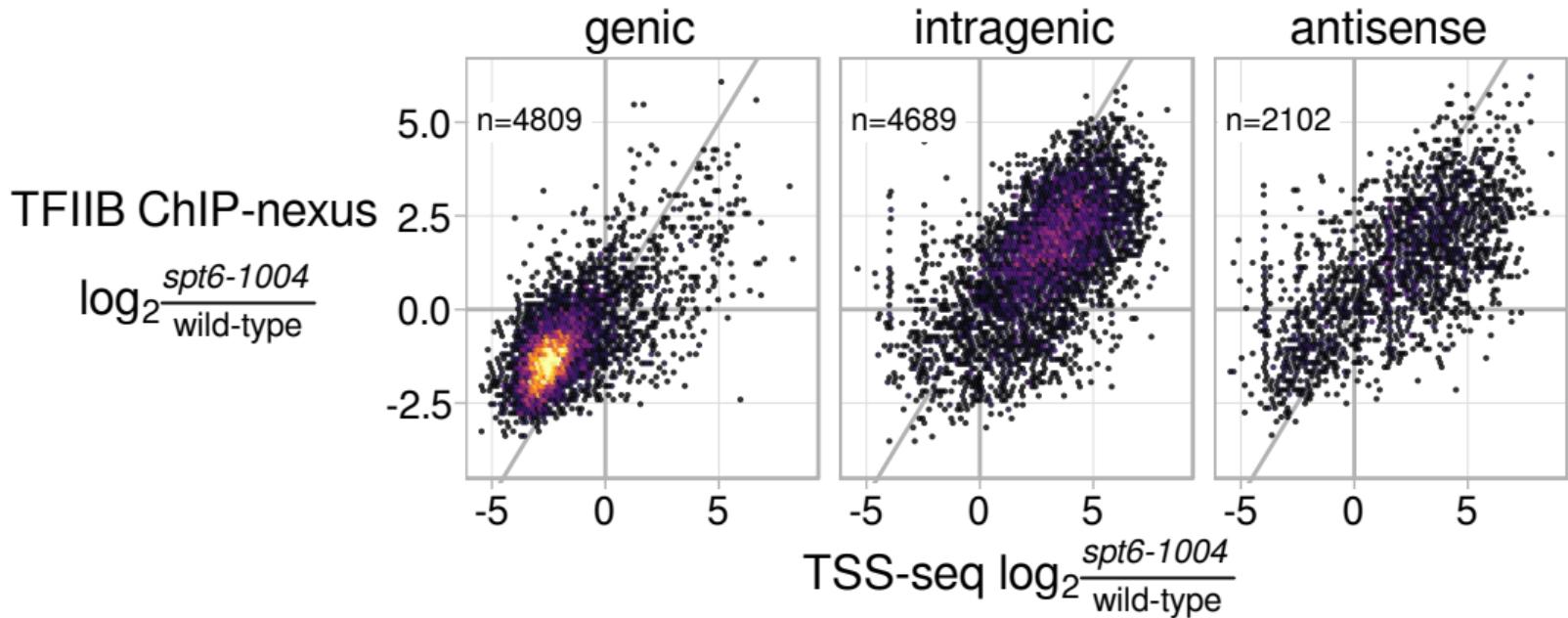
TFIIB protection



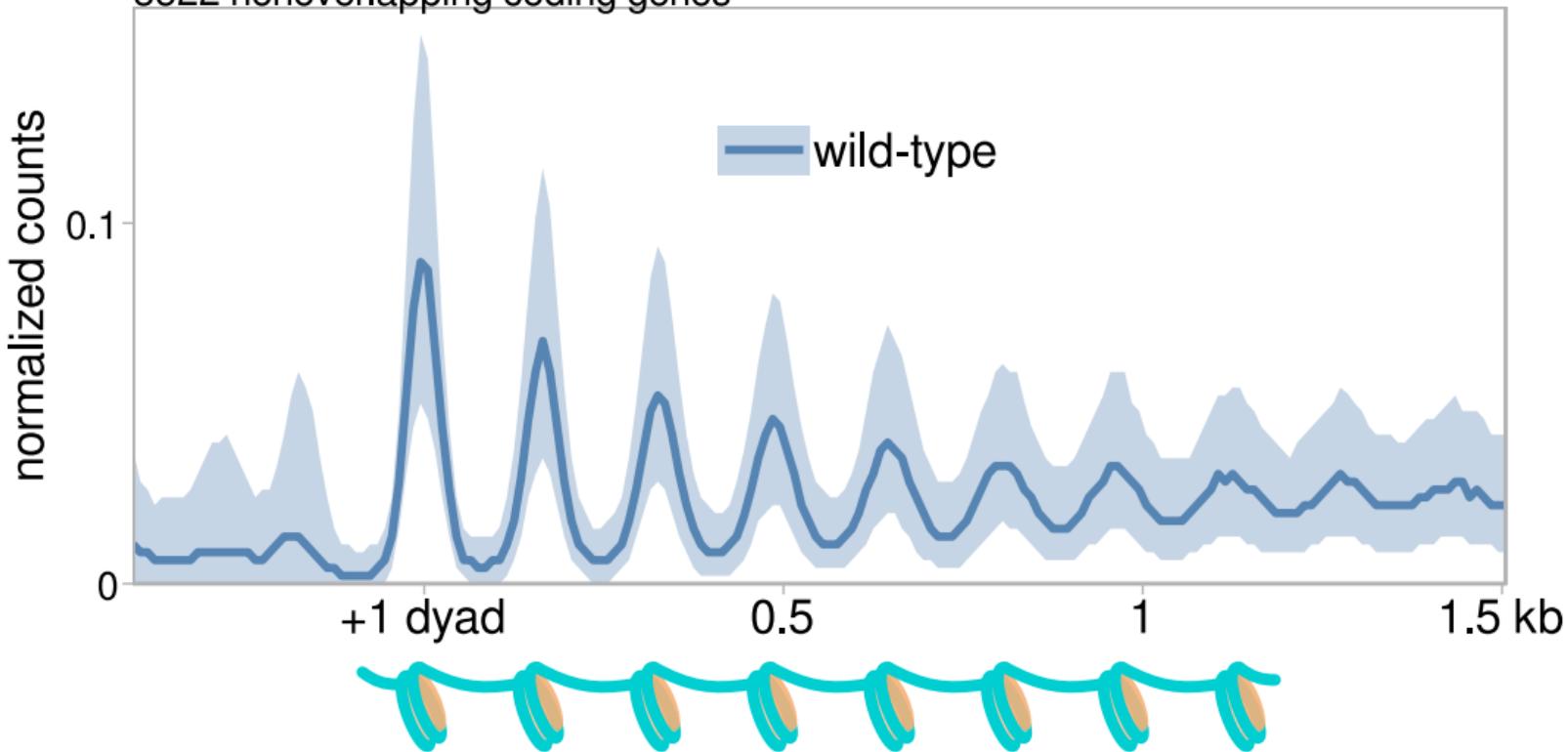
TFIIB binding changes dramatically in *spt6-1004*



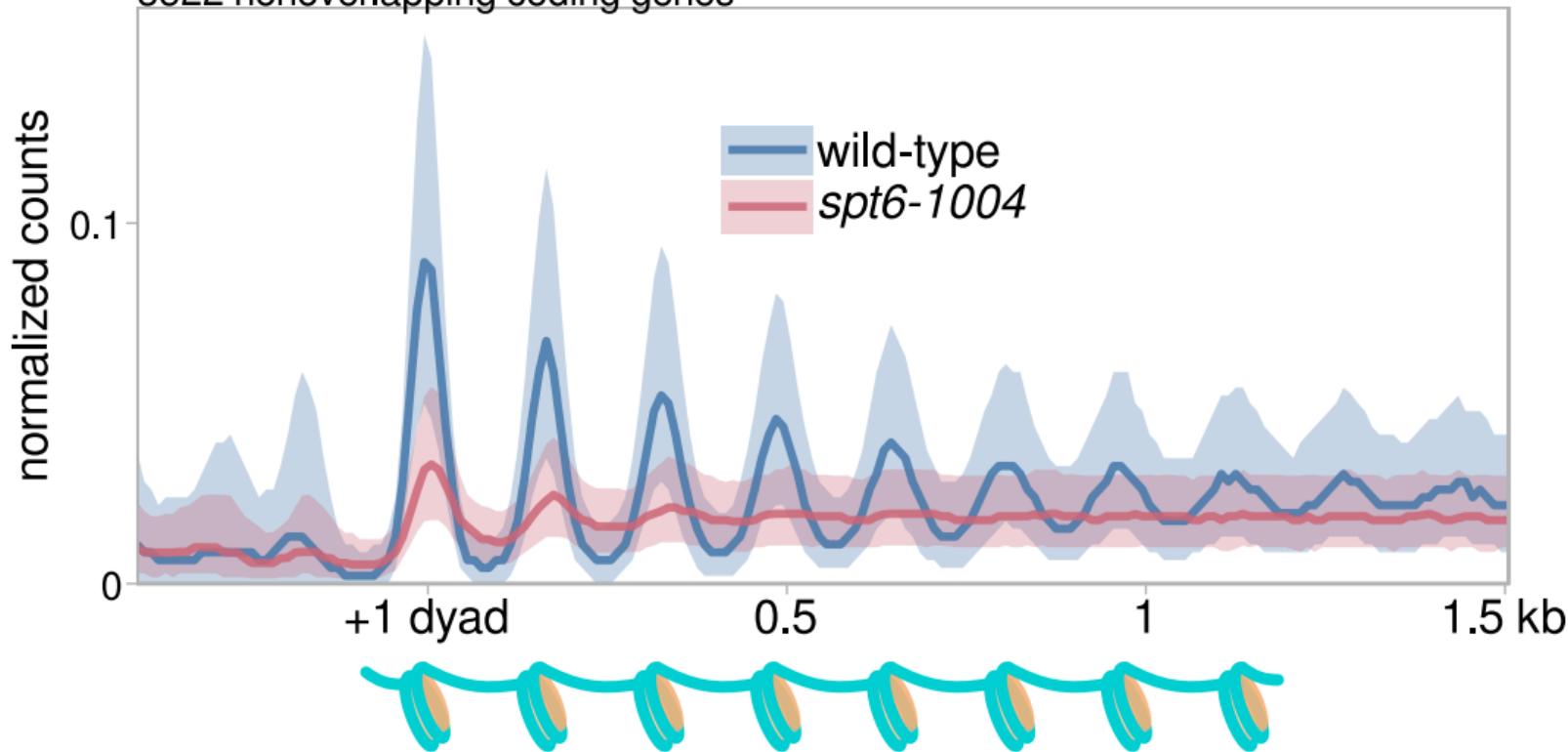
Transcriptomic changes are mostly explained by changes in initiation



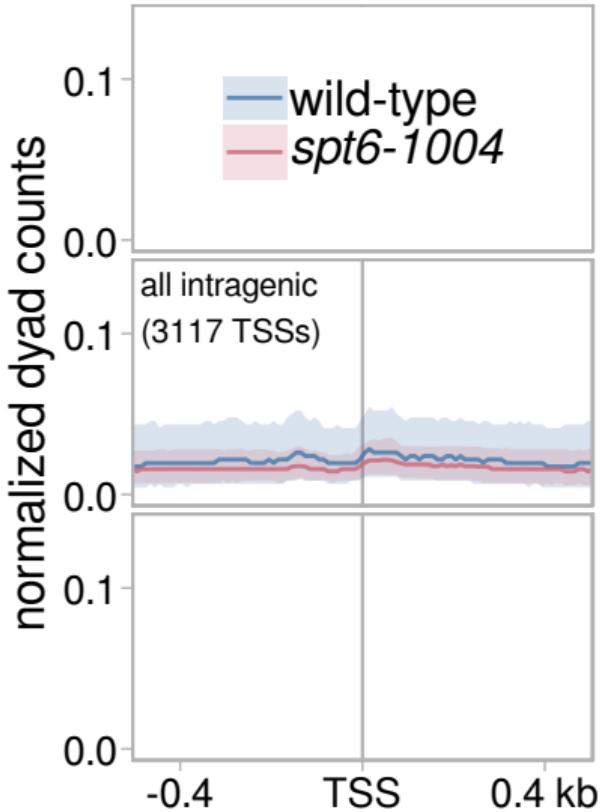
MNase-seq dyad signal
3522 nonoverlapping coding genes



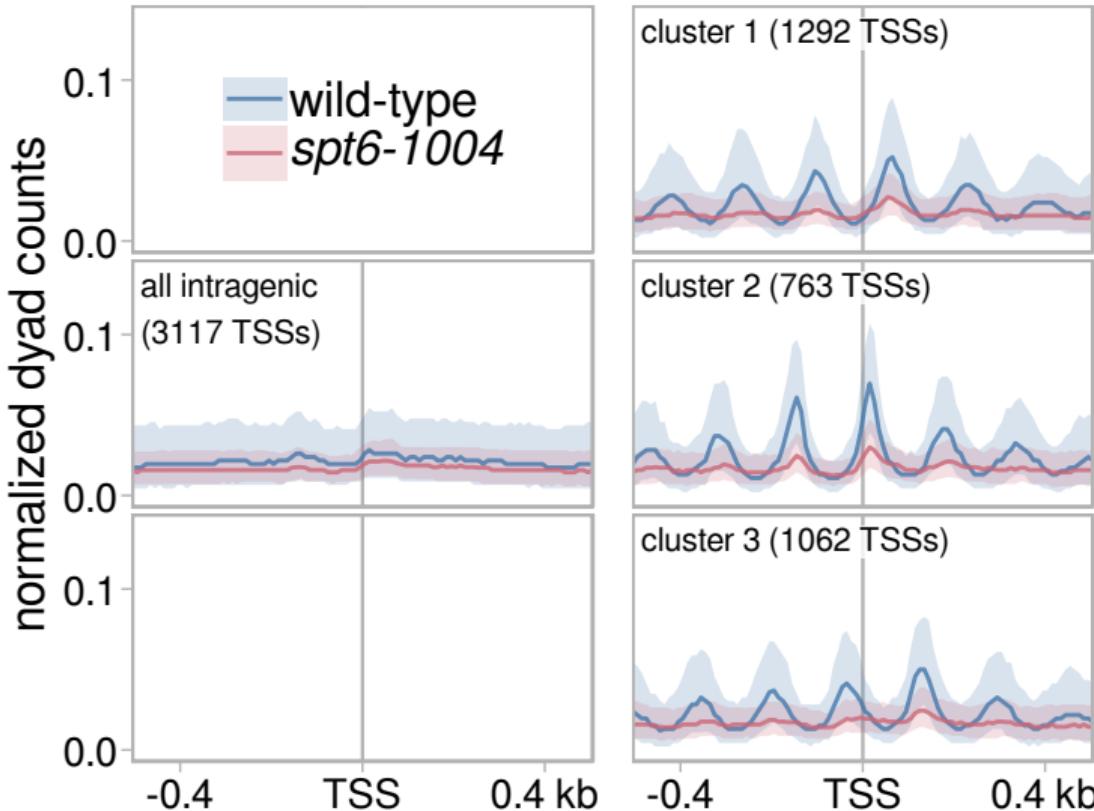
MNase-seq dyad signal
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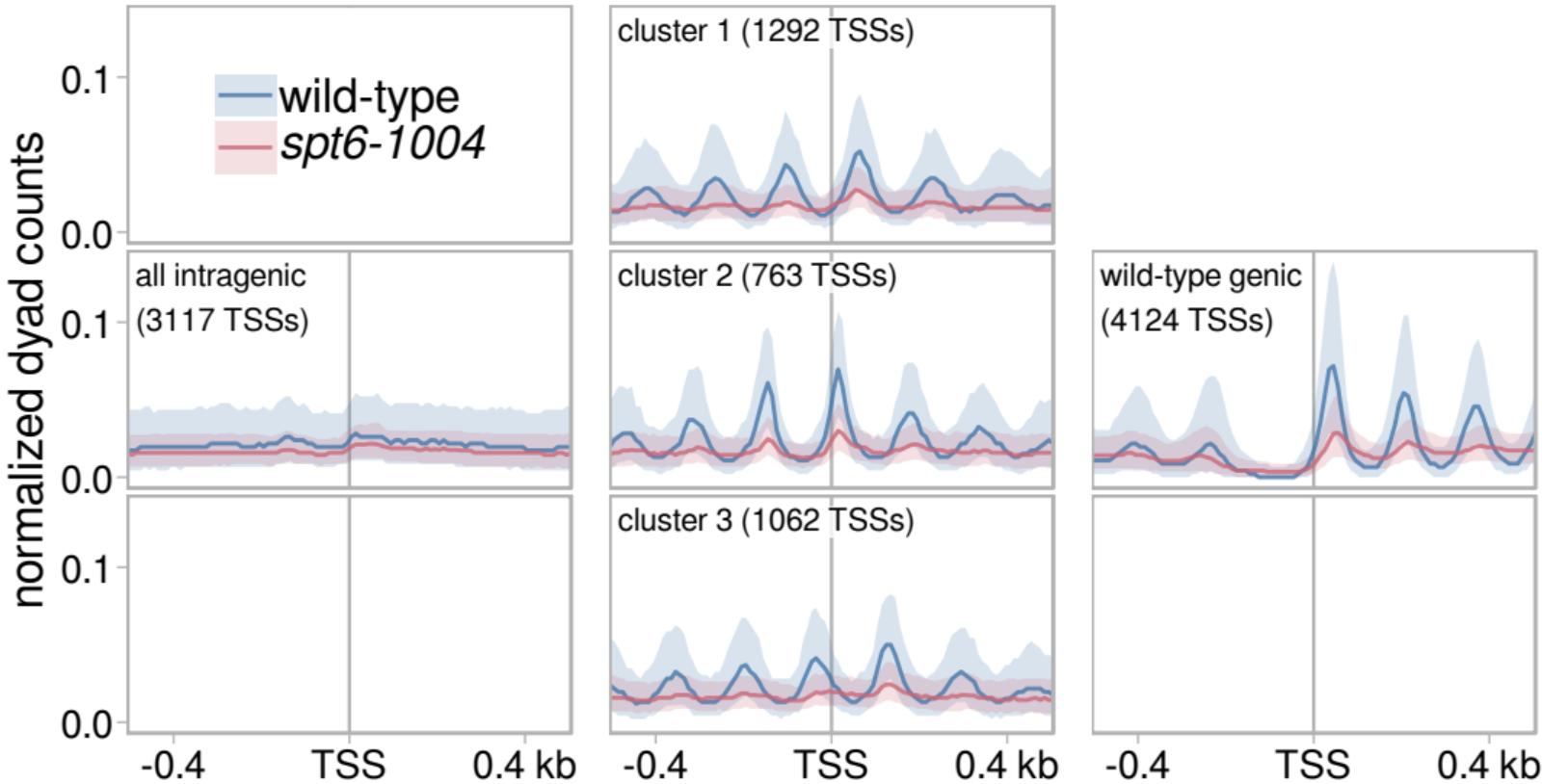
MNase-seq



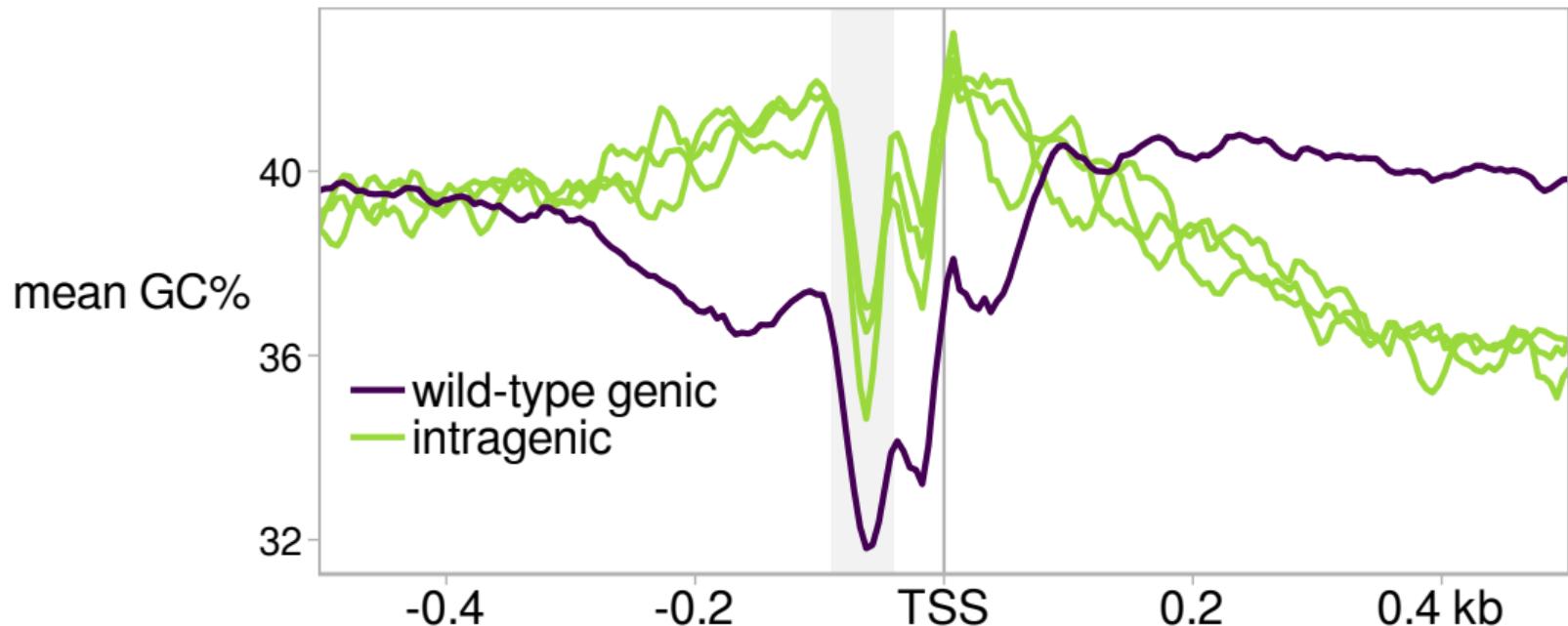
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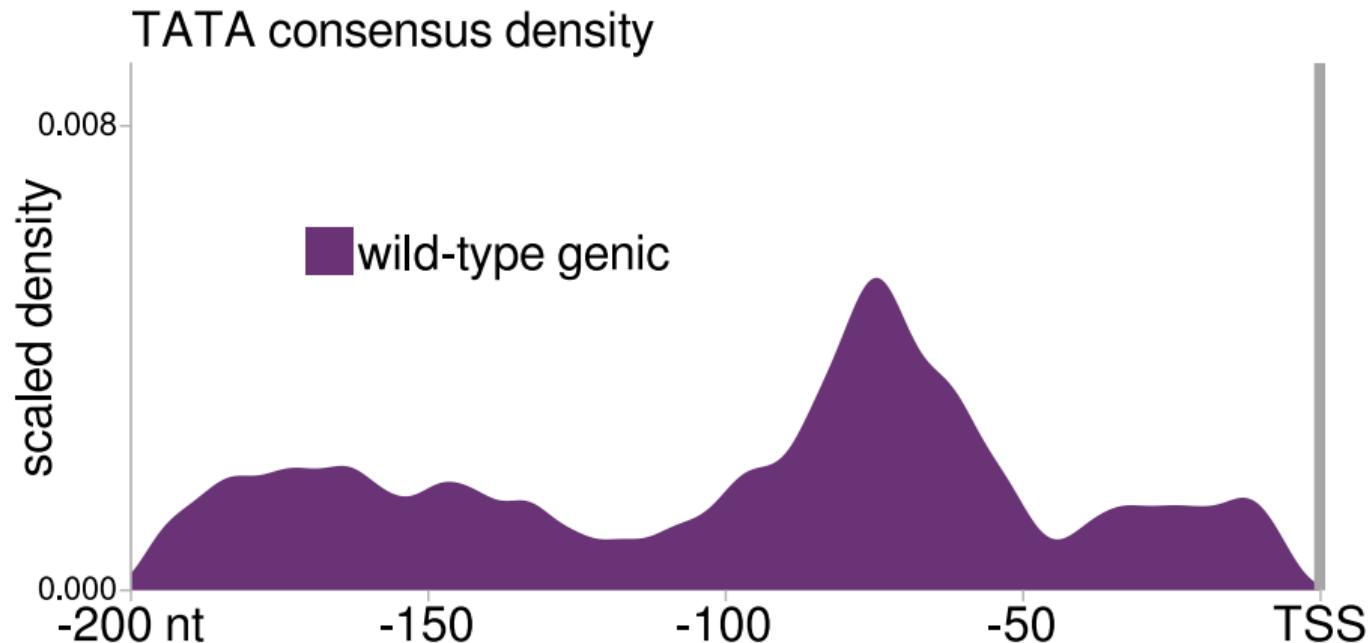
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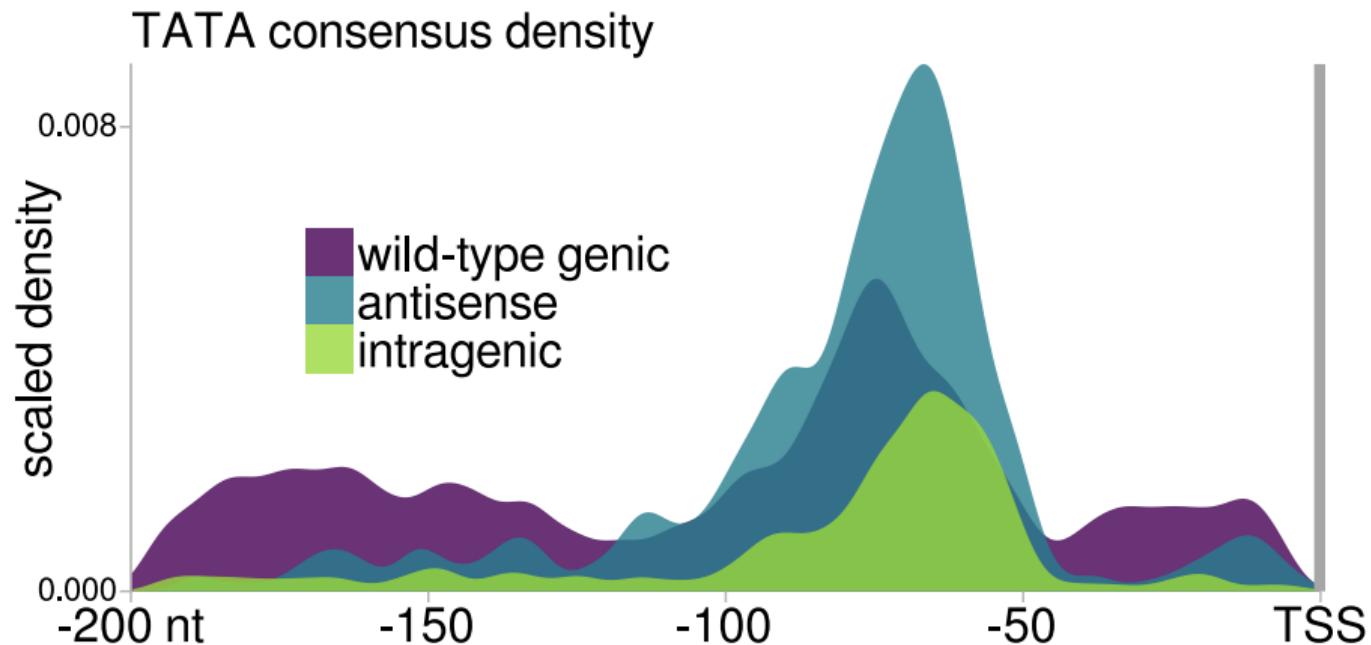
Intragenic promoters have features of genic promoters



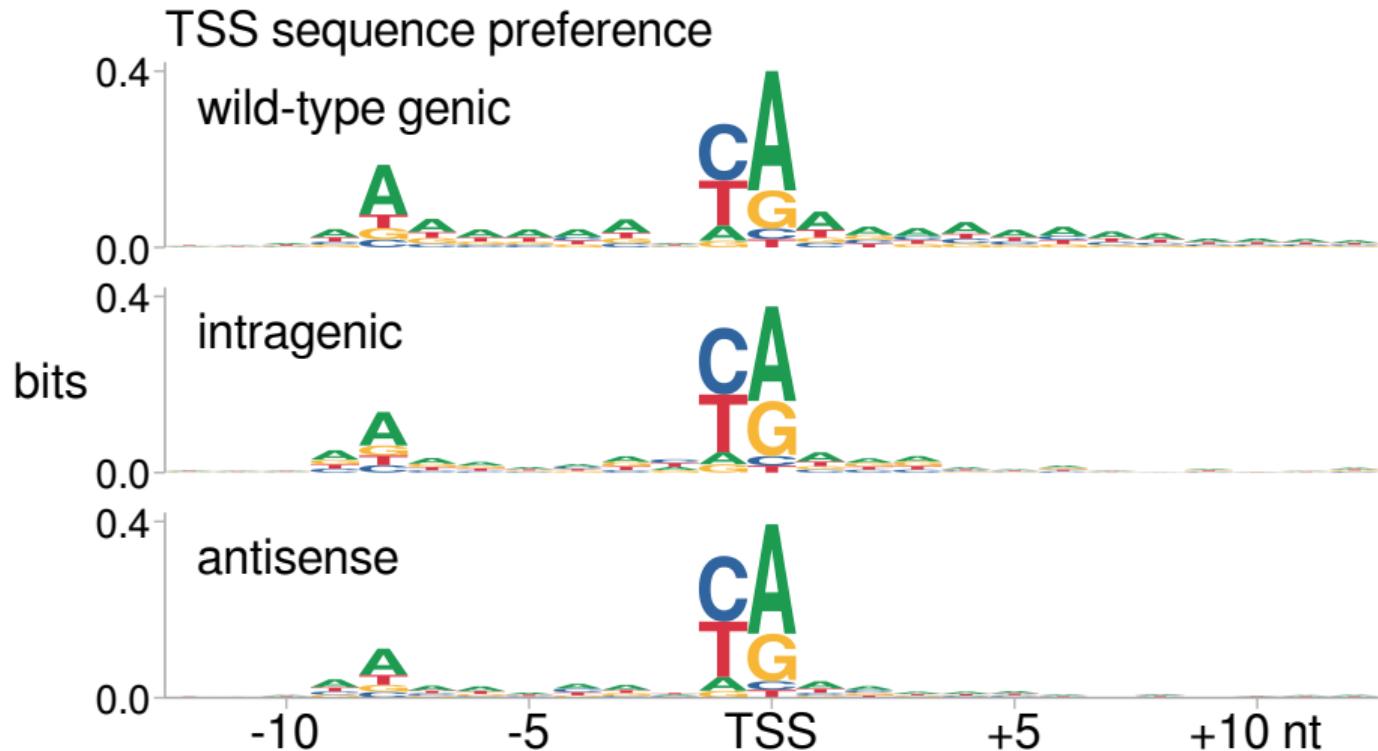
Intragenic promoters have features of genic promoters

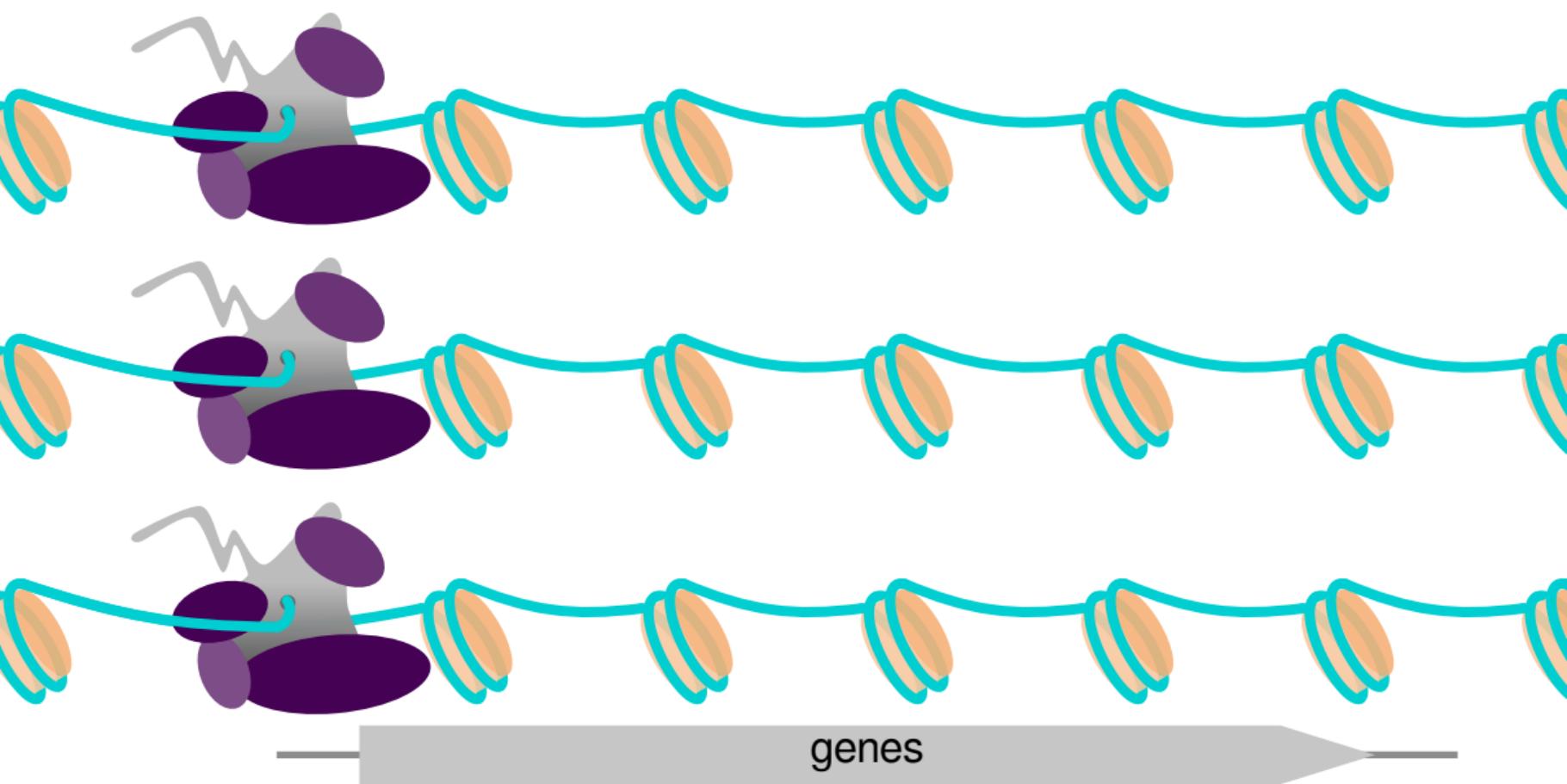


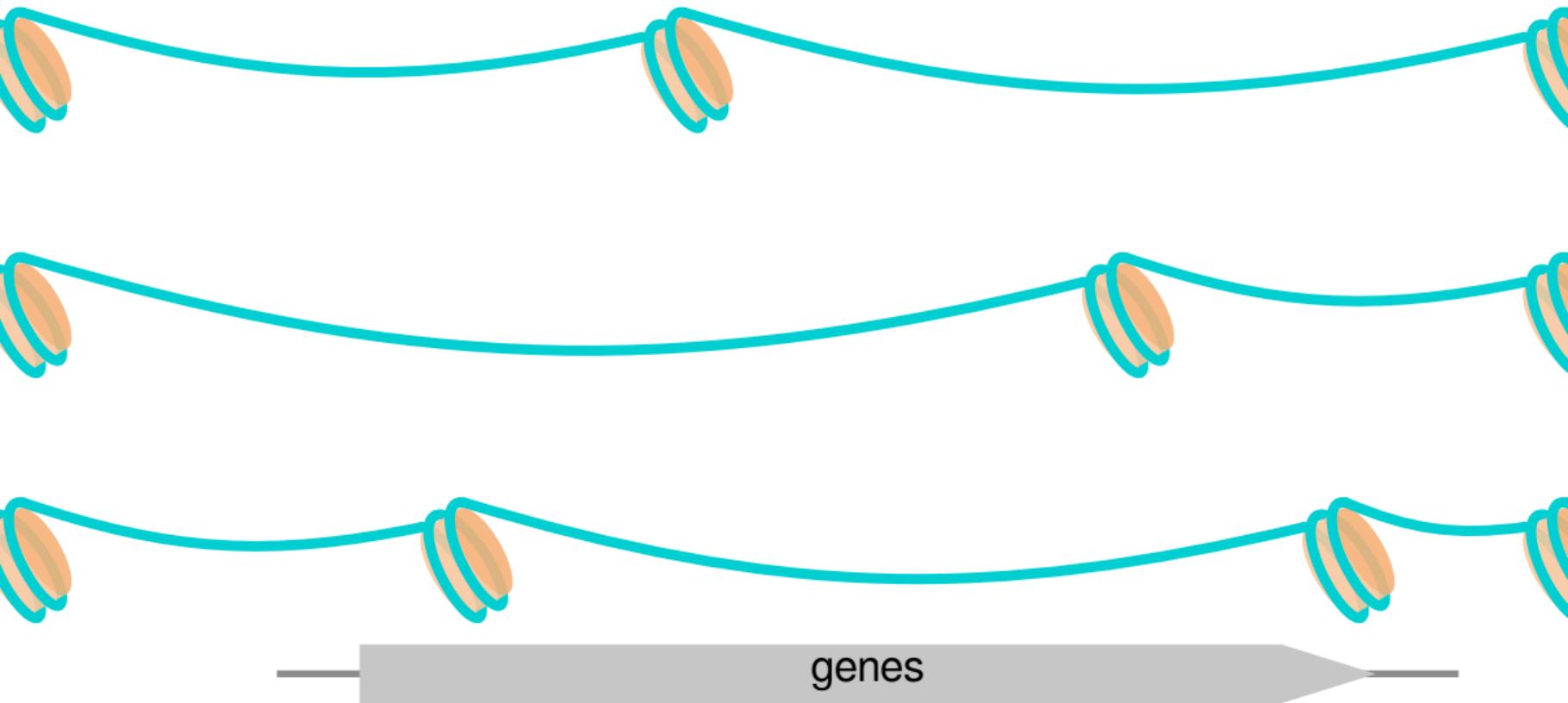
Intragenic promoters have features of genic promoters

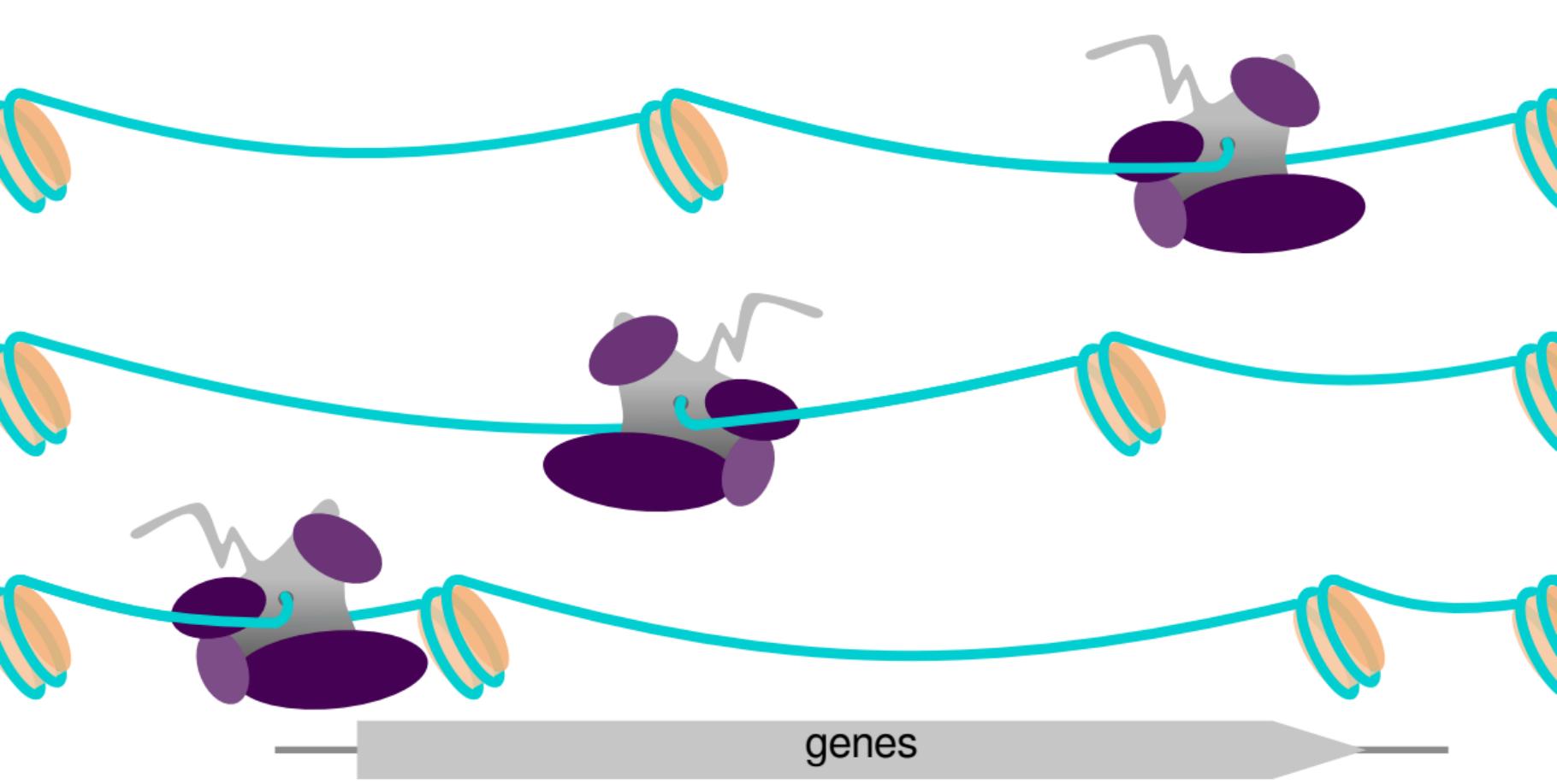


Intragenic promoters have features of genic promoters

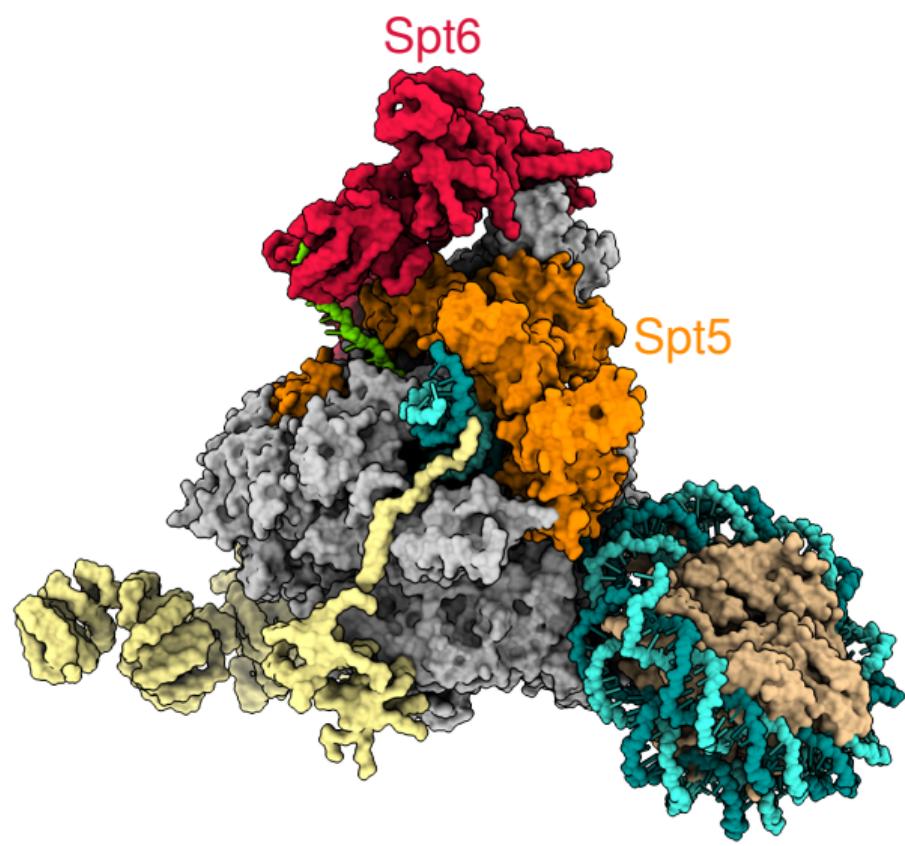




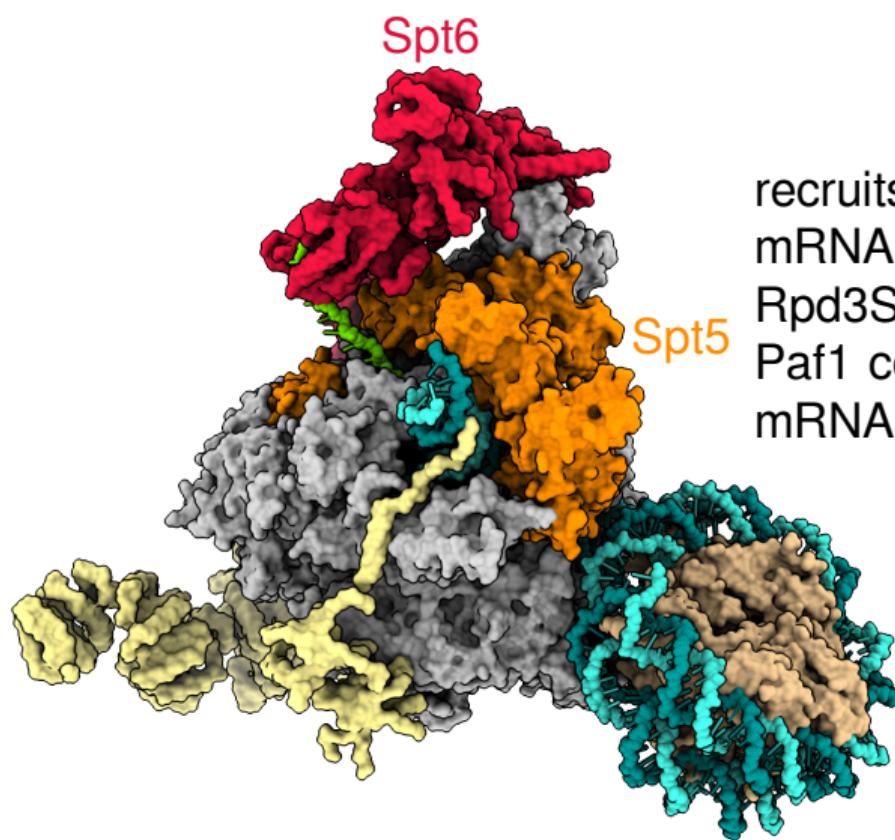




genes



Vos *et al.* (2018). *Nature*
Farnung *et al.* (2018). *Nat. Commun.*



recruits:

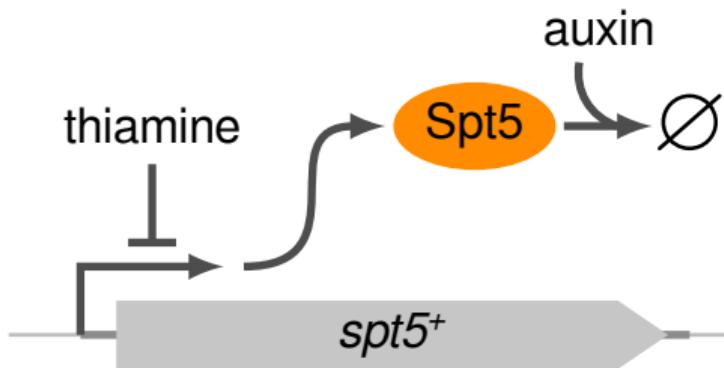
- mRNA capping enzyme
- Rpd3S HDAC complex
- Paf1 complex
- mRNA 3'-end processing factors

Vos *et al.* (2018). *Nature*
Farnung *et al.* (2018). *Nat. Commun.*

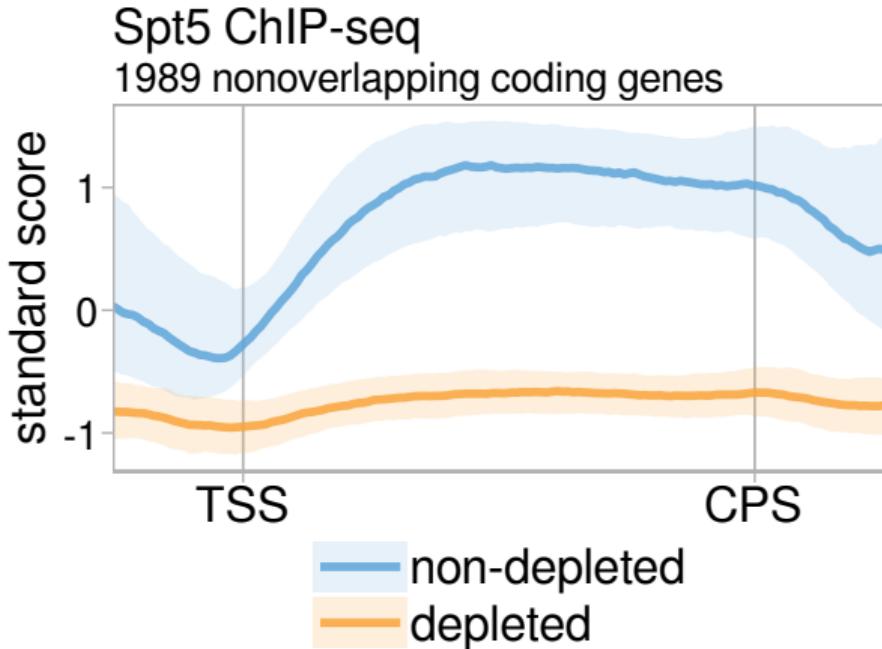
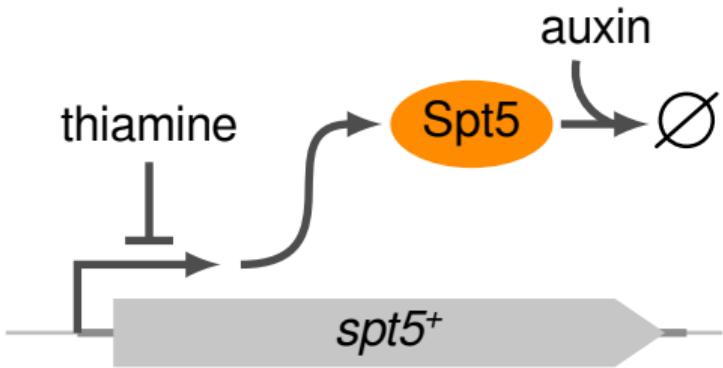
Spt5 project collaborators

Ameet Shetty NET-seq,
ChIP-seq,
RNA-seq,
TSS-seq,
MNase-seq,
etc.

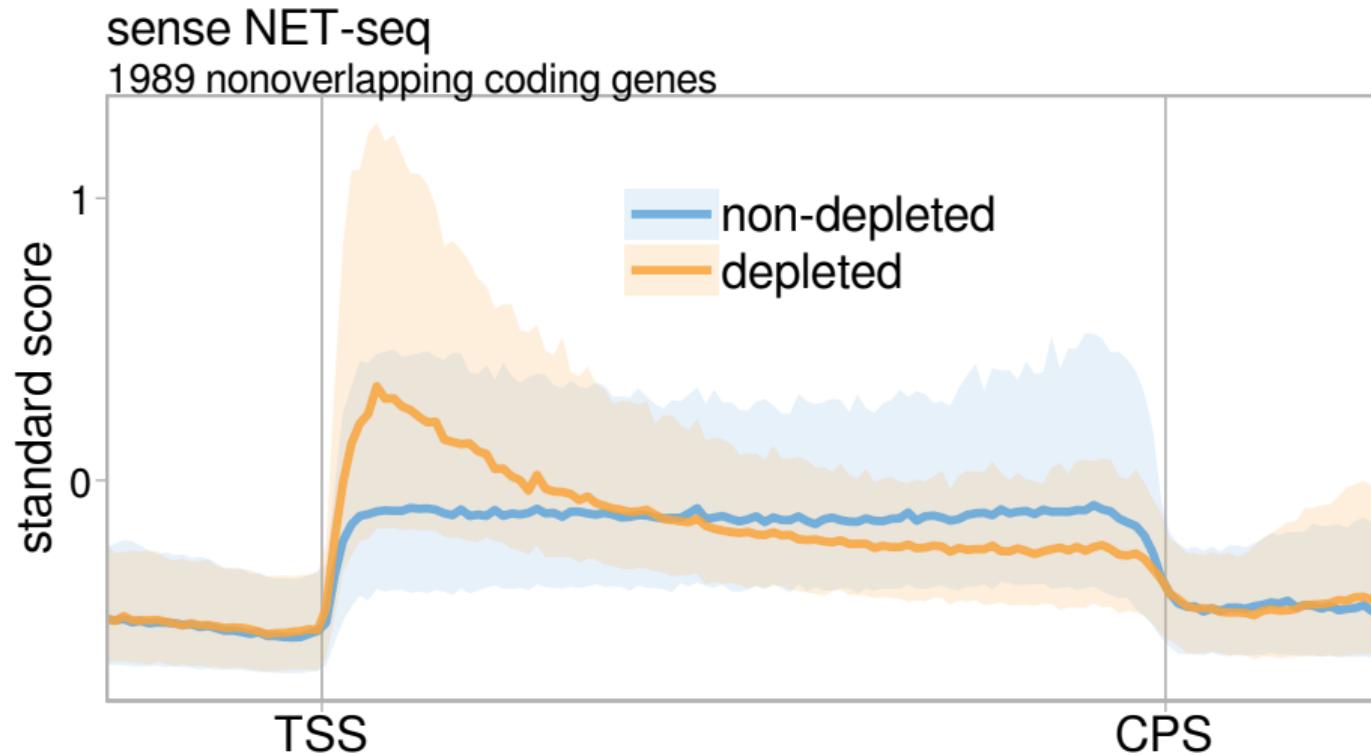
Spt5 depletion system



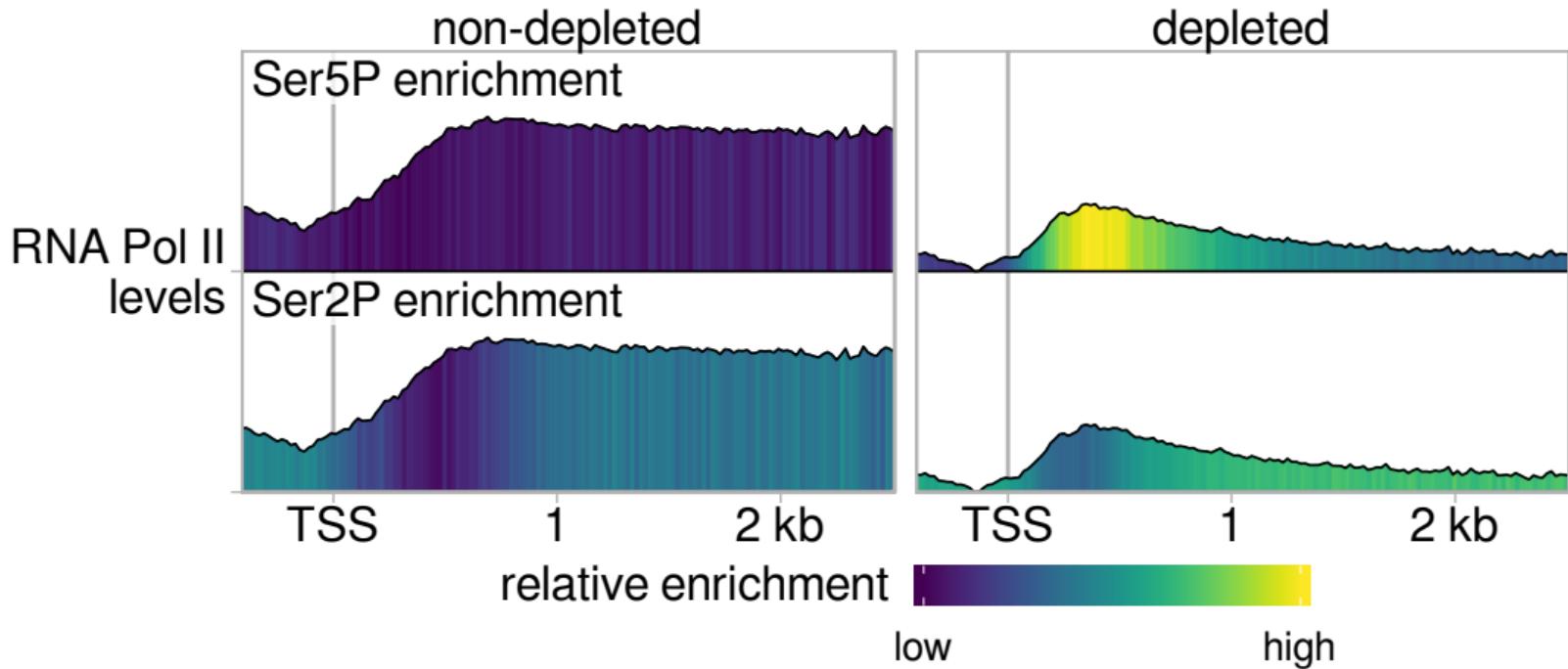
Spt5 depletion system



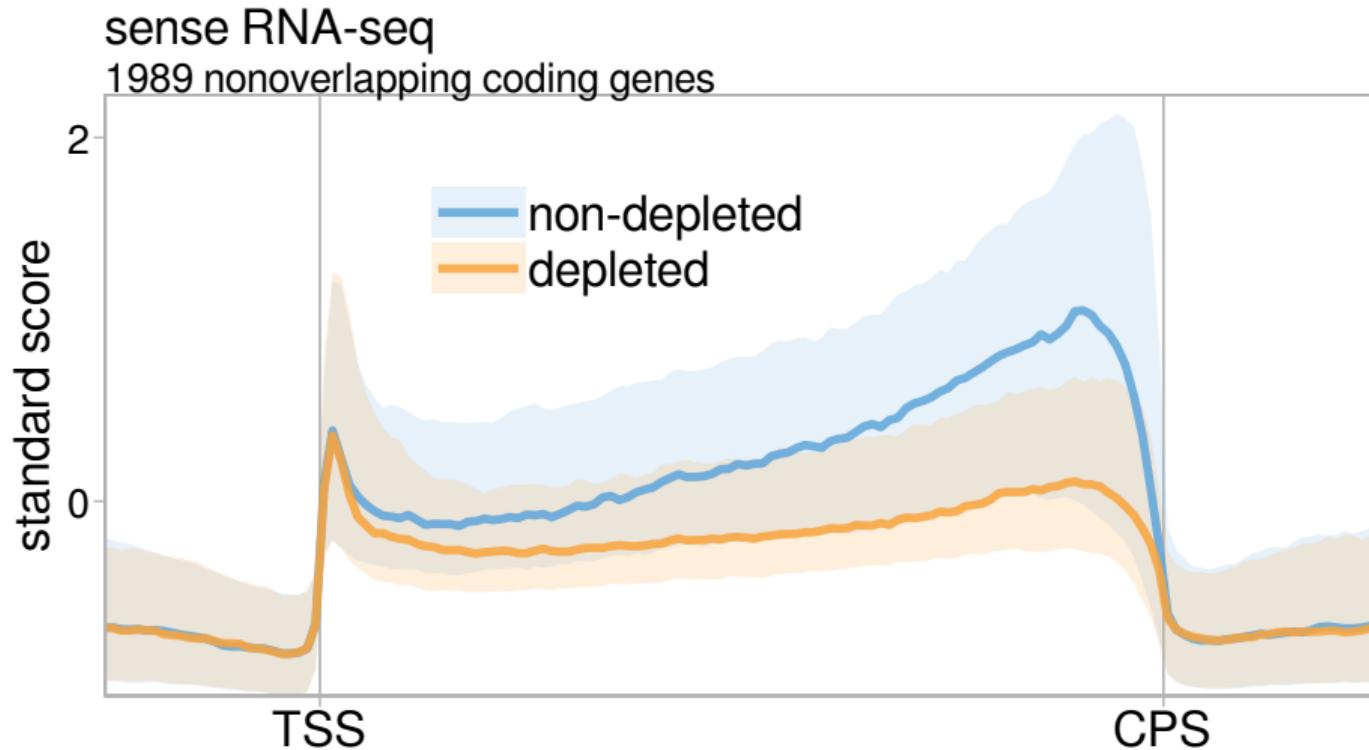
Elongation defects upon Spt5 depletion

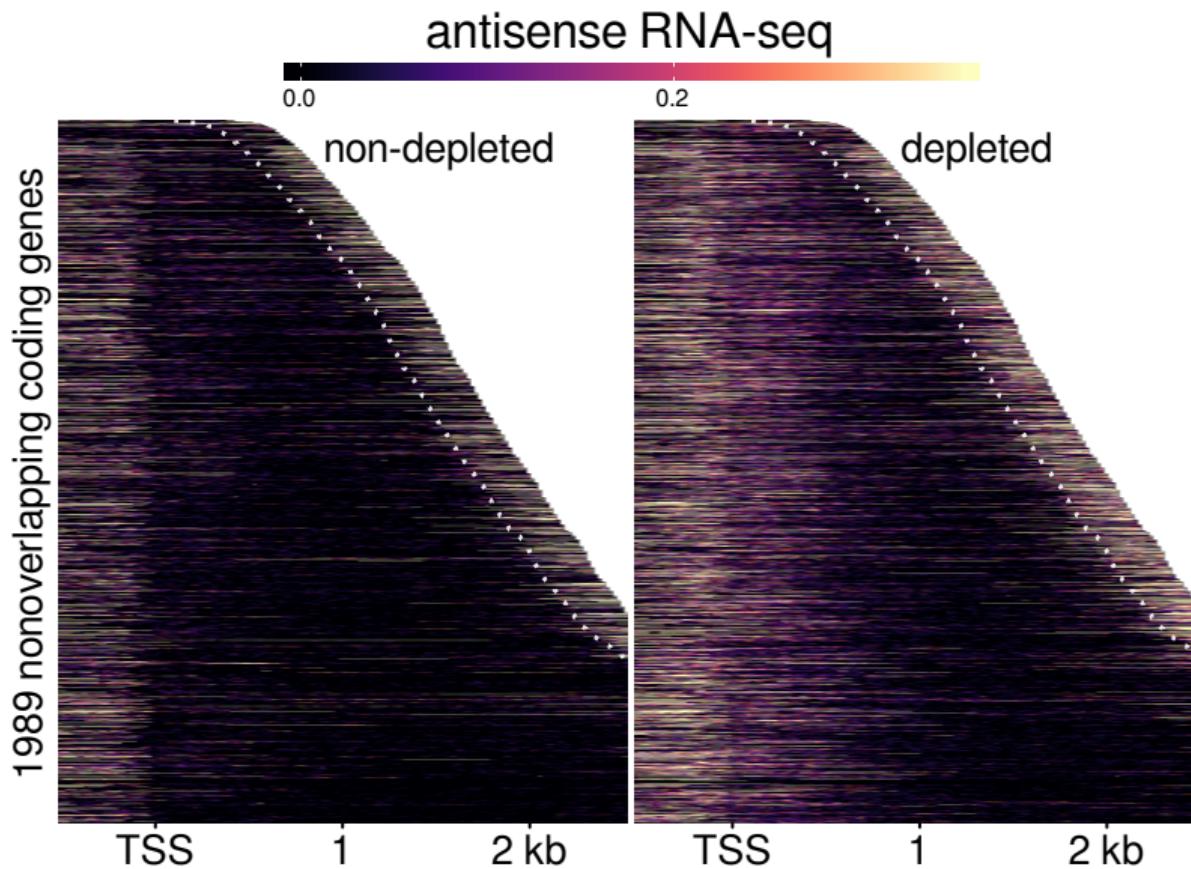


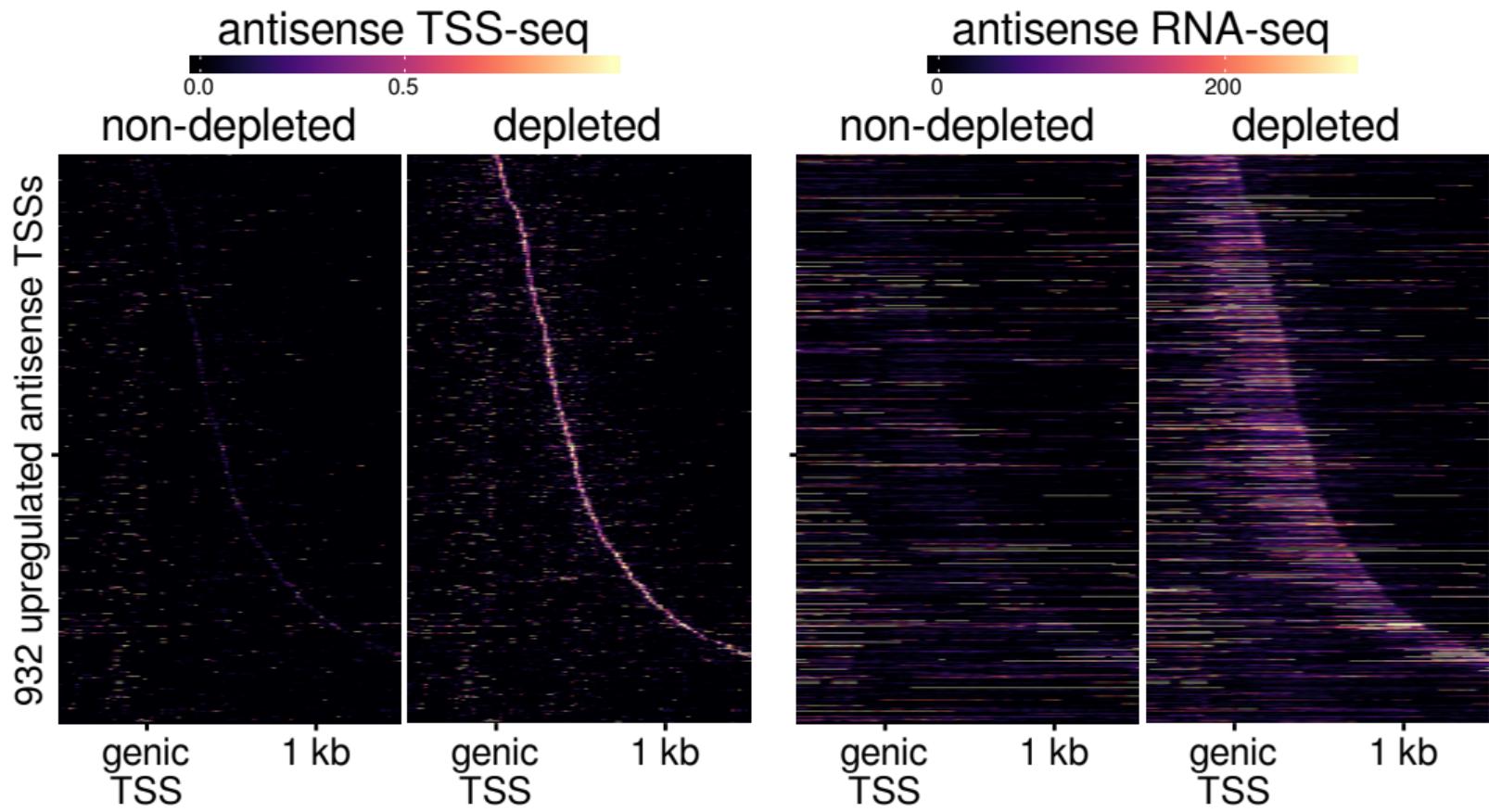
Trapped Pol II is enriched for CTD serine 5 phosphorylation



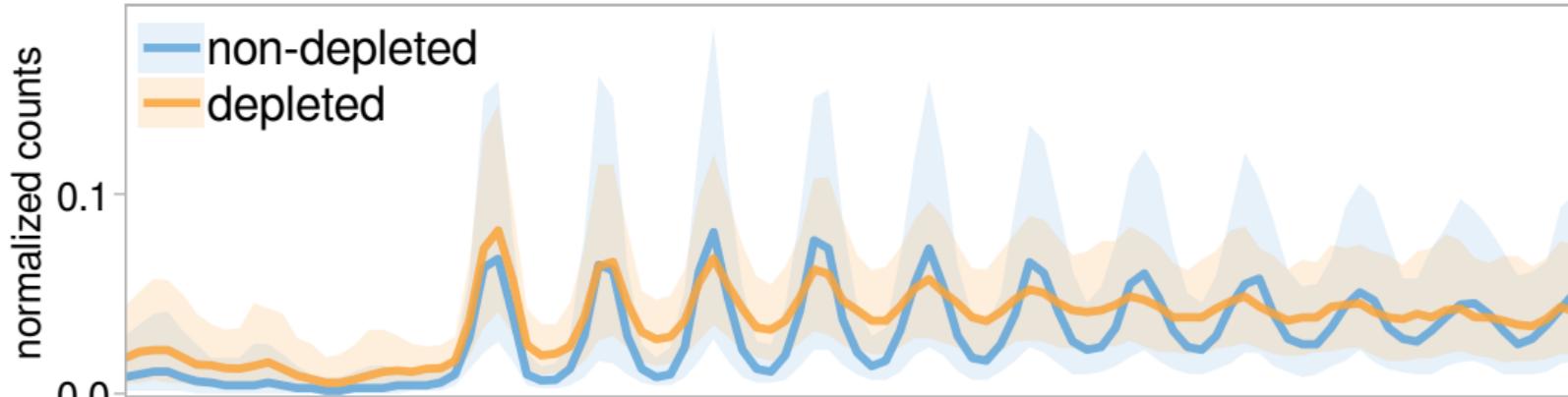
Evidence for premature termination upon Spt5 depletion



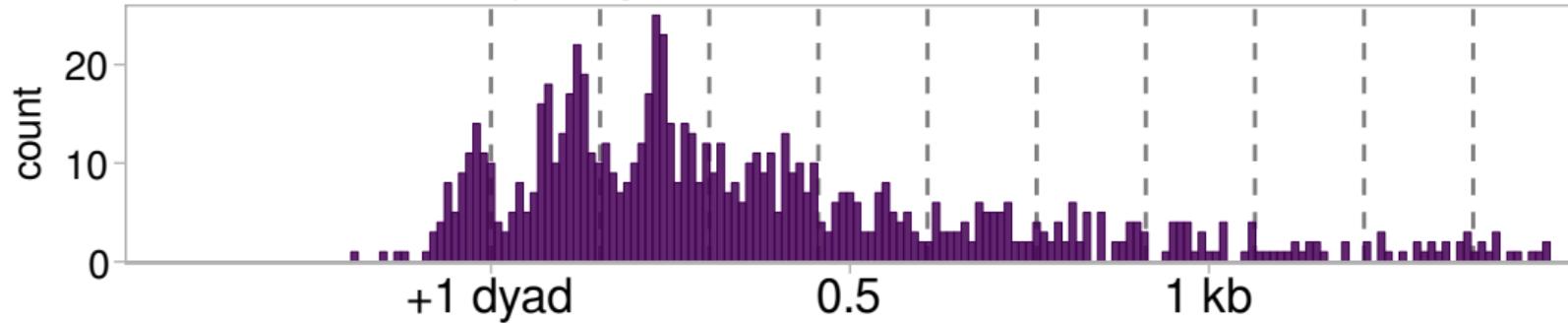




MNase-seq dyad signal

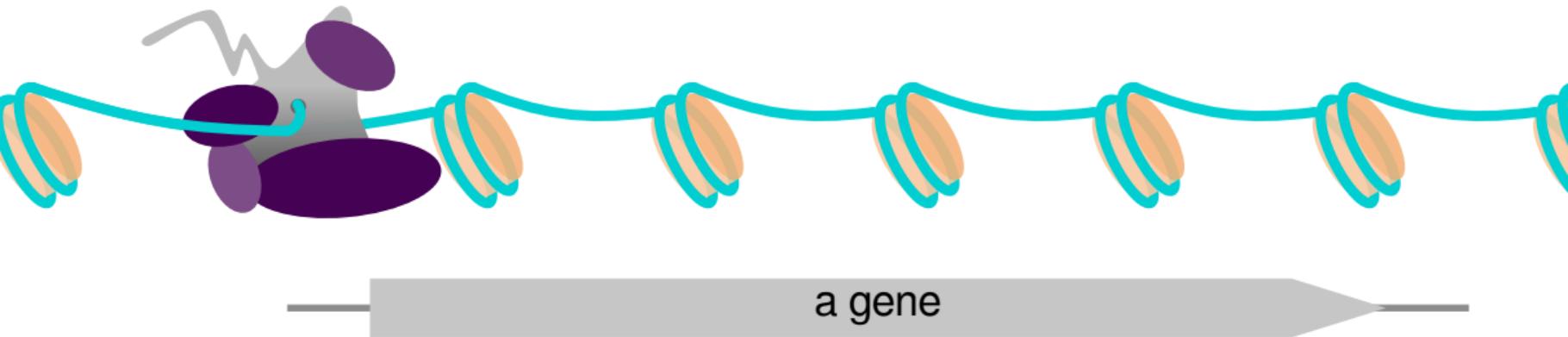


antisense TSS frequency

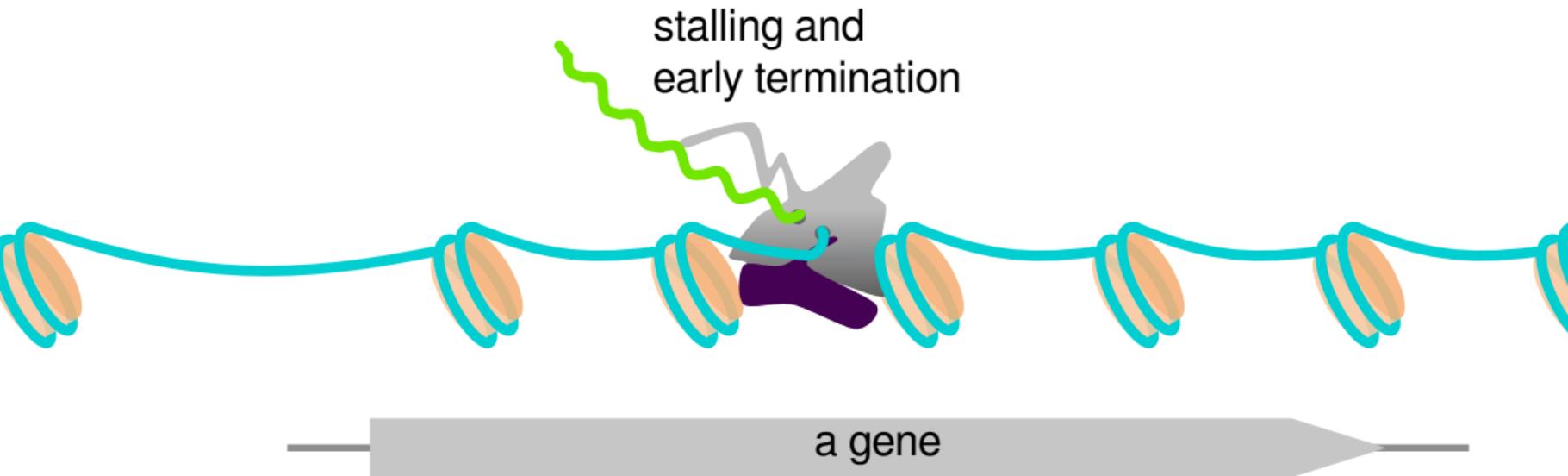


Transcription upon Spt5 depletion:

initiation

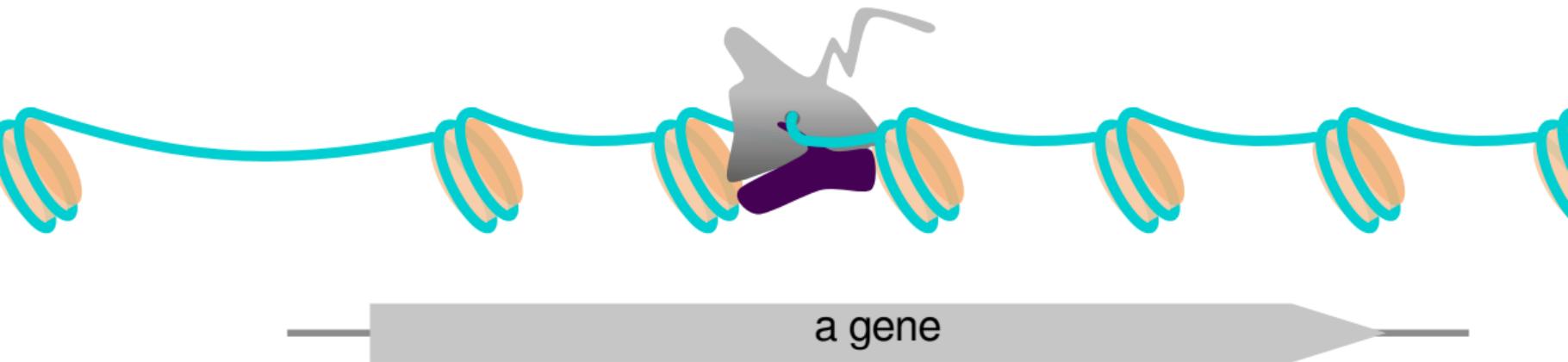


Transcription upon Spt5 depletion:



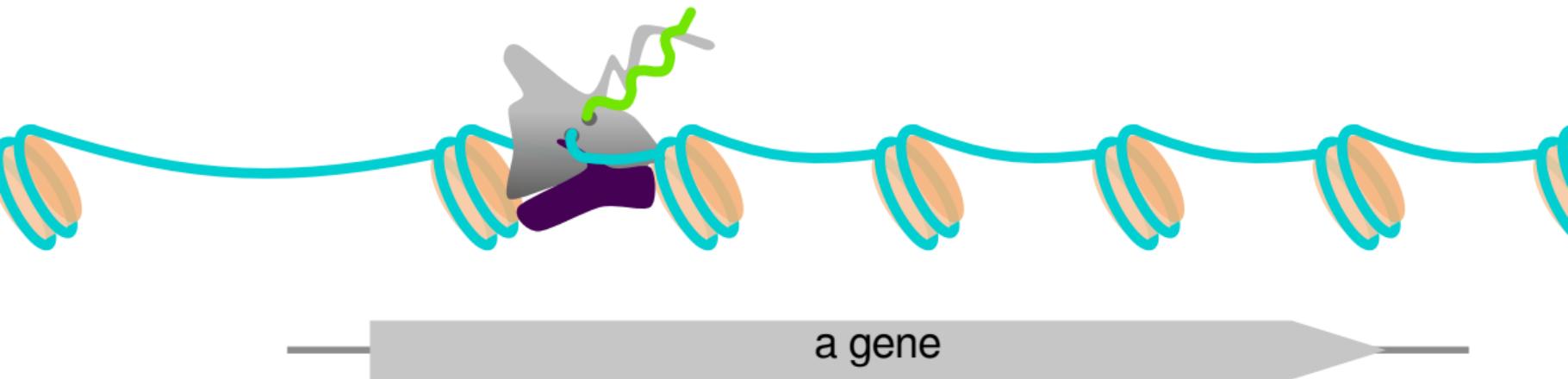
Transcription upon Spt5 depletion:

possible antisense re-initiation

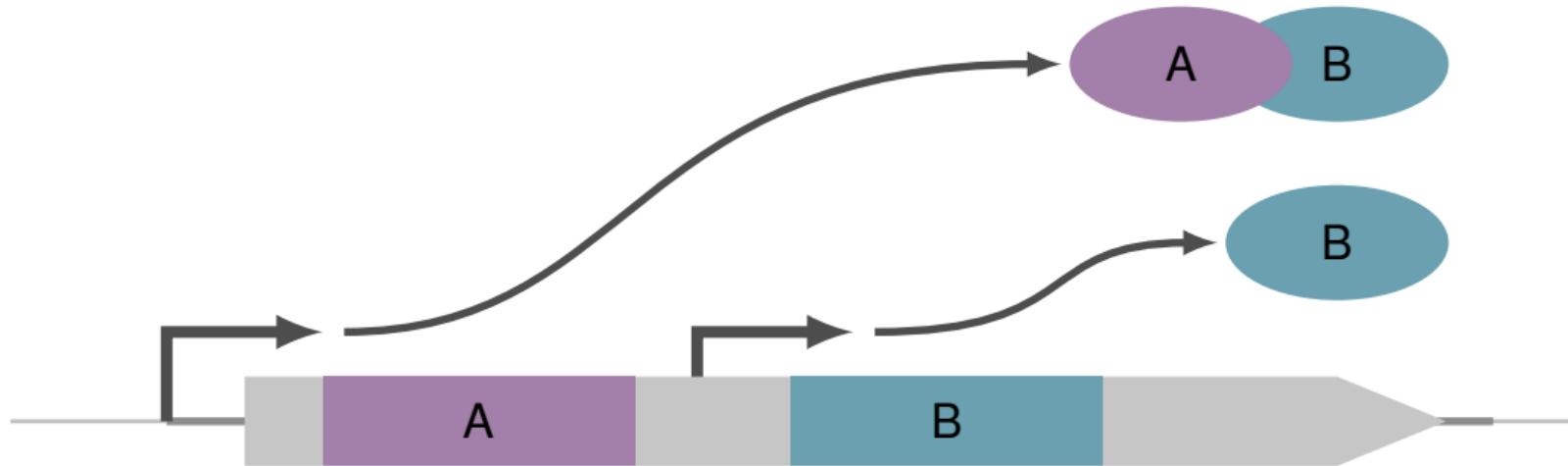


Transcription upon Spt5 depletion:

antisense transcription



Intragenic transcription in wild-type cells



project collaborators

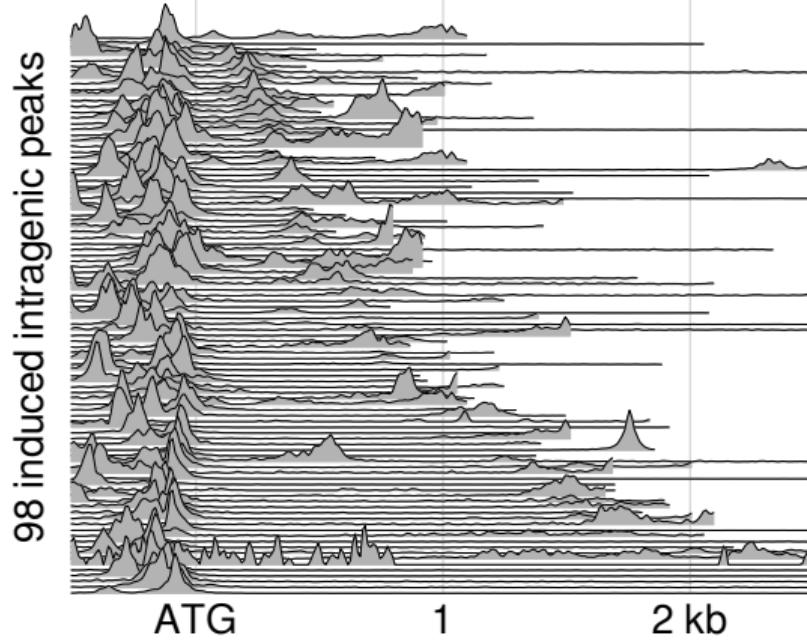
Steve Doris TSS-seq and ChIP-nexus

Dan Spatt polyribosome fractionation,
competitive growth assays,
and Northern blots

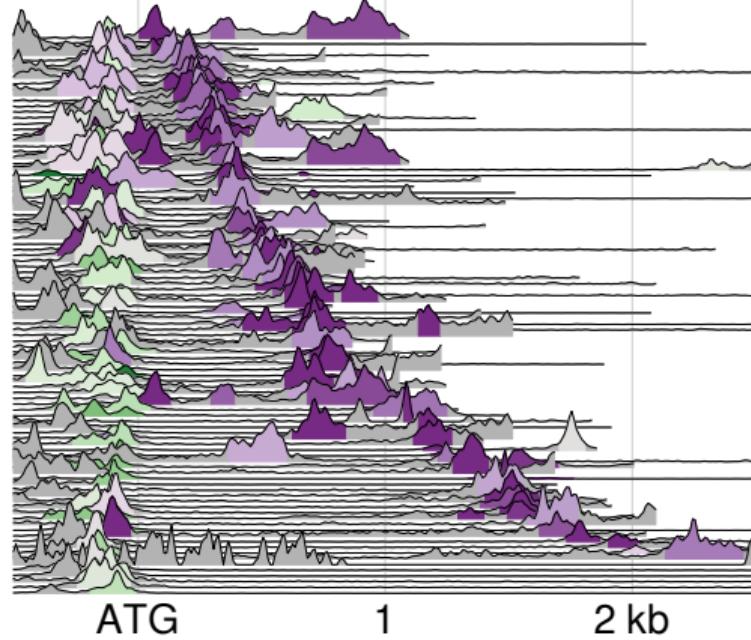
James Warner Northern blots

relative TFIIB protection

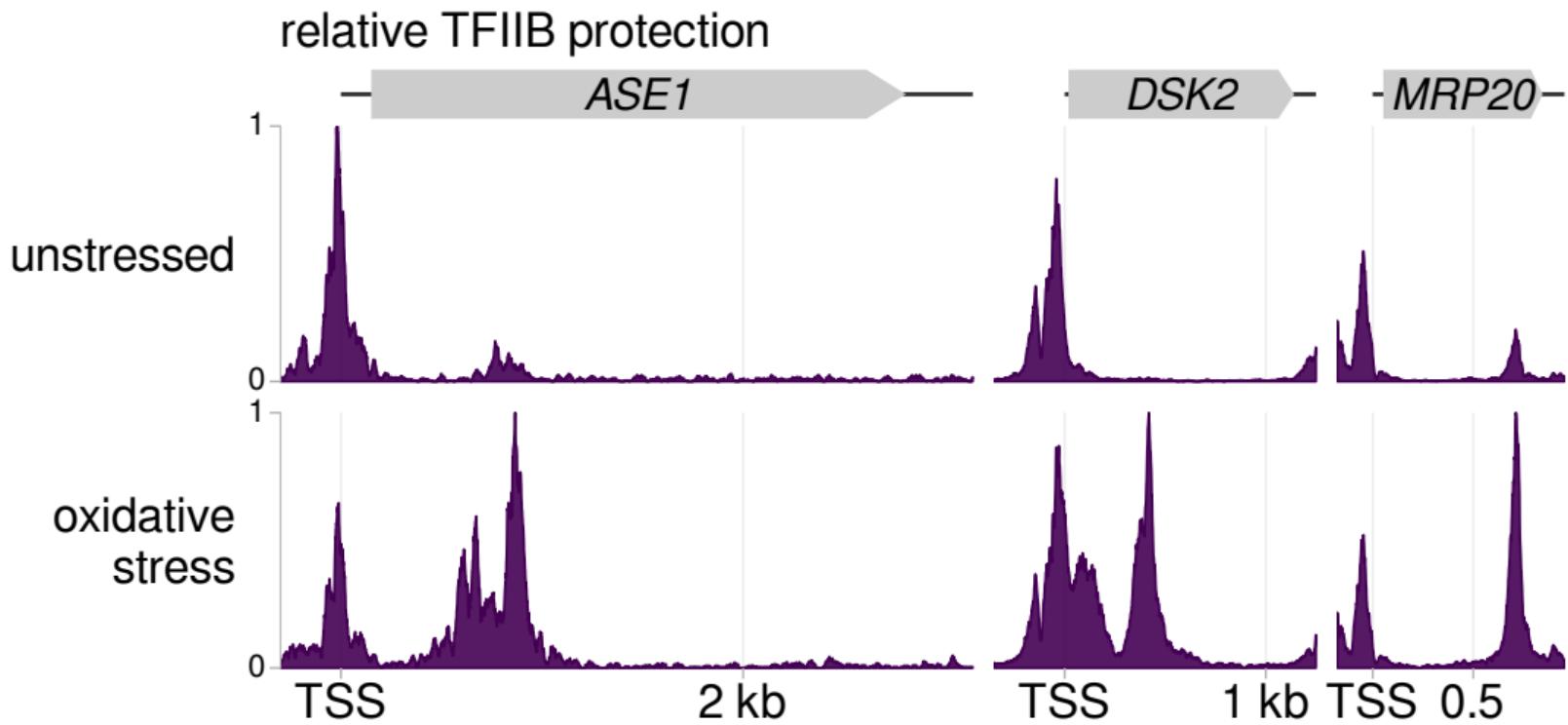
unstressed



oxidative stress

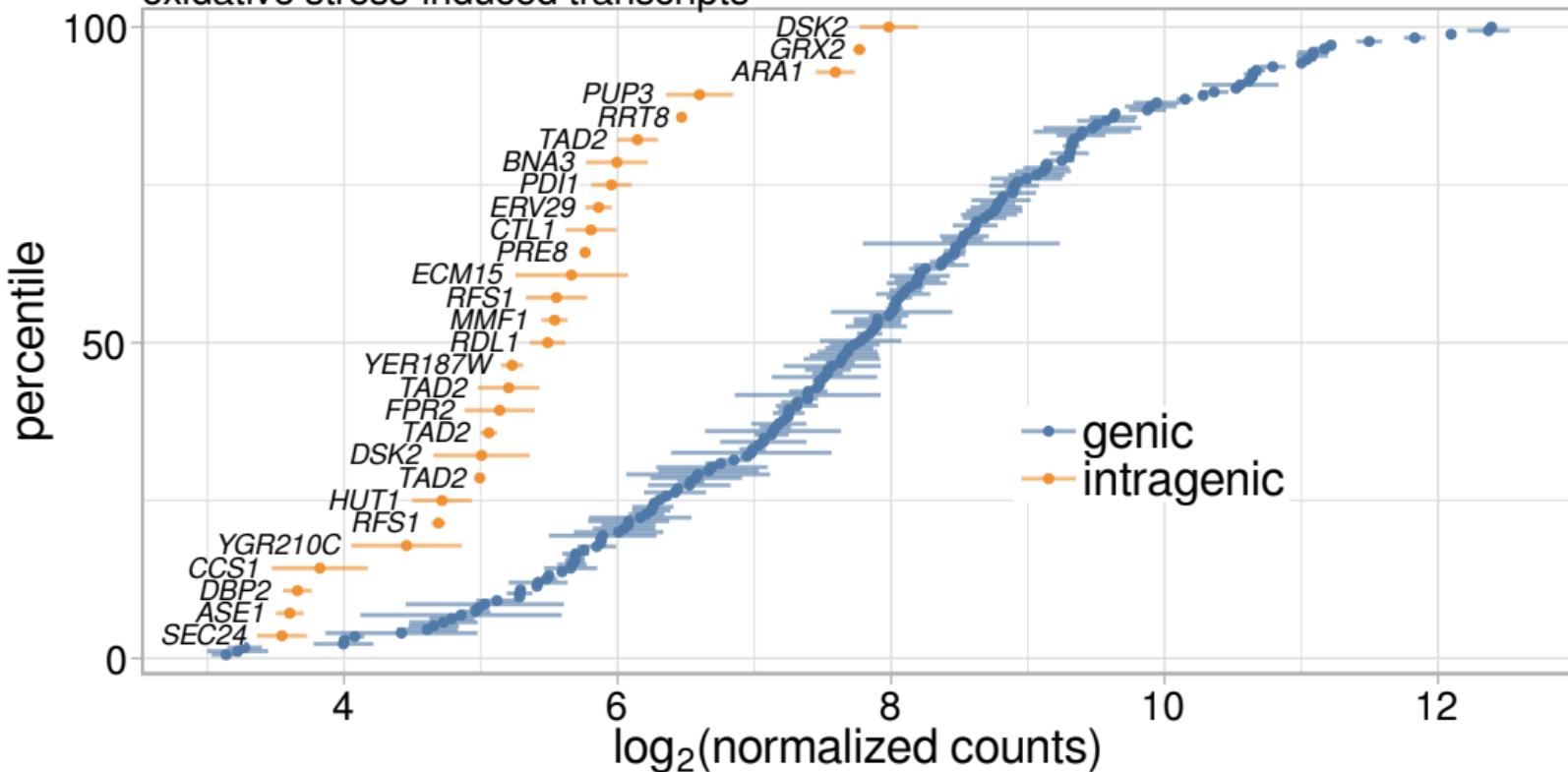


A horizontal color scale bar representing the ratio $\log_2 \frac{\text{oxidative stress}}{\text{unstressed}}$. The scale ranges from -2 to 2, with tick marks at -2, -1, 0, 1, and ≥ 2 . The color gradient transitions from dark green for negative values, through light green and yellow for zero, to orange and red for positive values.

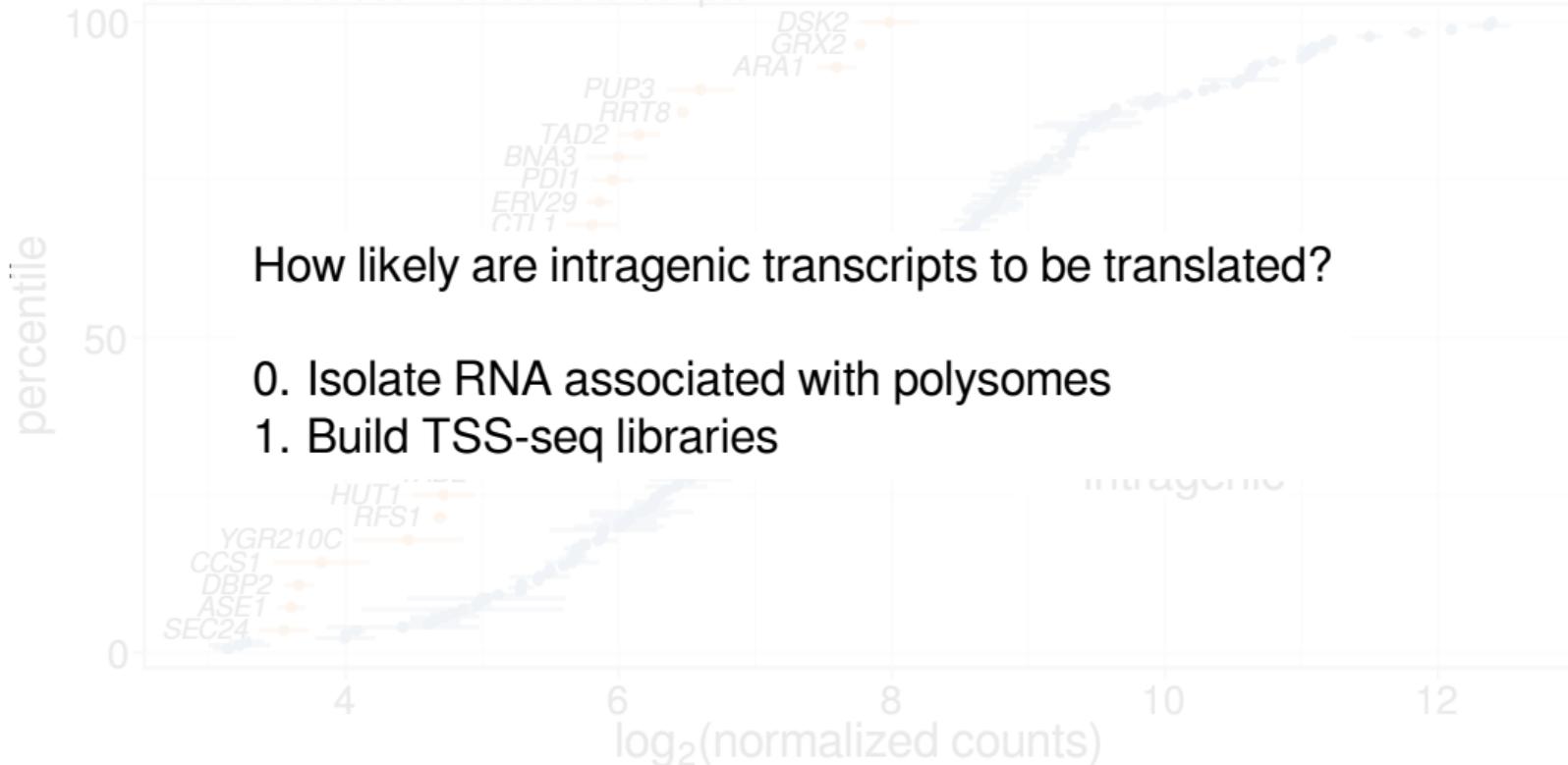


transcript abundances in oxidative stress

oxidative stress-induced transcripts

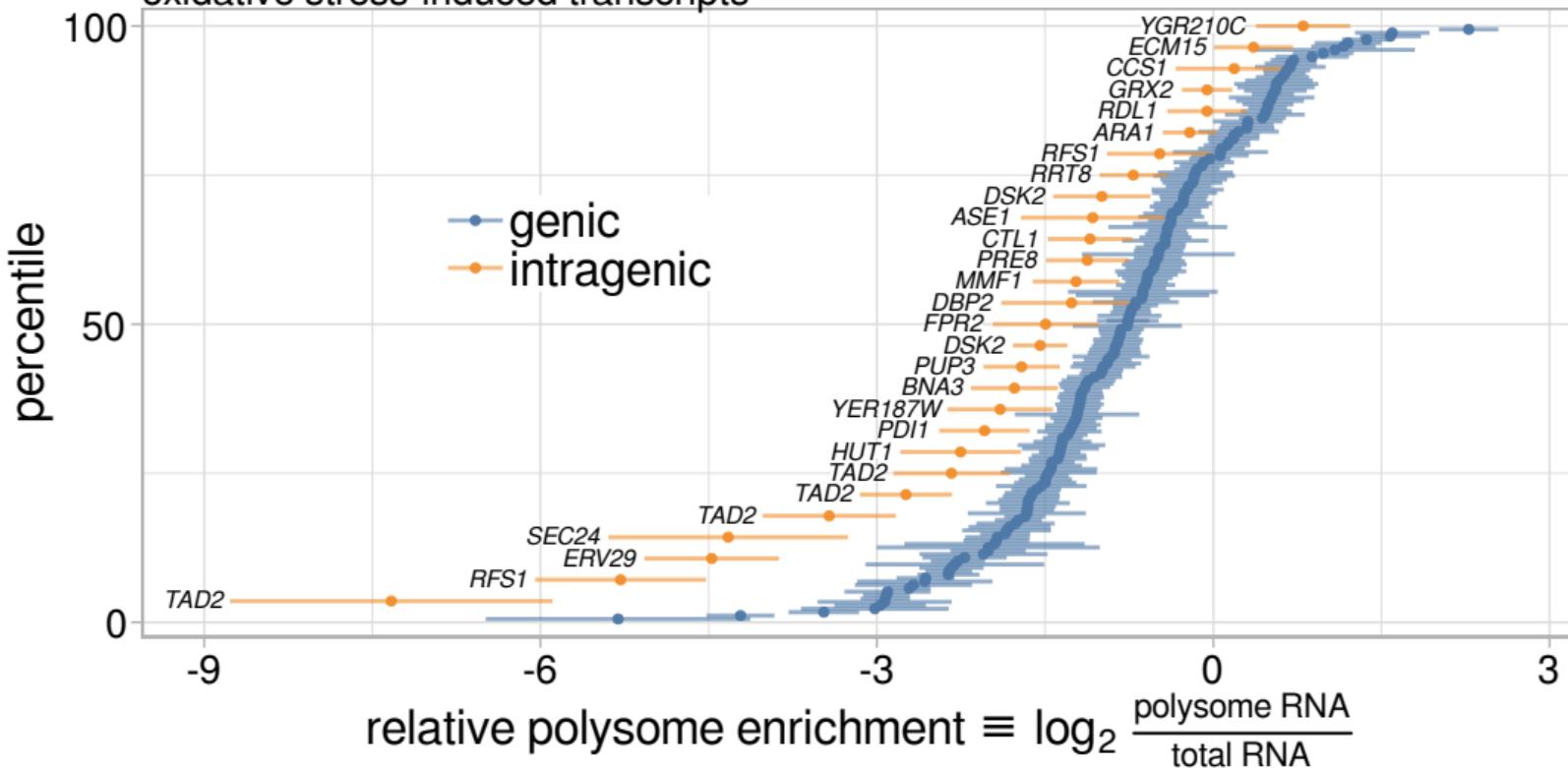


transcript abundances in oxidative stress oxidative stress-induced transcripts

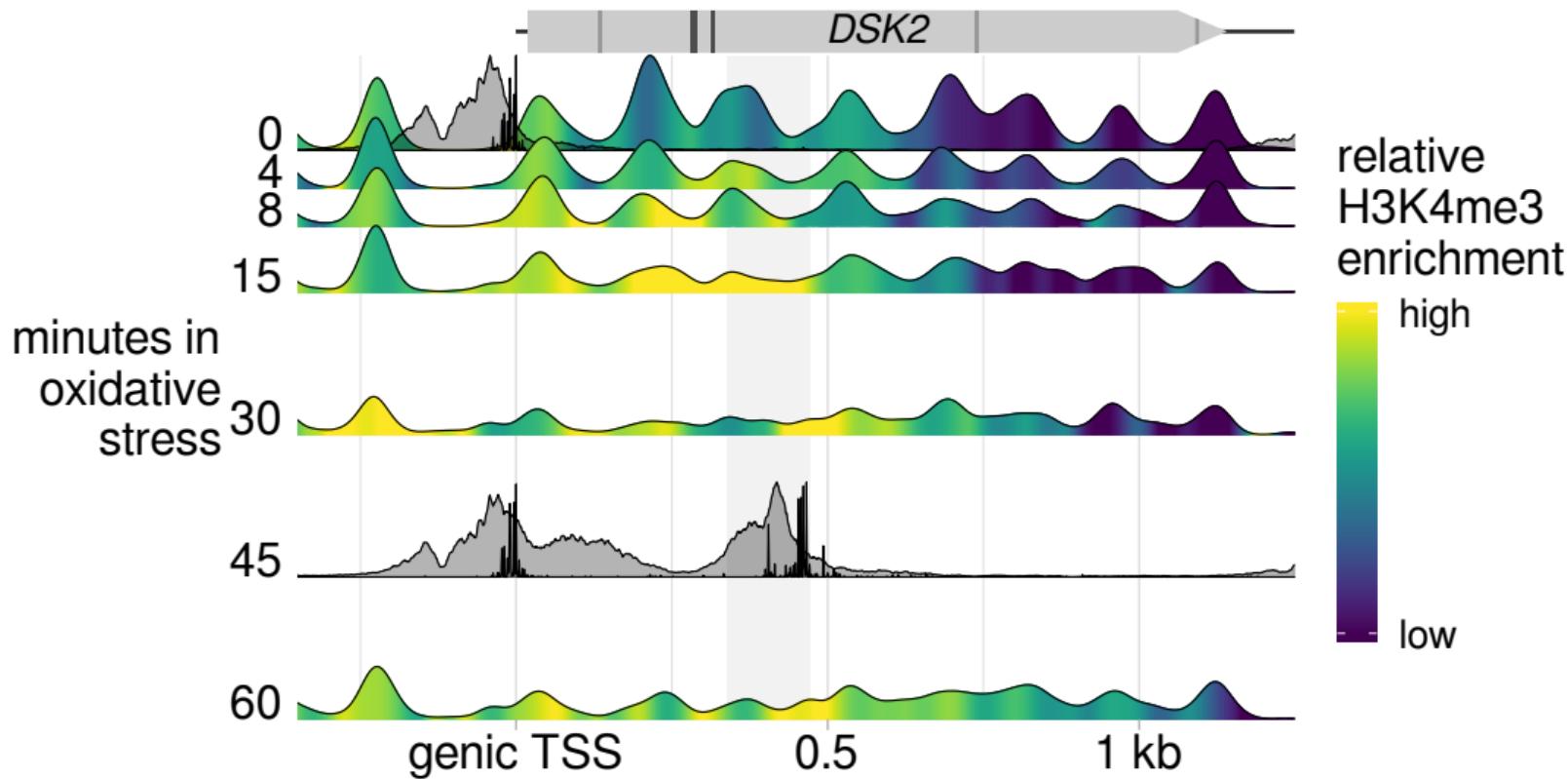


polysome enrichment in oxidative stress

oxidative stress-induced transcripts

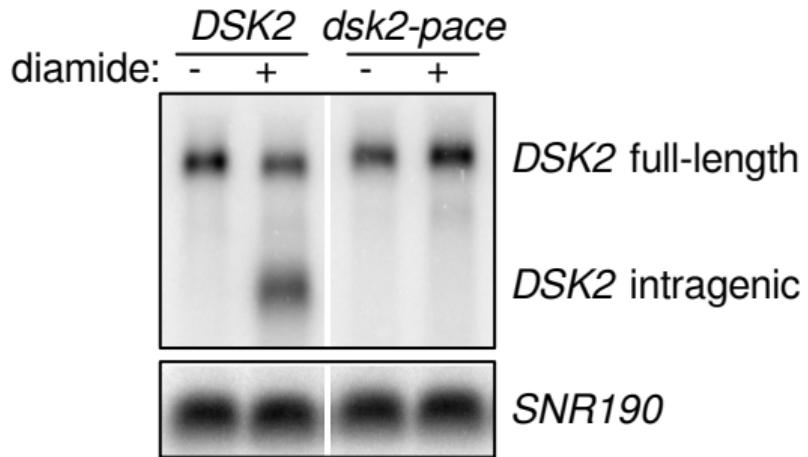


MNase dyads, TFIIB protection, and sense TSSs

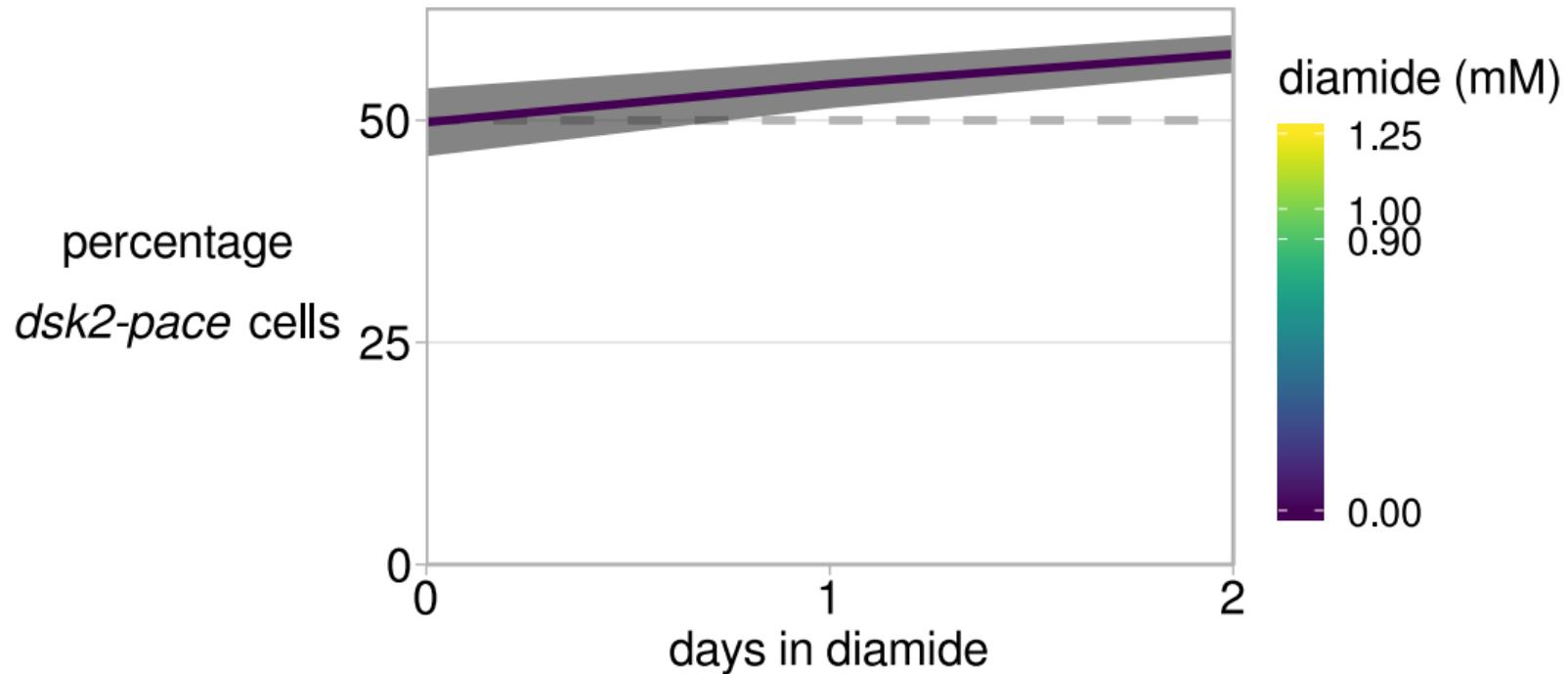


MNase-ChIP-seq: Weiner *et al.* (2015). *Mol. Cell*

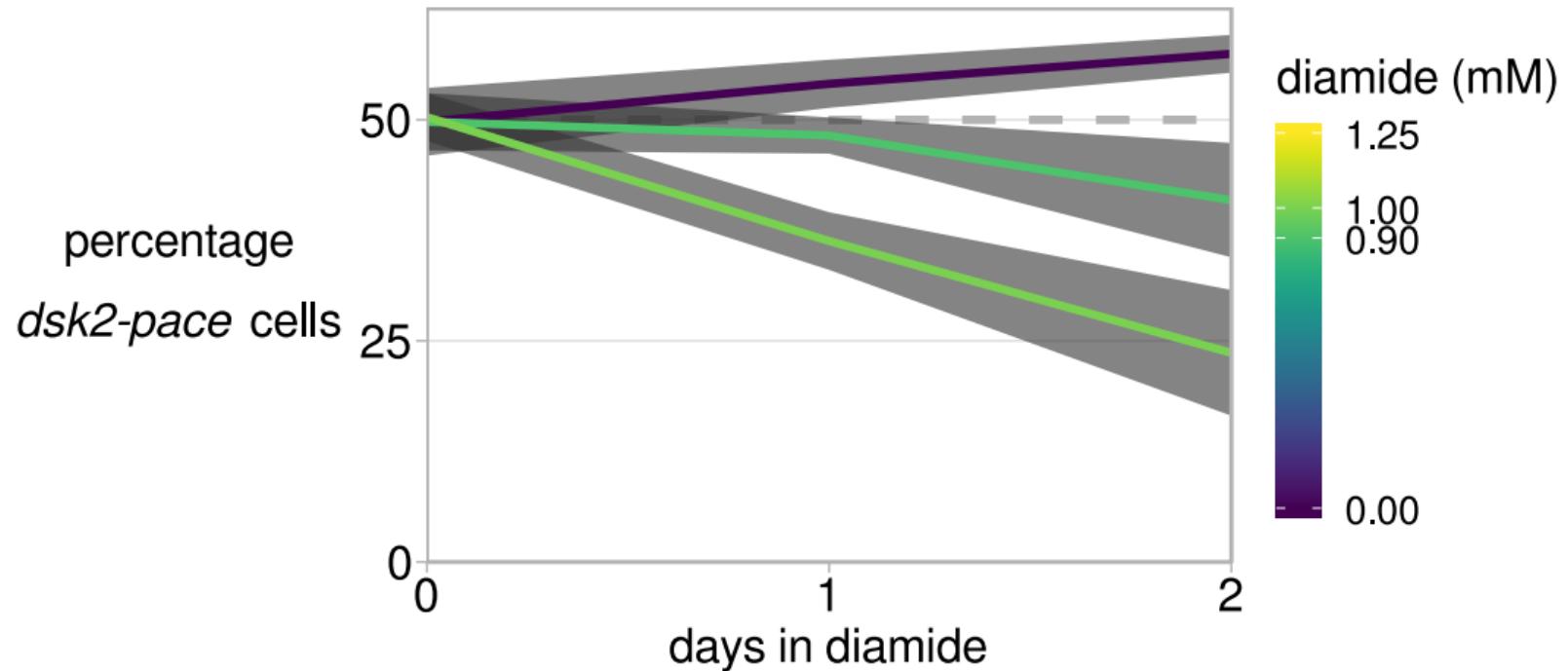
intragenic *DSK2* expression requires PACE elements



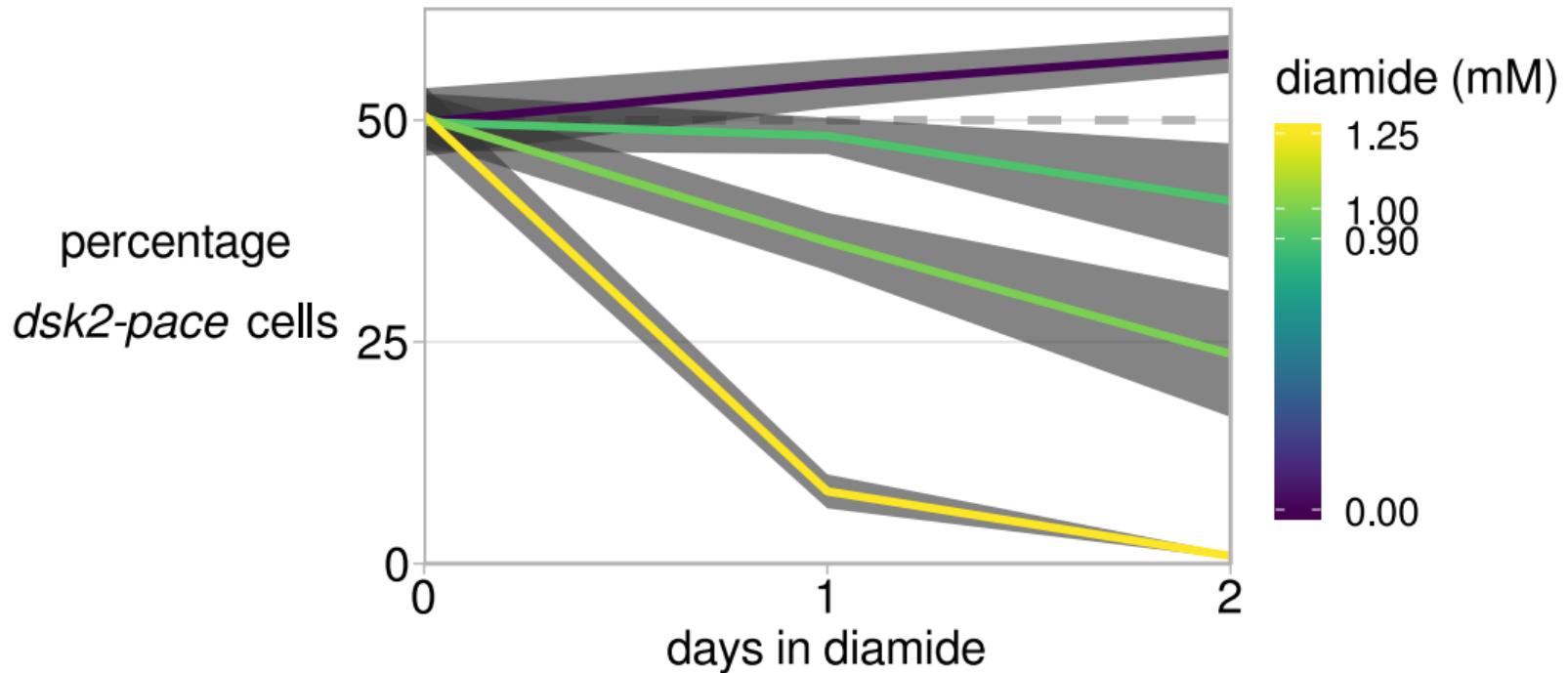
dsk2-pace cells respond poorly to oxidative stress



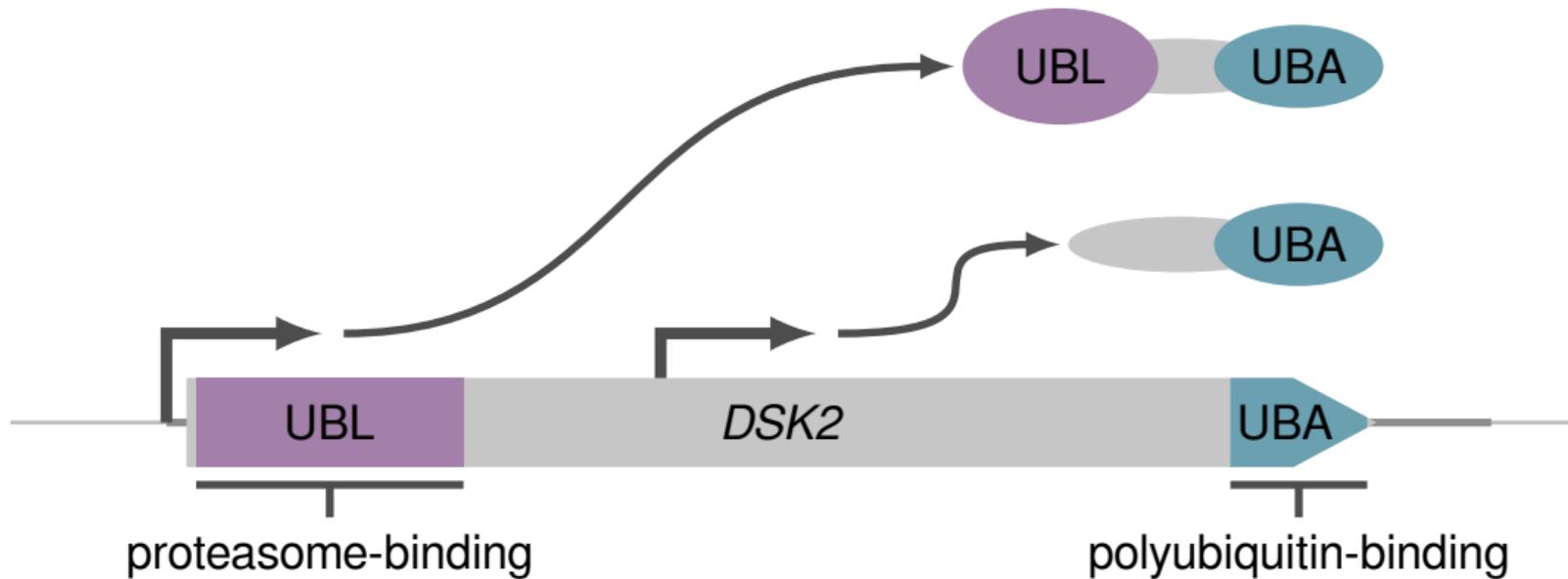
dsk2-pace cells respond poorly to oxidative stress



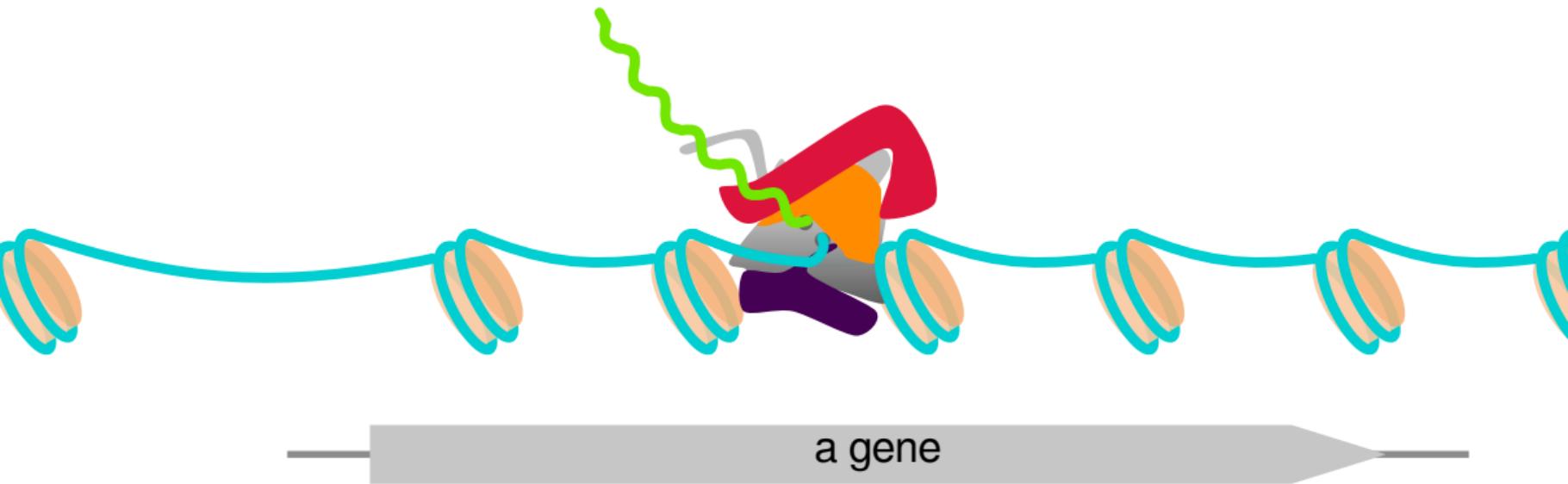
dsk2-pace cells respond poorly to oxidative stress



One possible mechanism for intragenic *DSK2* function



Summary



Acknowledgements

Winston lab

Fred Winston
Ameet Shetty
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Rajaraman Gopalakrishnan
Francheska Lopez Rivera
Katie Weiner
James Warner
Francis Apolinario
Mallory Rice

dissertation committee

Fred Winston
Mo Khalil
Stirling Churchman
John Ngo
Wilson Wong

HMS Research Computing
github.com/winston-lab